

ENDOSULFAN  
RISK CHARACTERIZATION DOCUMENT

VOLUME I

MEDICAL TOXICOLOGY and WORKER HEALTH AND SAFETY BRANCHES  
DEPARTMENT OF PESTICIDE REGULATION  
CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

Date: November 16, 2007

**CONTRIBUTORS AND ACKNOWLEDGEMENTS**

Principal Author: Marilyn H. Silva, Ph.D., D.A.B.T.  
Staff Toxicologist  
SB 950 Data Review Section  
Medical Toxicology Branch

Toxicology Reviews: Marilyn H. Silva, Ph.D., D.A.B.T.  
Staff Toxicologist  
SB 950 Data Review Section  
Medical Toxicology Branch

Dietary Exposure Assessment: Wesley C. Carr, Jr., M.S  
Associate Pesticide Review Scientist  
Health Assessment Section  
Medical Toxicology Branch

Worker Exposure Assessment: Sheryl Beauvais, Ph.D.  
Staff Toxicologist  
Exposure Assessment Section  
Worker Health and Safety Branch

Reviews By: Joyce Gee, Ph.D.  
Senior Toxicologist  
Health Assessment Section  
Medical Toxicology Branch

Keith Pfeifer, Ph.D. (Retired)  
Senior Toxicologist  
Health Assessment Section  
Medical Toxicology Branch

Jay P. Schreider, Ph.D.  
Primary State Toxicologist  
Medical Toxicology Branch

**TABLE OF CONTENTS**

I. SUMMARY .....	1
II. INTRODUCTION .....	21
A. Chemical Identification .....	21
B. Regulatory History .....	22
C. Technical and Product Formulations .....	23
D. Usage .....	23
E. Reported Illness .....	24
F. Physical and Chemical Properties .....	25
G. Environmental Fate .....	26
III. TOXICOLOGY PROFILE .....	34
A. Pharmacokinetics .....	34
B. Acute Toxicity .....	45
C. Subchronic Toxicity .....	54
D. Chronic Toxicity and Oncogenicity .....	63
E. Genotoxicity .....	67
F. Reproductive Toxicity .....	72
G. Developmental Toxicity .....	80
H. Neurotoxicity .....	85
IV. RISK ASSESSMENT .....	93
A. Hazard Identification .....	93
B. Exposure Assessment .....	103
C. Risk Characterization .....	147
V. RISK APPRAISAL .....	157
A. Introduction .....	157
B. Hazard Identification .....	157
C. Exposure Appraisal .....	161
D. Risk Characterization .....	169
E. Issues Related to the Food Quality Protection Act .....	173
VI. TOLERANCE ASSESSMENT .....	183
A. Introduction .....	183
B. Acute Tolerance Assessment .....	184
C. Chronic Tolerance Assessment .....	186
D. Tolerance Assessment - 2006 .....	186
VII. REFERENCE DOSES/CONCENTRATIONS .....	188
VIII. CONCLUSIONS .....	191
IX. REFERENCES .....	196
X. APPENDICES	
A. Endosulfan Dietary Exposure Assessment (1998) .....	Appendix A
B. Residue Data and Tolerance Assessment .....	Appendix B
C. Endosulfan Dietary Exposure Assessment – Addendum .....	Appendix C

D. Summary of Toxicology Data ..... Appendix D  
E. VOLUME II: Exposure Assessment Document ..... Appendix E  
F. USEPA Comments and DPR Response ..... Appendix F  
G. Office of Environmental Health Hazard Assessment Comments and DPR Response  
..... Appendix G  
H. Endosulfan Task Force Comments and DPR Response ..... Appendix H

**List of Tables and Figures**

Figure 1. Proposed Metabolic Pathway for Endosulfan . . . . .3 and 41

Table 1. Illnesses Reported in California Associated with Endosulfan Exposure (1992-2003) . . . 25

Table 2. Summary of Historical Surface Water Sampling Data for Endosulfan in California  
Between August 1990 and July 1996 . . . . . 29

Table 3. Clinical Signs of Neurotoxicity Observed in a Rat LC<sub>50</sub> Study (Hollander & Weigand, 1983) . . 48

Table 4. Effects of Endosulfan in a 13-Week Rat Subchronic Diet Study and 4 Week  
Recovery. . . . . 56

Table 5. Pathological effects of Endosulfan in a 13-Week Rat Subchronic Diet Study With 4-  
Week Recovery . . . . . 57

Table 6. Non-neoplastic Pathological effects in a 104-Week Dietary Rat Oncogenicity Study . . . 64

Table 7. Major Effects Observed in Dogs After 1 Year of Endosulfan Treatment . . . . . 66

Table 8. Chromosome aberrations in Mice Induced by Different Doses of Endosulfan. . . . . 69

Table 9. Developmental Effects Observed in Fetal Rats . . . . . 82

Table 10. Maternal Toxicity in Rabbits After Endosulfan Treatment . . . . . 84

Table 11. Rabbit Dam Toxicity in a Developmental Study, After Treatment Gestation Days 6-28. . 84

Table 12. The Acute Effects of Endosulfan and the NOELs and LOELs . . . . . 96

Table 13. The Subchronic Effects of Endosulfan and the NOELs and LOELs . . . . . 98

Table 14. The Chronic Effects of Endosulfan and the NOELs and LOELs . . . . .100

Table 15. Genotoxicity of Endosulfan (Gene Mutation Assays) . . . . . 101

Table 16. Genotoxicity of Endosulfan (Chromosome Aberration and DNA Damage Assays) . . . . 102

Table 17. Exposure of Open-Cab Airblast Applicators . . . . . 105

Table 18. Data Used in Estimates of Exposure and Short-Term Exposure Estimates for Workers  
Handling Endosulfan in Support of Aerial and Ground Applications. . . . . 108

Table 19. Seasonal and Annual Estimates for Workers Handling Endosulfan in Support of

Aerial and Ground Applications . . . . .	109
Table 20. Data Used and Short-Term Exposure Estimates for Handlers Using Handheld Equipment . . . . .	110
Table 21. Seasonal and Annual Exposure Estimates for Endosulfan Handlers Using Handheld Equipment . . . . .	111
Table 22. Short-term Exposures to Endosulfan Estimated for Reentry Workers . . . . .	117
Table 23. Seasonal and Annual Exposures to Endosulfan Estimated for Reentry Workers . . . . .	117
Table 24. Average Annual Endosulfan Use in California. . . . .	119
Table 25. Summary of Endosulfan ( $\alpha$ -, $\beta$ - and Sulfate) Expressed as Total Endosulfan Residues (July, 1998) . . . . .	124
Table 26. Comparative DPR and PDP Maximum Measured Endosulfan Residues . . . . .	127
Table 27. Comparison of Percent User Day Rates Between the 1989-92 and 1994-98 CSFII. . . . .	133
Table 28. Acute (daily) and Chronic (annual) Dietary Exposure to Anticipated Endosulfan Residues on Raw Agricultural Commodities. . . . .	137
Table 29. Ambient Air and Bystander Exposure Estimates for Persons Exposed to Endosulfan. . . . .	139
Table 30. Summary of Historical Surface Water Sampling Data for Endosulfan in California Between 1990 and July 2000 . . . . .	140
Table 31. Exposures to Endosulfan Estimated for Swimmers in Surface Waters . . . . .	141
Table 32. Potential Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic Exposure to Endosulfan by Either Aerial, Airblast or Groundboom Application. . . . .	144
Table 33. Potential Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic Exposure to Endosulfan for Handlers Using Handheld Equipment to Apply Endosulfan. . . . .	145
Table 34. Potential Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic Exposure to Endosulfan for Reentry Workers . . . . .	145

Table 35. Aggregate (Dietary + Ambient Air or Bystander) Exposure Estimates for Persons Exposed to Endosulfan ..... 146

Table 36. Endosulfan Exposure Swimmers in Surface Waters on a Short Term, Seasonal or Chronic Basis. .... 147

Table 37. Estimated Margins of Exposure for Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic for Aerial, Airblast and Groundboom Workers with Endosulfan ..... 149

Table 38. Estimated Margins of Exposure for Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic for Handlers Using Handheld Equipment to Apply Endosulfan ..... 150

Table 39. Estimated Margins of Exposure for Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic Endosulfan Exposure for Reentry Workers. .... 151

Table 40. Estimated Margins of Exposure for Non-Dietary and Aggregate (Non-Dietary + Dietary) Short Term, Seasonal and Chronic Endosulfan Exposure in Ambient Air and to Bystanders. .... 152

Table 41. Estimated Margins of Exposure for Non-Dietary and Aggregate (Non-Dietary + Dietary) Short Term, Seasonal and Chronic Endosulfan for Swimmers in Surface Waters. .... 152

Table 42. Dietary Margins of Exposure from Anticipated Endosulfan Residues on Raw Agricultural Commodities .....154

Table 43. Comparison of Aerial Applicator Exposure to Endosulfan Estimated from Surrogate Data by DPR and USEPA Policy. .... 163

Table 44. Comparison of critical no-observed-effect levels (NOELs) and endpoints for risk characterization between the Department of Pesticide Regulation and U.S. Environmental Protection Agency ..... 182

Table 45. Summary of Margins of Exposure Less Than 100 for Population Subgroups From Tolerance Levels of Endosulfan ..... 186

Table 46. Estimated MOEs for Endosulfan in Ambient Air and to Bystanders and their Corresponding Percent Reference Concentrations. .... 190

**List of Abbreviations**

AADD	Annual Average Daily Dosage
ADI	Acceptable Daily Intake
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
ADR	Absorbed Dose Rate (mg/day)
Bronzan Bill	California Assembly Bill 2161 (1989)
CalEPA	California Environmental Protection Agency
CAS	Chemical Abstracts Services
CERCLA	Comprehensive Environmental Response Compensation and Liability Act
ChE	Acetyl Cholinesterase Activity
CSFII	Continuing Survey of Food Intake by Individuals
CWA	Clean Water Act
CYP	Cytochrome P450
DEEM™	Dietary Exposure Evaluation Model
DFR	Dislodgeable Foliar Residues
DNT	Developmental neurotoxicity study
DPR	Department of Pesticide Regulation
EM & PM	Environmental Monitoring and Pest Management Branch of DPR
ENEL	Estimated No Effect Level
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOB	Functional Observational Battery
FQPA	Food Quality Protection Act
FSIS	Food Safety Inspection Service of the USDA
GABA	$\gamma$ -amino-butyric acid
HCDB	Hazardous Chemical Data Book
HSDB	Hazardous Substance Data Bank
LC <sub>50</sub>	Median Lethal Concentration
LD <sub>50</sub>	Median Lethal Dose
LOD	Endosulfan residue Limit of Detection
LOEL	Lowest Observed Effect Level
MDL	The FDA multiple residue screen minimum detection level for endosulfan
MOE	Margin of Exposure
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
ND	Non-Detected Residues, Non-Detects
NOEL	No Observed Effect Level
NRCC	National Research Council Canada
PAD	Population Adjusted Dose
PDP	Pesticide Data Program
PDR	Potential Dose Rate (mg/day dose absorbed from incidental, non-dietary ingestion)
PHI	Pre-harvest Interval

PPE	Personal Protective Equipment
PSIP	Pesticide Illness Surveillance Program
PHED	Pesticide Handler Exposure Database
ppm	Parts per million
PRD	Pesticide Residue Document
Proposition 65	Safe Drinking Water and Toxic Enforcement Act of 1986 (California)
PUR	Pesticide Use Report Database
RAC	Raw Agricultural Commodity
RBC	Red Blood Cell
RCD	Risk Characterization Document
RCRA	Resource Conservation and Recovery Act
RED	Reregistration Eligibility Decision Document
REI	Restricted Entry Interval
SADD	Seasonal Average Daily Dosage
SB950	Birth Defects Prevention Act of 1984
SARA	Superfund Amendments and Reauthorization Act of 1986
STADD	Short Term Average Daily Dosage
SWRCB	State Water Resources Control Board (of California)
TAC	Toxic Air Contaminant
TAS	Technical Assessment Systems, Inc., software program for dietary exposure analyses
TC	Transfer coefficients from chemicals with surrogate chemicals
TEF	Toxicity Equivalence Factor
Thiodan 2 CO/EC	Endosulfan formulation in cotton oil (emulsifiable concentrate)
Thionex 35 EC	Endosulfan formulation in emulsifiable concentrate form
Thiodan 50 WP	Endosulfan formulation in wettable powder form
USDA	U.S. Department of Agriculture
USEPA	U.S. Environmental Protection Agency
UB	Upper Bound, 95 <sup>th</sup> percentile (acute MOE calculations)
UCL	Upper Confidence Limit (PHED database)
UF	Uncertainty Factor
WHS	Worker Health and Safety Branch

## I. SUMMARY

### **Introduction:**

This report evaluates the potential for endosulfan exposure, and includes: 1) a review of the available scientific evidence on  $\alpha$ -endosulfan,  $\beta$ -endosulfan, and endosulfan sulfate regarding their physical properties, 2) and an occupational and dietary health risk assessment for technical endosulfan as currently used in California.

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide) is a pesticide, belonging to the chemical family of organochlorines, subclass chlorinated cyclodienes, and containing only one double bond. It is used to control more than 100 different insect pests (aphids, leafhoppers, borers, worms etc.) that infest a large number of crops in California. It serves as a contact and stomach insecticide for more than 60 food and non-food crops but has proven to be extremely toxic to fish and other aquatic organisms. In California, the food crops are primarily grapes, melons, lettuce and tomatoes, as well as cotton, both a food (cotton seed oil) and a non-food crop. Patented in 1956, it is usually included among pesticides of the “chlorinated hydrocarbons of the cyclodiene group.” CAS classifies it as a “dioxathiepin.”

### **Environmental Fate:**

Endosulfan hydrolysis increases with increased pH, binds tightly to some soils and is not mobile in soil. Surface and well water have not been sampled since 1996, as endosulfan is not considered to be a potential drinking water contaminant. Air monitoring shows that endosulfan can volatilize from water, soil and plant surfaces for 1 to 11 days post application. Endosulfan is translocated to roots after application to leaves and is metabolized within the plant. Bioaccumulation occurs in both aquatic (mussels, fish, shrimp, algae) and terrestrial (mosquito, snail) wildlife.

### **Pharmacology:**

The majority of endosulfan, regardless of exposure route, is excreted rapidly in feces, with virtually no retention in tissues, despite the lipophilicity of endosulfan and its primary metabolite, endosulfan sulfate. Enterohepatic circulation, conjugation and elimination in the urine, is not a major route for endosulfan metabolism. At 120 hours, 88% of  $\alpha$ -[<sup>14</sup>C]endosulfan and 87% of  $\beta$ -[<sup>14</sup>C]endosulfan had been eliminated. The default policy for DPR is that if oral absorption is 80% or greater, the absorption is assumed to be 100%. After endosulfan was dermally administered to rats, within 5 days 47.3% of the dose was absorbed and 95% of the absorbed material was eliminated. Fatty tissues had the highest endosulfan concentrations after dermal treatment. After oral treatment in rats, liver and kidney were the sites of greatest endosulfan concentration. These organs are likely the primary sites of biotransformation, since their weights increase after treatment, as do the concentrations and activities of xenobiotic metabolizing enzymes such as P450s and glutathione-transferases.

### **Biotransformation:**

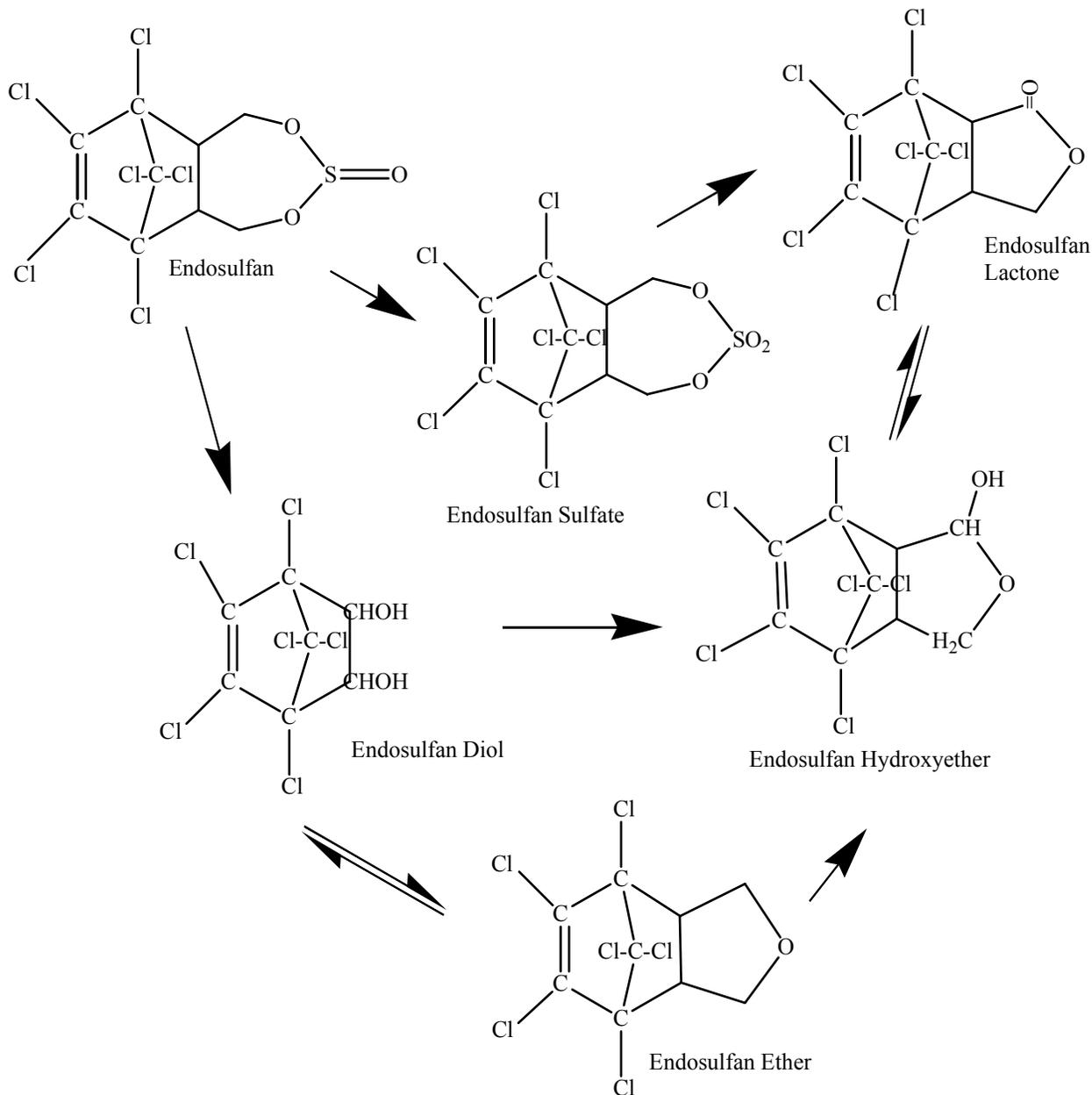
Endosulfan modifies the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT),

glutathione peroxidase (GPX) and glutathione (GSH) in rat liver, lung and erythrocytes when administered via aerosol, thereby potentially contributing to oxidative stress in some tissues.

Stereoselective endosulfan sulfate formation from human recombinant P450s showed that endosulfan is mediated by CYP2B6, CYP3A4 and CYP3A5 and  $\alpha$ -isomer by CYP3A4 and CYP3A5.

Endosulfan affected glutathione (GSSG), glutathione peroxidase (GPX), reductase (GTR) and S-transferase (GST) activities. GSSG and GPX were increased, and GTR and GST were decreased after treatment.

**Figure 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)** Phase I reactions on endosulfan are performed with P450s: CYP2B6, CYP3A4 & CYP3A5; Phase II reaction is with GST; Other enzymes involved with endosulfan metabolism are antioxidants: SOD, GPX and CAT



## **Toxicology:**

**NEUROTOXICITY:** The mode of action of endosulfan is to bind and inhibit  $\gamma$ -amino-butyric acid (GABA)-gated chloride channel receptor, thereby inhibiting GABA-induced chloride flux across membranes (Abalis et al., 1986; French-Constant, 1993; Sutherland et al., 2004). Neurotoxicity is the primary effect observed both acutely and chronically in both humans and animals (where clinical signs were recorded). Documented human data have shown the central nervous system to be the major target of endosulfan action. Endosulfan is a strong neurotoxin in lower animals (rats, dogs, mice, cows, cats, goats and sheep) as well as in humans.

**ENDOCRINE DISRUPTION:** Effects to testes and reproductive tract occurred at lower doses in prepubertal and neonatal rats than in adults following repeat exposures. These observations, however, were from studies in the open literature (not FIFRA Guideline studies) and they occurred at doses greater than those that induced neurotoxicity. The developmental neurotoxicity study recently received and reviewed by DPR (acceptable, according to FIFRA Guidelines) showed no indication of neurotoxicity or endocrine disruption in rats treated with endosulfan in diet during both pre- and post-natal development. Dams, fetuses and pups showed a decrease in body weight during treatment and male pups had a slight delay (4-5%) in preputial separation at 10.8 mg/kg/day and greater. Therefore endocrine disruption is not considered by DPR to be a sensitive endpoint for endosulfan.

**TARGET ORGANS:** Liver and kidney are the primary target organs. Endosulfan induced xenobiotic metabolizing enzymes. Hepatocyte gap junctional intercellular communication was inhibited by endosulfan, as well as by the sulfate, lactone and ether metabolite. Gap junctional intercellular communication was also inhibited by both  $\alpha$ - and  $\beta$ - isomers in primary Sprague-Dawley rat hepatocytes, as well as WB-F344 rat liver cell lines.

Neither in FIFRA Guideline acceptable animal studies nor in open literature studies was endosulfan found to be oncogenic. There were inconclusive findings from contradictory results of genotoxicity induced by endosulfan technical as measured by gene mutation, chromosomal aberration and other genotoxic effects tests in studies submitted to DPR and found in the open literature.

## **Hazard Identification:**

For regulatory purposes under SB950 it is necessary for DPR to designate which studies are acceptable according to FIFRA Guidelines. Studies that are not acceptable, but contain useful information or are studies from the open literature are considered to be supplemental and will be so designated in the toxicity section.

### **ACUTE TOXICITY:**

#### **a) Acute Oral NOEL**

The adverse effects observed in laboratory animals following acute oral exposure to endosulfan include clinical signs of neurotoxicity, deaths, neurobehavioral effects, reductions in body weight, and increased gross and histopathological effects. The possible acute oral effects from endosulfan included effects observed in the LD<sub>50</sub>/LC<sub>50</sub> studies and in a rabbit developmental study. The effects observed in

the LD<sub>50</sub>/LC<sub>50</sub> studies included death, clinical signs, and liver, kidney, intestine, lung and adrenal toxicity. Liver changes were a granular-appearance, degeneration of hepatocytes with foamy cytoplasm and bile duct proliferation. Kidneys appeared congested and proximal convoluted tubules were necrotic and desquamated. Adrenal cortex showed swollen foamy cytoplasm, with eccentric nuclei. Congested lungs containing hemorrhagic areas were observed, along with irritation of the small and large intestine. Clinical signs were increased preening, salivation, excessive masticatory movements, lacrimation, exophthalmia, hyperresponsiveness to sudden sound and tactile stimuli, hyperexcitability, dyspnea, decreased respiration, ataxia, depression of righting reflex, discharge from eyes, nasal discharge, sprawling of the limbs, decreased reflexes (placement, pain, corneal, pupillary light, righting, startle, paw, cutaneous) and tremors, tonic and clonic convulsions and death.

The acute oral effects observed in a developmental toxicity study performed in the rabbit, included maternal signs within the first day of treatment (in the absence of fetal effects). Various clinical signs were observed in dams/does, including abortions, phonation, coughing, cyanosis, convulsions/ thrashing, noisy/rapid breathing, hyperactivity, salivation, and nasal discharge and death (Nye, 1981). Clinical signs began on gestation day 6 (day 1 of treatment) at 1.8 mg/kg/day. In particular, hyperactivity was observed only at 1.8 mg/kg/day. The NOEL for this study was 0.7 mg/kg/day. Similar effects were observed in 2 rangefinding studies also performed in pregnant New Zealand rabbits (Fung, 1981a, b). In these studies the LOELs were 1.0 mg/kg/day, based on neurotoxicity and deaths beginning day 8 of gestation (treatment day 2). There were no major deficiencies in this study and it provided the lowest acute oral NOEL for evaluating exposure and to calculate the MOE for potential acute single-day (non-inhalation) human exposures to endosulfan.

#### b) Acute Dermal NOEL

There were no FIFRA Guideline acceptable studies nor were there acceptable studies available in the open literature for determination of an acute dermal NOEL with endosulfan technical. Therefore, the oral acute NOEL (0.7 mg/kg) was used for determinations of MOEs for acute dermal occupational exposure and for swimmer exposure in surface water.

#### c) Acute Inhalation NOEL

An acceptable acute inhalation LC<sub>50</sub> exposure study was performed but did not achieve a NOEL. However an acceptable subchronic rat inhalation study with a NOEL of 0.0010 mg/L (0.194 mg/kg/day) was used to calculate the potential for acute single-day inhalation exposure to workers, and for exposure to endosulfan in ambient air or to bystanders (Hollander et al., 1984). In this study, endosulfan was administered by aerosol (nose-only) for 21 days at 6 hours per day, followed by a 29-day recovery. The NOEL of 0.194 mg/kg/day is lower than the oral NOEL of 0.7 mg/kg/day from the rabbit developmental study and more importantly, it is route-specific. The study was therefore selected as the definitive study for the critical inhalation NOEL of 0.0010 mg/L (0.194 mg/kg/day) and a LOEL of 0.0020 mg/L (0.3873 mg/kg/day). This NOEL was used to estimate the MOE (MOE) for acute inhalation (occupational and (non-occupational) ambient air and bystander exposure).

### **SUBCHRONIC TOXICITY:**

#### a) Subchronic Oral NOEL

For the definitive subchronic oral NOEL a rat dietary reproduction study was selected. In this study parental effects were observed after an exposure of 24 weeks throughout pre-mating, mating, gestation, lactation and weaning for 2 generations (Edwards et al., 1984). The oral, systemic NOEL was 1.18 mg/kg/day based on increased relative liver and kidney weights, decreased food consumption, and decreased body weights. The NOEL was used to estimate the subchronic dietary exposure to endosulfan.

b) Subchronic Dermal NOEL

There were no FIFRA Guideline acceptable studies nor were there acceptable studies available in the open literature for determination of a subchronic dermal NOEL with endosulfan technical. Therefore, the oral rat reproduction NOEL (1.18 mg/kg/day) was used for determinations of MOEs for seasonal dermal occupational exposures and for exposures to swimmers in surface water.

c) Subchronic Inhalation NOEL

The definitive study for subchronic inhalation exposure was a study performed in rat, where endosulfan was administered by aerosol (nose-only) for 21 days at 6 hours per day, followed by a 29 day recovery (Hollander et al., 1984). The NOEL for inhalation was 0.0010 mg/L based on emaciation, pale skin, squatting position and high-legged position, decreased bodyweight gain and food consumption, increased water consumption, and clinical chemistry parameters (reversed during recovery). This study was acceptable according to FIFRA Guidelines and was the only study available for evaluation of endosulfan exposure by inhalation. It was therefore selected as the definitive study for the critical inhalation NOEL of 0.0010 mg/L (0.194 mg/kg/day) to estimate the MOE for seasonal (non-occupational) ambient air and bystander exposure.

**CHRONIC TOXICITY:**

a) Chronic Oral NOEL

Chronic dietary endosulfan exposure to dogs showed that neurotoxicity was the most sensitive endpoint for chronic oral endosulfan toxicity. The NOEL was 0.57 mg/kg/day for males and 0.65 mg/kg/day for females, based on clinical signs of violent contractions of the upper abdomen and convulsive movements, extreme sensitivity to noise, frightened reactions to optical stimuli and jerky or tonic contractions in facial muscles, chaps and extremities and impairment of the reflex excitability and postural reactions (Brunk, 1989). It was necessary to sacrifice some of the dogs prematurely due to the clinical signs of neurotoxicity. In addition, body weights and food consumption were decreased. This study was acceptable according to FIFRA Guidelines and the NOEL of 0.57 was used to determine MOE for both dietary and worker exposure.

b) Chronic Dermal NOEL

There were no FIFRA Guideline acceptable studies nor were there acceptable studies available in the open literature for determination of an chronic dermal NOEL with endosulfan technical. Therefore, the procedure is to use the chronic oral NOEL in dog (0.57 mg/kg/day) for determinations of MOEs for chronic dermal occupational exposures and for exposures to swimmers in surface water.

c) Chronic Inhalation NOEL

An acceptable chronic inhalation exposure study was **not available either from the open literature or from studies submitted by the registrants** to obtain a chronic inhalation NOEL. Therefore, an acceptable subchronic rat inhalation study with a NOEL of 0.0010 mg/L (0.194 mg/kg/day) was used to calculate the potential for chronic inhalation exposure to workers, and for exposure to endosulfan in ambient air or to bystanders (Hollander et al., 1984). In this study, endosulfan was administered by aerosol (nose-only) for 21 days at 6 hours per day, followed by a 29-day recovery. The NOEL for inhalation was based on emaciation, pale skin, squatting position and high-legged position, decreased bodyweight gain and food consumption, increased water consumption, and clinical chemistry parameters (reversed during recovery). A 10x uncertainty factor for extrapolation from subchronic to chronic was added to the NOEL of 0.194 mg/kg/day to give a final critical Estimated No Effect Level (ENEL) of 0.0194 mg/kg/day. This dose is lower than the chronic oral NOEL of 0.57 mg/kg/day from the chronic dog dietary study and more importantly, it is route-specific. The study was therefore selected as the definitive study for the critical NOEL with 0.0194 mg/kg/day and a LOEL of 0.03873 mg/kg/day. This NOEL will be used to estimate the MOE for chronic occupational and (non-occupational) ambient air and bystander exposure.

**ONCOGENICITY:** When considering the results of all available *in vivo* studies performed in rats and mice, there is no evidence indicating endosulfan is oncogenic. There were acceptable chronic and combined (chronic/oncogenicity) studies performed in dog (chronic) and rat and there was no indication of oncogenicity. **Endosulfan is categorized as “A4” (not classifiable as a human carcinogen) by the American Conference of Governmental Industrial Hygienists (Substances and Physical Agents and Biological Exposure Indices, Cincinnati, OH, 2005).** There were inconclusive findings with contradictory results from genotoxicity induced by endosulfan (technical), as measured by the gene mutation, chromosomal aberration and other genotoxic effects in tests submitted to DPR.

**Exposure Assessment:**

Assumptions for all exposure scenarios, unless otherwise indicated, were 47.3% dermal absorption, based on a rat study (Craine, 1988), a 70 kg body weight (Thongsinthusak et al., 1993), and inhalation absorption of 100% (USEPA, 2001b).

**OCCUPATIONAL EXPOSURE ASSESSMENT:**

- Acute, short-term exposures: For short-term exposures, DPR estimates the highest exposure an individual may realistically experience during or following legal endosulfan uses. For this “upper bound” of daily exposure the estimated population 95<sup>th</sup> percentile of daily exposure is used. A higher percentile is not used because the higher the percentile the less reliably it can be estimated and the more it tends to overestimate the population value (Chaisson et al., 1999).

- Seasonal (1 week to 1 year) and annual (1 year): To estimate seasonal and annual exposures, the average daily exposure is of interest because over these periods of time, a worker is expected to encounter a range of daily exposures (i.e., DPR assumes that with increased exposure duration, repeated daily exposure at the upper-bound level is unlikely). To estimate the average, DPR uses the arithmetic mean of daily exposure (Powell, 2003). In most instances, the mean daily exposure of individuals over time is not known. However, the mean daily exposure of a group of persons observed in a short-term study is believed to be the best available estimate of the mean for an individual over a longer period.
- Surrogate Data (short-term, seasonal and annual): Although no acceptable studies were available in which handler exposure to endosulfan was monitored, one acceptable study was submitted in which dermal and inhalation exposure of airblast applicators to the surrogate compound, carbaryl, was monitored (Smith, 2005). This study provided acceptable data for estimating exposure of airblast applicators driving open-cab tractors. Carbaryl was applied in three orchard crops (peaches, apples, and citrus) in three states (Georgia, Idaho, and Florida). With the exception of airblast applicators and handlers dipping nursery stock, exposure estimates were derived using the Pesticide Handler Exposure Database (PHED, 1995).

When using surrogate data to estimate short-term exposure, DPR uses the 90% upper confidence limit (UCL) on the 95<sup>th</sup> percentile. The UCL is used to account for some of the uncertainty inherent in using surrogate data and to increase the confidence in the estimate

When using surrogate data to estimate seasonal or annual exposure, DPR uses the 90% UCL on the arithmetic mean. The 90% UCL is used for the reasons listed in the previous paragraph. As with short-term exposure estimates based on PHED subsets, a multiplier corresponding to the median sample size over body regions is used. If the median sample size is greater than 15, the multiplier is 1 (Powell, 2002).

Surrogate data from the PUR also were used to estimate intervals for seasonal and annual exposures. However, PUR data show that in many parts of the state and in many crops endosulfan use does not occur throughout the year, and that at other times relatively few applications are made. It is reasonable to assume that an individual handler is less likely to be exposed to endosulfan during these relatively low-use intervals. Thus, rather than assume that handlers are exposed throughout the year, annual use patterns are plotted based on monthly PUR data from one or more counties with the highest use. Annual exposure to endosulfan is assumed to be limited to the months when use is relatively high (defined as 5% or more of annual use each month). The occupational exposure values reported below are for total (dermal + inhalation), when applicable, for STADD, SADD and AADD.

USEPA (2002b) assumed that handler exposure durations would only be one day to one month. The basis for this assumption was not explained.

**AERIAL AND GROUND APPLICATIONS:** STADD for aerial applications ranged from 0.021 mg/kg (airblast M/L-WSP) to 2.63 mg/kg (aerial M/L-WP). SADD exposure values ranged from 0.005 (groundboom applicators) to 0.385 mg/kg/day (aerial M/L-WP). AADD values ranged

from 0.001 mg/kg (airblast M/L-WSP and EC) to 0.128 mg/kg (aerial M/L-WP). Mitigation measures proposed by USEPA (2002) would require all WP to be packaged in WSP.

**BACKPACK, HIGH AND LOW PRESSURE HANDWAND APPLICATIONS AND NURSERY STOCK DIP:** PHED data were used in exposure estimates for handlers applying endosulfan with a backpack sprayer, and both high and low pressure handwands. High and low-pressure handwands can be used to apply endosulfan to the same crops as backpack sprayers. Due to infrequent use, seasonal and annual exposures to endosulfan are not anticipated to occur by nursery stock dip, and only short-term exposures were estimated. STADD exposure estimates range from 0.00003 mg/kg/day (Dip, M/L-EC) to 41.4 mg/kg/day (Dip applicator). SADD exposures range from 0.003 mg/kg/day (LPHW M/L-EC) to 0.153 mg/kg/day (HPHW M/L/A) and AADD ranges were from 0.0005 mg/kg/day (LPHW M/L-EC) to 0.026 mg/kg/day (HPHW M/L/A).

**REENTRY EXPOSURE:** Representative exposure scenarios for reentry workers were selected as described in the document provided by the DPR Worker Health and Safety Branch (Beauvais, 2007). No exposure data were available for workers reentering crops treated with endosulfan. Because of this, exposures of workers reentering crops treated with endosulfan were estimated from dislodgeable foliar residue (DFR) values and from transfer coefficients (TCs) from studies with surrogate chemicals (residue transfer assumed not chemical-specific) (Beauvais, 2007).

Most reentry activities are not expected to result in pesticide exposure throughout the year. Annual exposure to endosulfan is assumed to be limited to the months when use is relatively high (defined as 5% or more of annual use each month). It was assumed that scouting occurred after all applications were completed.

STADD for reentry exposures ranged from 0.009 mg/kg/day (almond, thinning and ornamental plants, hand harvesting) to 0.533 (sweet corn, hand harvesting); SADD reentry exposures ranged from 0.004 mg/kg/day (potato, scouting; lettuce, scouting) to 0.141 mg/kg/day (grape, cane turning) and for AADD, ranges went from 0.001 mg/kg/day (cucumber, hand harvesting) to 0.047 mg/kg/day (grape, cane turning).

**AMBIENT AIR and BYSTANDER EXPOSURES:** Ambient air and application site air monitoring detected endosulfan, suggesting that the public may be exposed to endosulfan in air. Individuals might be exposed to endosulfan if they are working adjacent to fields that are being treated or have recently been treated (bystander exposure). In addition, air monitoring conducted in Fresno County suggests that airborne endosulfan exposures are possible in areas that are far from application sites (ambient air). Public exposure to airborne endosulfan was estimated based on monitoring studies of endosulfan at application sites and in ambient air.

- **Ambient Air:** Short-term exposures to ambient air are anticipated to be equal to or less than the acute bystander exposure, which addresses exposure of an individual who is adjacent to an application. Ambient air STADD was not estimated as the highest short-term ambient air exposure is anticipated to be adjacent to an application, which is estimated by bystander STADD. SADD is 0.000037 mg/kg/day for infants and 0.000017 mg/kg/day for adults. AADD is 0.00002 mg/kg/day for infants and 0.00001 mg/kg/day for adults.

- **Bystanders at application sites:** STADD for bystanders was 0.0016 mg/kg/day for infants and 0.00076 mg/kg/day for adults. Seasonal ADD estimates for bystander exposures to endosulfan were 0.00056 mg/kg/day for infants and 0.00027 mg/kg/day for adults. Annual ADD estimates for bystanders were 0.000047 mg/kg/day for infants and 0.000022 mg/kg/day for adults.

## Water

**SURFACE WATER:** Historically, endosulfan has been detected numerous times in California surface waters. Endosulfan sulfate has been detected more frequently in surface water samples than  $\alpha$ - or  $\beta$ -endosulfan, and generally at higher concentrations. Endosulfan residues have been detected in California surface waters in the Central Valley (Ross et al., 1996 and 2000) and in the Sierra Nevada Mountains (Fellers et al., 2004). Movement of endosulfan into surface water via rainfall runoff and irrigation drainage has been documented (Gonzalez et al., 1987; Fleck et al., 1991).

In surface water systems, endosulfan residues have also been detected in sediment (Gonzalez et al., 1987; Fleck et al., 1991; Ganapathy et al., 1997; Weston et al., 2004); mussels (Singhasemanon, 1996; Ganapathy et al., 1997); amphibians (Sparling et al., 2001); and fish (Singhasemanon, 1995; Brodberg and Pollock, 1999). Because endosulfan has been detected in surface water, sediment and aquatic organisms, and in response to concerns about endosulfan's toxicity, in 1991 DPR began requiring permit conditions to prevent use of endosulfan where it might be allowed to reach surface water (Okumura, 1992).

**SWIMMER EXPOSURES:** Exposures of adults and children swimming in surface waters were estimated based on equations listed in U.S. EPA (2003). Both STADD and SADD were calculated from absorbed dose rate and potential dose rate by dividing by default body weights of 70 kg for an adult (Thongsinthusak *et al.*, 1993) and 24 kg for a 6 year-old child (USEPA, 1997c). Inhalation exposure was assumed to be negligible, and was not included in swimmer exposure estimates. The total exposure was calculated by summing dermal and non-dietary ingestion exposure estimates. Total STADD was 0.00027 mg/kg/day for adults and 0.00156 mg/kg/day for children. Total SADD was 0.00000468 mg/kg/day for adults and 0.000048 mg/kg/day for children. Total AADD was 0.00000128 mg/kg/day for adults and 0.0000131 mg/kg/day for children.

## Dietary Exposure:

DPR evaluates the risk of human exposure to an active ingredient in the diet using two processes: (1) use of residue levels detected in foods to evaluate the risk from total exposure, and (2) use of tolerance levels to evaluate the risk from exposure to individual commodities. For evaluation of risk to detected residue levels, the total exposure in the diet is determined for all label-approved raw agricultural commodities, processed forms, and animal products (meat and milk) that have established USEPA tolerances. The potential exposure from residues in the water and certain commodities without tolerances are also assessed in some cases. Tolerances may be established for the parent compound and associated metabolites. DPR considers these metabolites and other degradation products that may be of toxicological concern in the dietary assessment.

The dietary exposure to endosulfan and its metabolites was assessed initially in 1998 by Medical Toxicology Branch staff. The 1998 assessment used the TAS, Inc EX<sup>TM</sup> acute and chronic dietary exposure software (TAS, 1996a, b). All of the acute and chronic dietary margins-of-exposure

(MOEs) exceeded 100 at the 95<sup>th</sup> percentile. A revised DPR dietary exposure assessment was assessed and it was concluded that the previous 1998 assessment was the more health protective (Carr, 2006).

**ACUTE (and short term):** The potential acute dietary exposure of endosulfan from all labeled uses ranged from 1.37 ug/kg/day, males 13-19 years (females 13-19 years = 1.37) to 3.30 ug/kg/day, children 1-6 years for the 95<sup>th</sup> percentile of user-days exposures. Male and female values (13-19 years), when rounded to two significant figures, were both 1.37 ug/kg/day. The complete acute dietary exposure analysis includes all current USEPA label approved endosulfan uses.

The exposure to endosulfan through the diet was also considered for pesticide workers in combination with occupational exposure. For acute dietary exposure, the value for Females (13+), nursing, was used for adult acute occupational, adults in the general public for ambient air and bystanders and for adult swimmers in surface water. This population subgroup was selected, since it was a relatively high exposure in a population that would be found amongst all exposure scenarios for adults. The potential acute dietary exposure was estimated to be 2.06 ug/kg/day, based on the 95<sup>th</sup> percentile of user-day exposure for females age 13+ years, nursing. The acute dietary exposure levels for infants (non-nursing, < 1 year) was selected to represent infants exposed to endosulfan in ambient air and to bystanders (95<sup>th</sup> percentile, 3.18 ug/kg/day). Children exposed to endosulfan while swimming in surface water had the acute dietary component of 3.30 ug/kg/day from the population subgroup of Children (1 - 6 years).

**SUBCHRONIC (seasonal) AND CHRONIC (annual) EXPOSURE:** The TAS program does not perform a subchronic dietary analysis; therefore, potential subchronic dietary exposures were estimated using the chronic exposure data (average measured residue values of all values for each commodity). The subchronic NOEL, however, was different from the chronic. Therefore, subchronic dietary exposure is likely different even when using chronic RAC residues. For commodities with residues at "below detection limit," a value equal to one-half (50%) of the MDL was assigned to each commodity. When the residue values are derived from monitoring programs, the assumption is that the data represent annual average level in the diet (%CT). Therefore, for subchronic dietary exposure, the chronic value for Females (13+), nursing, was used for adult subchronic occupational, adults in the general public for ambient air and bystanders and for adult swimmers in surface water. The potential subchronic dietary exposure was estimated to be 0.17 ug/kg/day, based on the %CT annualized average for females age 13+ years, nursing. The dietary subchronic exposure levels for infants (non-nursing, < 1 year) was selected to represent infants exposed to endosulfan in ambient air and to bystanders (0.28 ug/kg/day). Children exposed to endosulfan while swimming in surface water had the subchronic dietary component of 0.41 ug/kg/day from the subgroup of Children (1 - 6 years). Chronic dietary exposure data were the same as those used for subchronic exposure estimations.

### **Aggregate (Occupational or Public + Dietary) Exposure**

**AGGREGATE EXPOSURE:** For aggregate (occupational plus dietary) exposure in occupational scenarios the STADD, SADD and AADD exposure components were derived from the occupational exposure total of the dermal plus the inhalation values (Tables 16-21). In addition, for this aggregate combination of occupational plus dietary, the oral NOELs for acute, subchronic and chronic studies were used in the STADD, SADD and AADD determinations for occupational and swimmer in surface water scenarios. This is because for these particular "combined" exposures, the dietary and dermal routes comprise the primary routes. An oral NOEL is used for dermal exposure

(no acceptable dermal study).

a) Occupational Aggregate (Dermal + Inhalation + Dietary) Exposure

The predominant factor for mitigating human exposure to endosulfan is the occupational exposure. For example, in more than half of all aggregate occupational exposure scenarios (acute, subchronic, chronic), the dietary component comprised less than 3% (49/89 = 55%) of the aggregate exposure. The majority of the aggregate occupational exposures where diet comprised a higher percentage (3% or greater) was observed for STADD (18/35; 51%) and AADD (16/27; 59%). SADD total occupational aggregate exposures had a dietary component of 22% (6/27) which was less than half the other scenarios. The highest percentages for dietary contribution of aggregate occupational exposure were re-entry scenarios where STADD was 60% (9/15), SADD was 30% (3/10) and AADD was 80% (8/10).

b) Aggregate (Dietary + Inhalation) Exposure in Ambient Air and to Bystanders

For adults and children with aggregate exposure to endosulfan in ambient air or as bystanders plus diet showed that the dietary component for STADD, SADD and AADD is the major exposure. All of the non-dietary exposure components for all air scenarios are very low and, therefore, that is why the dietary contribution (while also quite low) appears to be so much greater. The dietary percentage of exposure was lowest in SADD infant bystanders (33%; non-dietary exposure was 0.00046 mg/kg/day). The dietary exposure was highest in ambient air for adults (AADD, 94%), where the non-dietary exposure was 0.00001 mg/kg/day. **However, since the majority of the aggregate MOEs were less than 1000 (all except SADD ambient air--infants and adults and AADD for infants) endosulfan should be considered as a toxic air contaminant.**

d) Aggregate (Diet +( Dermal + Non-Diet Ingestion) Exposure to Swimmers in Surface Water

STADD for child non-diet ingestion (and total) had the lowest dietary component for aggregate exposure (68%). The non-dietary exposure was 0.00156 mg/kg/day and was the highest exposure of all scenarios. STADD for adult non-dietary ingestion (and total) was 0.00027 mg/kg/day and the dietary comprised 88% of the aggregate exposure. For SADD for child non-dietary and total, there was an 89% dietary contribution. For all other groups, the non-dietary exposure was so comparatively low that the dietary comprised 97% to 100% of the aggregate exposure.

**Risk Characterization: Margins of Exposure**

The risks for potential adverse human health effects with occupational, public (swimmers in surface water, dermal and non-dietary ingested), ambient air and dietary exposure to endosulfan were evaluated using margins of exposure (MOE) estimates. The MOEs for acute, subchronic and chronic exposure were calculated using no-observed-effect levels (NOELs) from the available guideline and literature toxicity studies for endosulfan. Generally, an MOE greater than 100 is considered sufficiently protective of human health when the NOEL for an adverse effect is derived from an animal study. The MOE of 100 allows for humans being 10 times more sensitive than animals and for a 10-fold variation in sensitivity between the lower distribution of the overall human population and the sensitive subgroup.

**Short Term Margins of Exposure (MOE):**

**OCCUPATIONAL SCENARIOS:** The majority of occupational exposure scenarios (33/ 35, 94%) for STADD had MOEs that were less than 100. Of those, 17% of the MOEs (6/35) were less than or equal to 1 (Aerial M/L-WP, inhalation and dermal; applicator dermal; HPHW M/L/A EC dermal; dip applicator, dermal; sweet corn hand-harvesting). STADD MOEs were greater than 100 for root dip M/L (both EC and WP), ranging from 233 (M/L WP) to 23,333 (M/L EC).

**NON-DIETARY BYSTANDER SCENARIOS:** All short term MOEs for non-dietary infant and adult bystander scenarios were greater than 100, ranging from 121 to 255 for infant and adult bystanders, respectively. It must be noted that since the bystander, infant scenario has an MOE of less than 1000 endosulfan may be listed as a potential toxic air contaminant (California Food and Agricultural Code: 14021-14027).

**NON-DIETARY INTAKE FOR SWIMMERS IN SURFACE WATER:** All short term non-dietary MOEs for swimmers in surface water were greater than 100 and ranged from 449 (child non-diet ingested and total) to 321,101 (adult dermal).

**Seasonal Margins of Exposure**

**OCCUPATIONAL SCENARIOS:** Approximately half of occupational exposure scenarios (14/27, 52%) had SADD MOEs that were less than 100. None was less than or equal to 1. Aerial M/L B WP (3), Aerial Applicator (7), HPHW M/L/A - EC (8) and grape, cane turning (8), all less than 10, were the lowest MOE values. The remaining MOEs below 100 ranged from 13 to 98. All aerial scenarios (M/L, applicator and flagger) had MOEs of less than 100. MOEs that were more than 100 ranged from 107 (backpack sprayer M/L/A EC) to 393 LPHW, M/L/ACEC.

**NON-DIETARY AMBIENT AIR and BYSTANDER SCENARIOS:** All seasonal exposure MOEs for the infant and adult ambient air and bystander scenarios were greater than 100, ranging from 346 (bystander, infant) to 11,415 (ambient air, adult). Note that since the bystander scenarios have MOEs of less than 1000, endosulfan may be listed as a potential toxic air contaminant (California Food and Agricultural Code: 14021-14027).

**NON-DIETARY INTAKE FOR SWIMMERS IN SURFACE WATER:** All seasonal MOEs for swimmers in surface water were greater than 100 and ranged from 24,583 (child: non-diet ingested + dermal) to 31,216,931 (adult dermal).

### **Annual Margins of Exposure**

**OCCUPATIONAL SCENARIOS:** More than half of occupational exposure scenarios (16 of 27, 59%) had AADD MOEs of greater than 100, however there were 11 (41%) that were less than 100. All aerial scenarios (M/L, applicator and flagger) had MOEs of less than 100 but no scenario had an MOE of less than or equal to 1. Aerial M/L B WP (4) had MOE of less than 10. MOEs that were more than 100 ranged from 114 (2 scenarios: reentry workers scouting broccoli and peach, thinning) to 1140 for LPHW M/L/A-EC.

**NON-DIETARY AMBIENT AIR and BYSTANDER SCENARIOS:** All annual exposure MOEs for the infant and adult ambient air and bystander scenarios were less than 1000 (range: 413 bystander infant to 970 ambient air infant) except ambient air adult (1940).

**NON-DIETARY INTAKE FOR SWIMMERS IN SURFACE WATER:** All annual MOEs for swimmers in surface water were greater than 100 and ranged from 43,511 (child: non-diet ingested and total) to 55,339,806 (adult dermal).

### **Dietary Exposure Estimates and Margins of Exposure (MOEs)**

**ACUTE and SHORT TERM DIETARY EXPOSURE:** Acute dietary MOEs were calculated for the various population subgroups using the NOEL for acute toxicity (0.7 mg/kg). Estimates of exposure ranged from 1.37 ug/kg in Females (13- 19 years), not pregnant, not nursing to 3.30 in Children (1-6 years). Females (13+ years, nursing) was selected for the acute dietary exposure group for adults (based on the 95<sup>th</sup> percentile of user-day exposure). Acute dietary exposure for infants (non-nursing, < 1 year) was 3.18 (based on the 95<sup>th</sup> percentile of user-day).

All population subgroups have MOEs (acute 95<sup>th</sup> percentile) greater than 100 and these dietary MOEs are based on anticipated endosulfan residues on RAC. None of the MOEs for categories involving acute dietary exposure of infants and children is greater than 1000 (all are greater than 100), as recommended under the FQPA (1996), however all are greater than 1000 for chronic dietary exposure.

The MOEs for acute dietary exposure ranged from 212 in children (1 -6 years) to 513 in males (13-19). Acute MOE for Females (13+, nursing) was 340. For infants (non-nursing, < 1 year old) it was 220 and for children (1-6 years) it was 212. All MOEs in these population subgroups were greater than 100.

**SUBCHRONIC and CHRONIC DIETARY EXPOSURE:** The chronic dietary exposures ranged from 0.08 ug/kg/day in infants (nursing, < 1 year old) to 0.041 in children (1 - 6 years). Since there are no subchronic dietary data for endosulfan, chronic data were used for subchronic calculations. Chronic dietary exposure for infants (non-nursing, < 1 year) was 0.28 ug/kg/day; 0.41 ug/kg/day was used for children (1 - 6 years) exposed to endosulfan (dermal and

non-dietary ingestion) by swimming in surface water and 0.17 ug/kg/day (Females (13+, nursing)) was used to represent adults, both occupational and in the general public. There were no percent crop treated (%CT) adjustments used in these calculations.

MOEs for chronic dietary exposure were calculated from data for the various population subgroups and the definitive NOEL from the chronic dog study (0.57 mg/kg/day). The MOEs ranged from 1407 in children (1 - 6 years) to 7,421 in infants (nursing < 1 year of age). Percent crop treated (%CT) adjustments were used in these calculations. The chronic dietary exposures were the same as the subchronic subpopulations used for adults (Females (13+ years, nursing = 340), infants (infants non-nursing, < 1 year = 220) and children (children 1 - 6 = 212).

Drinking water is not a likely source of uncertainty with regard to endosulfan dietary exposure. Surface and well water samplings have been negative for endosulfan residues since 1996. In addition, the PDP samples from 2001 to 2003 (PDP, 2003, 2004, 2005) have been negative for endosulfan in drinking water.

### **Aggregate (non-dietary plus dietary) Exposure for Occupational, or Public (ambient air; bystander; swimmers in surface water) Scenario**

#### a) Occupational Aggregate Exposure

The predominant factor for mitigating human exposure to endosulfan is the occupational exposure. In more than half of all aggregate occupational exposure scenarios (acute, subchronic, chronic), the dietary component comprised less than 3% (49/89 = 55%) of the aggregate exposure. The majority of the aggregate occupational exposures where diet comprised a higher percentage ( $\geq 3\%$ ) was observed for STADD (18/35; 51%) and AADD (16/27; 59%). **Aggregate STADD MOEs of less than or equal to 1 are Aerial (inhalation and dermal) M/L-WP; applicator; HPHW M/L/A EC; dip applicator, dermal; sweet corn hand-harvesting).** SADD total occupational aggregate exposures had a dietary component of 22% (6/27) was (less than half the other scenarios). The highest percentages for dietary contribution of aggregate occupational exposure were re-entry scenarios where STADD was 60% (9/15), SADD was 30% (3/10) and AADD was 80% (8/10).

#### b) Aggregate Dietary and Exposure in Ambient Air and to Bystanders

For adults and children, aggregate exposure to endosulfan in ambient air or as bystanders plus diet showed that the dietary component for STADD, SADD and AADD is the major exposure. However, all of the non-dietary exposure components for all air scenarios are very low and that is why the dietary contribution (while also quite low) appears to be so much greater. The dietary percentage of exposure was lowest in SADD infant bystanders (38%; non-dietary exposure was 0.00046 mg/kg/day). The dietary exposure was highest in ambient air for adults (AADD, 97%), where the non-dietary exposure was 0.000005 mg/kg/day.

#### c) Aggregate Dietary and Exposure to Swimmers in Surface Water

STADD for child non-diet ingestion (and total) had the lowest dietary component for aggregate exposure (68%). The non-dietary exposure for this was 0.00156 mg/kg/day and was the highest exposure of all scenarios. STADD for adult non-dietary ingestion (and total) was 0.00027 mg/kg/day

and the dietary comprised 88% of the aggregate exposure. The SADD for child non-dietary and total had an 89% dietary contribution. For all other groups, the non-dietary exposure was so comparatively low that the dietary comprised 97% to 100% of the aggregate exposure.

### **RISK CHARACTERIZATION: Margins of Exposure**

The acute, subchronic and chronic NOELs employed for the characterization of the risk for exposure to endosulfan were derived from studies performed on laboratory animals. Consequently a calculated MOE of 100 was considered prudent for protection against endosulfan toxicity. The benchmark of 100 includes an uncertainty factor of 10 for interspecies sensitivity and 10 for intraspecies variability.

For aggregate (occupational + dietary) exposure in occupational scenarios the STADD, SADD and AADD exposure components were derived from the occupational exposure total of the dermal plus the inhalation values. In addition, for this aggregate combination of occupational plus dietary, the oral NOELs for short-term, subchronic and chronic studies (0.7 mg/kg, 1.18 mg/kg/day and 0.57 mg/kg/day, respectively) were used in the STADD, SADD and AADD determinations for occupational and swimmer in surface water scenarios. This is because for these particular “combined” exposures, the dietary and dermal routes comprise the primary routes. An oral NOEL is used for dermal exposure (no acceptable dermal study).

For endosulfan exposure to the public in ambient air or for bystanders the same NOELs are used for calculations for short term and subchronic MOEs (0.194 mg/kg/day) from the subchronic, rat inhalation study (Hollander et al., 1984). The NOEL used for the chronic MOE calculations is also from the Hollander et al. (1984) study with an additional 10x uncertainty factor to extrapolate from subchronic to chronic (ENEL = 0.0194 mg/kg/day) is used.

### **OCCUPATIONAL RISK (Dermal, Inhalation and Total = Dermal + Inhalation)**

#### **Short Term Margins of Exposure (MOE):**

For dermal occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) STADD had 18 of 20 (90%) exposures with MOEs less than 100. Of those, 25% of the dermal MOEs (5/20) were less than or equal to 1 (Aerial M/L-WP; applicator; HPHW M/L/A EC; dip applicator; sweet corn hand-harvesting).

Inhalation scenarios that were less than or equal to 1 was aerial M/L-WP. STADD MOEs dermal were greater than 100 for root dip M/L (both EC and WP), ranging from 2333 (M/L WP) to 23,333 (M/L EC). Inhalation scenarios that were greater than 100 were airblast (M/L-EC, and applicator), groundboom (M/L-EC and applicator), backpack sprayer (M/L/A), LPHW (M/L/A EC), and dip (M/L EC and M/L WP).

All STADD re-entry worker exposure scenarios had MOEs that were less than 100. Sweet corn hand harvesting had an MOE of 1.

**Seasonal Margins of Exposure**

For dermal occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) SADD had 12 of 17 (59%) exposures with MOEs less than 100. SADD MOEs were greater than 100 for airblast M/L-EC (197), airblast M/L-WSP, all of groundboom scenarios except M/L-WP (15), backpack sprayer (107) and LPHW (M/L/A EC).

For inhalation occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) SADD had 5 of 17 (29%) exposures with MOEs less than 100. SADD inhalation MOEs were greater than 100 were aerial (M/L-EC, applicator, flagger), airblast (M/L-EC, M/L WSP and applicator) all groundboom but M/L WP, backpack sprayer (M/L/A), and LPHW (M/L/A EC and M//L/A WP).

The SADD re-entry worker exposure scenarios had 4 of 10 MOEs of less than 100 (broccoli, scouting--98; sweet corn, hand harvesting--16; grape, cane turning--8; and peach, thinning—42) and the remainder was 131 or greater.

**Annual Margins of Exposure**

For dermal occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) AADD had 10 of 17 (59%) exposures with MOEs less than 100. The dermal MOEs remaining that were greater than 100 ranged from 143 (groundboom M/L-WSP) to 1140 (low pressure handwand M/L/A-EC).

For inhalation occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) AADD had 8 of 17 (47%) exposures with MOEs less than 100. AADD MOEs were greater than 100 for the remaining scenarios and they ranged from 194 (airblast M/L-WSP, groundboom M/L-EC and applicator and low pressure handwand M/L/A-WP) to 6467 for both backpack sprayer and low-pressure handwand M/L/A-EC.

The AADD re-entry worker exposure scenarios had 2 of 10 MOEs of less than 100 (sweet corn, hand harvesting--95; and grape, cane turning--12) and the remainder were 114 or greater.

**AGGREGATE (COMBINED) MOE VALUES:**

a. Aggregate MOE (oral route or inhalation + dietary):

When exposure routes use the same NOEL and the same uncertainty factors, then the following calculation is used for MOE, regardless of scenario.

$$\text{Aggregate MOE (oral route)} = \frac{\text{NOEL (oral)}}{\text{Occupational or Non-Dietary Exposure Dose} + \text{Dietary Exposure Dose}}$$

b. Aggregate Exposure (oral + inhalation + dietary):

The potential for health hazard associated with the use of endosulfan was considered to determine MOEs for occupational (inhalation + dermal + oral) and oral (non-dietary); bystanders and ambient air (inhalation + oral (dietary) and for swimmers in surface water (non-dietary ingested or dermal) in combination with oral dietary exposure. For aggregate exposure, the risk was determined by a total MOE approach (USEPA, 2001e). This approach is used when there is a common effect with different NOELs for the different routes of exposure but with the same uncertainty factor (UF) applied for both routes. The magnitude of the total MOE expressed only the risks for specified endpoints. The calculations are as follows:

$$\text{Aggregate Total MOE (MOE}_T\text{)} = \frac{1}{\frac{1}{\text{MOE dermal}} + \frac{1}{\text{MOE inhal}} + \frac{1}{\text{MOE diet}}}$$

For aggregate (occupational<sub>dermal/inhalation</sub> + dietary) MOE determinations in occupational scenarios the STADD, SADD and AADD exposure components were derived from the dermal and the inhalation values previously provided and from dietary MOEs studies (0.7 mg/kg, 1.18 mg/kg/day and 0.57 mg/kg/day, respectively) used in the STADD, SADD and AADD determinations for occupational and swimmer in surface water scenarios. This is because for these particular aggregate exposures, the dietary and dermal routes comprise the primary routes, and because an oral NOEL is used for dermal exposure (no acceptable dermal study).

For endosulfan exposure to the public in ambient air or for bystanders the same NOELs are used for calculations for short term and subchronic MOEs (0.194 mg/kg/day) from the subchronic, rat inhalation study (Hollander et al., 1984). The NOEL used for the chronic MOE calculations is also from the Hollander et al. (1984) study with an additional 10x uncertainty factor to extrapolate from subchronic to chronic (NOEL = 0.0194 mg/kg/day) is used.

The dietary MOE contribution to aggregate estimations were determined for acute MOE (340, 95<sup>th</sup> percentile for females (13+ years), nursing), and chronic (used also for subchronic; 3448 (females (13+ years), nursing) exposures.

### c. Aggregate MOEs Occupational Exposure

- Aggregate MOEs for Aerial and Ground Application

Aerial application MOEs for all aggregate STADD scenarios were less than 100, ranging from less than 1 (aerial M/L WP and applicator) to 25 (airblast M/L WSP). SADD aggregate MOEs were less than 100, except for airblast M/L EC (156), groundboom M/L EC (123) and applicator (180). AADD aggregate MOEs were less than 100, except for airblast M/L EC (156), airblast M/L WSP (139) and applicator (112) (Table 35).

- Aggregate MOEs for Handlers Using Handheld Equipment.

All aggregate STADD MOEs were well below 100 (<1 to 45) for handlers using handheld equipment except dip M/L EC (335) and dip M/L WP (280) (Table 36). SADD and AADD aggregate MOEs less than 100 were for HPHW M/L/A-EC (7 and 10, respectively) and LPHW M/L/A-WP (66 and 93, respectively). Other MOEs for SADD and AADD were greater than 100 and ranged from 103

(SADD backpack sprayer M/L/A EC) to 757 (LPHW M/L/A EC).

- Aggregate MOEs for Reentry Workers

All scenarios for STADD for reentry workers had aggregate MOEs that were less than 100, with a range of 1 for sweet corn, hand harvesting to 64 for hand harvesting ornamentals and for almond thinning (Table 37). For SADD, 4/10 aggregate MOEs were less than 100, however one of the MOEs was 97 (range = 8 for grape, cane turning to 97 for broccoli, scouting). The highest SADD MOE was 283 for both lettuce, scouting and for potato, scouting. The only AADD MOEs less than 100 were sweet corn, hand harvesting (92) and grape, cane turning (12). All other AADD MOEs (8/10) were greater than 100 (110 = peach, thinning to 487 for cucumber, hand harvesting).

d. Data for Aggregate MOEs in Non-Occupational Scenarios

- Aggregate MOEs for Ambient Air and for Bystanders

Aggregate bystander scenarios (no short-term ambient air exposure values) had STADD MOEs of 78 (infants) and 146 (adults). SADD MOEs were all greater than 100, ranging from 296 (bystander infants) to 2648 (ambient air adults). For AADD, aggregate scenarios for ambient air and for bystanders were all greater than 100, ranging from 343 (bystander infants) to 1241 (ambient air adults). The majority of the aggregate MOEs were less than 1000 and must be flagged for further evaluation under the Food Quality Protection Act (1996). (See page 173 for a discussion of the 10x safety factor.)

e. Aggregate MOEs for Swimmers in Surface Water

All aggregate scenarios for swimmers in surface water had STADD, SADD and AADD MOEs of greater than 100 (Table 39). Aggregate MOEs for STADD ranged from 144 for child non-dietary ingestion and for child total (144; non-dietary ingestion and dermal) to 350 for adult dermal. Within scenarios for STADD, SADD and AADD the aggregate MOEs for adults or for children, (that is, aggregate MOEs for (dermal + dietary); (non-dietary ingestion + dietary) and [dermal + non-dietary ingest] + dietary) did not vary significantly. For example MOEs for STADD aggregate scenarios had adult MOEs of 308 to 350 and child MOEs of 144 to 212. SADD aggregate MOEs for adults ranged from 6755 to 6940 and child MOEs or 2634 to 2949. AADD aggregate MOEs for adults ranged from 3328 to 3353 and for children ranged from 1380 to 1425.

## **Tolerance Assessment**

**ACUTE:** There are currently more than 72 human consumption commodities that have endosulfan tolerances (CFR, 2006). A total of 20 commodities, including milk, were analyzed for tolerance level acute dietary exposure. There were 15 commodities that had MOEs of less than 100 for 1 or more population subgroups when assessed using tolerance level values. The MOEs were based on tolerance levels of endosulfan. RACs (apple, melon and tomato) acute 95<sup>th</sup> percentile MOEs ranged from 5 for apples (nursing infants < 1 year) to greater than 100 for tomatoes (seniors 55+). All commodities for all population subgroups listed had acute 95<sup>th</sup> percentile MOEs less than 100 for apples, melons and tomatoes, except seniors (55+). Apples and melons are the only two commodities

with endosulfan tolerances that have all 20 of their analyzed populations with MOEs less than 100. Tomatoes had 19 of the analyzed populations with MOE values of less than 100.

**CHRONIC TOLERANCE ASSESSMENT:** A chronic exposure assessment using residues equal to the established tolerances for individual or combinations of commodities has not been conducted because it is highly improbable that an individual would chronically consume single or multiple commodities with pesticide residues at the tolerance levels. This conclusion is supported by data from both federal and DPR pesticide monitoring programs which indicate that less than one percent of all sampled commodities have residue levels at or above the established tolerance (DPR, 1994,1995,1997).

**TOLERANCE ASSESSMENT - 2007:** In 1998 there were 72 commodities with human consumption that had USEPA endosulfan tolerances (USEPA, 1999a) but since then 9 commodity tolerances have either been canceled or proposed for cancellation by the registrants of technical endosulfan. The USEPA draft endosulfan RED also decreased the maximum label application rates by approximately 17-33%, depending on commodity, for a number of commodities that will still have tolerances. These reductions in maximum annual application rates may be reflected in a corresponding decrease in the magnitude of the residues detected on endosulfan treated commodities. In 2006, the USEPA announced in the Federal Register (September 15, 2006 (Volume 71, Number 179)] the final ruling on endosulfan tolerance actions that carried out the proposed tolerance changes that were described in the RED for endosulfan (USEPA, 2002).

## **Conclusions**

**OCCUPATIONAL & PUBLIC RISK MARGINS OF EXPOSURE (MOEs):** In each occupational (dermal, oral, inhalation) there were MOEs less than 100 (primarily for short term) and in several cases the MOEs were less than 1. MOEs for ambient air and bystander inhalation and for swimmer in surface water were all greater 100 for all scenarios.

**DIETARY MOEs:** The MOEs from anticipated endosulfan residues for acute toxicity (95<sup>th</sup> percentile, UB) were all well above 100; however, the acute 95<sup>th</sup> percentile MOEs from tolerance levels of endosulfan for apple, melon and tomato in selected population groups were all, except for seniors 55+ years, less than 100. For dietary exposure, all population subgroups have MOEs (acute 95<sup>th</sup> percentile and chronic) greater than 100 for acute and 1000 for chronic.

**AGGREGATE (Combined Occupational plus Dietary) MOEs:** There is a preponderance of short-term seasonal and annual scenarios (both aggregate and route-specific) where the MOEs fall well below 100. However, there are also some MOEs that are close to or greater than 100.

**PUBLIC RISK MARGINS OF EXPOSURE (MOEs):** MOEs for all route-specific and aggregate scenarios (inhalation exposure to bystanders and in ambient air and to swimmers in surface water) were greater than 100 (except for bystander infants, short term).

## II. INTRODUCTION

This report evaluates the potential for endosulfan exposure, and includes: 1) a review of the available scientific evidence on  $\alpha$ -endosulfan,  $\beta$ -endosulfan, and endosulfan sulfate regarding their physical properties, 2) and an occupational and dietary health risk assessment for technical endosulfan as currently used in California.

The risk assessment for endosulfan was performed by the California Department of Pesticide Regulation (DPR) because of the chemical's low No-Observed-Effect-Levels (NOELs) in a rabbit teratology, in a combined (chronic toxicity/oncogenicity) study in the rat and in a chronic dog study. These studies were submitted to DPR under the Birth Defect Prevention Act of 1984. An assessment of dietary risk was also conducted (Bronzan and Jones, 1989) to determine risk for pesticides with food crop uses. Therefore, the purpose of this document is to address the potential adverse health effects for agricultural workers exposed to endosulfan and for the general public exposed to endosulfan through potential dietary sources and ambient air under the Toxic Air Contamination Act (California Food and Agricultural Code: 14021-14027). The State Water Resources Control Board (SWRCB) requested that DPR place endosulfan into formal reevaluation for the purpose of mitigating fish toxicity (DPR, 1994). Currently, endosulfan has been included on the U.S. Clean Water Act Section 303(d) list for registered pesticides 2002 (finalized by SWRCB, February 2003, Singhasemanon, 2003).

### A. CHEMICAL IDENTIFICATION

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide) is a pesticide, belonging to the chemical family of organochlorines, subclass chlorinated cyclodienes, and containing only one double bond. It is used to control more than 100 different insect pests (aphids, leafhoppers, borers, worms etc.) that infest a large number of crops in California. It serves as a contact and stomach insecticide for approximately 50 food and non-food crops but has proven to be extremely toxic to fish and other aquatic organisms (USEPA, 2002). In California, the food crops are primarily grapes, melons, lettuce and tomatoes, and cotton, both a food (cotton seed oil) and a non-food crop (Carr, 2006).

Although endosulfan, patented in 1956 (Ware, 1994), is usually included among pesticides of the “chlorinated hydrocarbons of the cyclodiene group,” or organochlorines, Maier-Bode (1968) considered endosulfan, as a sulfite ester of a cyclic diol, to be sufficiently different from other cyclodiene insecticides in its chemical properties, physiological effects and fate on the surface of living plants, that it should not be included in this group. CAS classifies it as a “dioxathiepin.” Endosulfan has not been separated from the group primarily because the acute effects it produces in laboratory animals or in humans are indistinguishable from those caused by other cyclodiene compounds.

#### 1. Mechanisms of Toxicity

The mode of action of endosulfan is to bind and inhibit  $\gamma$ -amino-butyric acid (GABA)-gated chloride channel receptor, thereby inhibiting GABA-induced chloride flux across membranes (Abalis et al., 1986; Ffrench-Constant, 1993; Sutherland et al., 2004). A point mutation in a family of GABA receptor subunits is associated with resistance to endosulfan in insects (Ffrench-Constant, 2000).

Neurotoxicity has been attributed to several actions such as an inhibition of the calmodulin-dependent  $\text{Ca}^{+2}$ -ATPase activity (Srikanth, et al., 1989), alterations in the serotonergic system (Agrawal et al., 1983) and inhibition of GABA. Endosulfan functions as a non-competitive GABA antagonist at the chloride channel within the GABA receptor in mammalian brain synaptosomes (Abalis, et al., 1986; Cole and Casida, 1986; Gant et al., 1987; Ozoe and Matsumura, 1986). Antagonism of GABAergic neurons within the central nervous system causes generalized brain stimulation. When GABA binds to its receptor ( $\text{GABA}_A$ ), the chloride-selective ion channels are opened, leading to an influx of chloride into neurons through an electrochemical gradient. This process results in hyperpolarization of the cellular membrane and inhibited neuron firing. Endosulfan may prevent chloride ions from entering neurons, thus inhibiting GABA from binding to its  $\text{GABA}_A$  receptor, resulting in uncontrolled excitation.

Influx of chloride ion into rat brain microsacs (in the presence of both  $\alpha$ - and  $\beta$ - endosulfan) by GABA was measured (Gant et al., 1987). The  $\alpha$ -isomer ( $\text{IC}_{50}$  0.19 +/- 0.07  $\mu\text{M}$ ) was significantly more potent at inhibiting chloride influx than the  $\beta$ -isomer ( $\text{IC}_{50}$  8.09 +/- 2.0  $\mu\text{M}$ ). Ablais et al. (1986) also measured chloride influx across rat brain membranes and found that 1  $\mu\text{M}$   $\alpha$ -endosulfan completely inhibited influx, the same concentration of the  $\beta$ -isomer inhibited only 70%. Another study showed that  $\alpha$ -endosulfan blocked chloride uptake induced by GABA in primary cultures of cortical neurons from fetal mice (15 days old) by interacting with the GABA antagonist t-butylbicyclophosphorothionate binding site (Pomes et al., 1994). The effects on the GABA-receptor complex are similar to those of lindane, dieldrin and endrin (Lawrence and Casida, 1984; Casida and Lawrence, 1985; Cole and Casida, 1986).

## 2. Chemical Interactions

Animals exposed chronically to low doses of endosulfan respond more markedly to the pharmacological actions of diazepam, chlorpromazine, pentobarbital and ethanol, when compared to controls (Paul and Balasubramaniam, 1997). Changes in potency and duration of action may be due to the enzyme-inducing action of endosulfan, since the drugs in question are biotransformed by mixed function oxidases (MFO). There is a concern about hazards caused by the interaction of endosulfan and therapeutic agents that act on the central nervous system, since endosulfan is a potent MFO inducer.

## B. REGULATORY HISTORY

Farbwerke Hoeschst A.G. registered endosulfan in the United States, in 1954. The initial trademark was “Thiodan” (Maier-Bode, 1968). It is currently registered by the United States Environmental Protection Agency (USEPA, 2002) as a broad-spectrum insecticide and acaricide. Endosulfan is listed under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA, 1980) and Superfund Amendments and Reauthorization Act of 1986 (SARA) as a hazardous substance (USEPA, 1996). A hazardous substance is defined as something that, upon exposure, will result in adverse effects on health and safety of humans in an occupational setting (USDOL, 1988). The statutory source for this designation for the  $\alpha$ - and  $\beta$ -isomers is Section 307 of the Clean Water Act (CWA). In addition, the designation for endosulfan is based on section 311(b)(4) of the CWA and Section 3001 of the Resource Conservation and Recovery Act (RCRA)(USEPA, 1995). The USEPA Office of Pesticide Programs anticipates the submission of a subchronic rat neurotoxicity study by the registrant for review (USEPA, 2002). All study requirements have been met in California under The Birth Defects Prevention Act (1984). Endosulfan is not currently listed

under California's Safe Drinking Water and Toxics Enforcement Act of 1986 that requires the identification of chemicals "known to the state to cause cancer or reproductive toxicity," (Proposition 65, 1987). Endosulfan is under consideration for listing as a toxic air contaminant under the Toxic Air Contamination Act (California Food and Agricultural Code: 14021-14027).

In 1991, the technical registrants amended labels to incorporate a 300-foot spray drift buffer zone for aerial applications between treated areas and water bodies. This setback was adopted in order to address concerns about contamination of water and risks to aquatic organisms. In 2000, the registrants amended technical product labels to remove all residential use patterns (USEPA, 2002).

### **C. TECHNICAL AND PRODUCT FORMULATIONS**

Technical endosulfan consists of two isomers,  $\alpha$ - (64 - 67%) and  $\beta$ - (29 - 32%) forms, as well as some impurities and degradation products (Maier-Bode, 1968; NRCC, 1975). One of the degradation products, endosulfan sulfate, which has chemical properties similar to pure endosulfan, results from photolysis (in solid or gas phase) or biotransformation (Callahan et al., 1979).

Endosulfan is a broad-spectrum foliar insecticide and miticide, with product trade names such as: Thionate, Endochem and Thionil (more names can be found in the Crop Protection Handbook, 2006, Meister and Sine eds., page D169 and USEPA, 2002). As of April 2007, two formulations were registered in California, an emulsifiable concentrate (EC) containing 34% AI (sold in two products), and a wettable powder (WP) containing 50% AI (sold in three products). In addition to these five products, a 95% AI technical endosulfan is registered solely for manufacturing use. The EC formulation contains 3 lbs AI/gallon (0.36 kg AI/L). Both EC and WP formulations are registered for use on several crops, all of which are listed in Beauvais (2007; Appendix D). Endosulfan may be applied by aerial or ground methods; application by any irrigation method is prohibited in California.

A proposed new product has been submitted for registration in California, an ear tag consisting of impregnated material containing 30% endosulfan. This product is proposed for use on cattle, to protect against the hornfly. Information is still being obtained for this product, and it is not considered further in the Exposure Assessment Document (Beauvais, 2007).

### **D. USAGE**

From 1997 to 1999, approximately 571,296 total pounds of endosulfan were used on more than 60 crops in California (DPR, 1999, 2001a, b). In 1999, 88.2% of endosulfan applied (179,584 lbs) was used on cotton, grapes (table and raisin), lettuce, alfalfa, cantaloupe and tomatoes (processing) (DPR, 2001b). Currently there are 13 active products containing endosulfan registered with the USEPA (compared with 50 in 1998). This reduction in product registrations has not significantly impacted the amount of endosulfan used nationally. In 2000, registrants for technical endosulfan changed the technical product labels to remove all residential use patterns (USEPA, 2002).

In California, there are 6 endosulfan products with active registrations (DPR, 2006). The total amount of endosulfan used in California decreased between 1998 and 2004 (total in PUR 1999: 179,584 lbs. vs. 2004: 153,339 lbs.). In addition to a decreased annual average endosulfan use in California, the spectrum of highest use crops has changed. From 1993-1995, average annual use was 356,970 lbs and the highest use crops were cotton (192,000 lbs), grapes (47,000 lbs), cantaloupe

(20,000 lbs) and head lettuce (20,000 lbs). In 1999-2003, the average annual use was 152,445 lbs and the highest use crops were cotton (38,085 lbs), alfalfa (32,981 lbs), lettuce (25,265 lbs) and tomatoes (21,713 lbs) that together accounted for 75% of endosulfan use in 2003 (Beauvais, 2007).

#### **E. REPORTED ILLNESSES (Complete report in Appendix E. [Volume II], Beauvais, 2007)**

Reports of illness and injury with definite, probable, or possible exposure to pesticide products are recorded in a database maintained by the Pesticide Illness Surveillance Program (PISP) at DPR. The PISP database contains information about the nature of the pesticide exposure and the subsequent illness or injury. In California between 1992 and 2004, 63 illnesses were reported to the Pesticide Illness Surveillance Program that suggested the involvement of endosulfan, alone or in combination with other pesticides (Verder-Carlos, 2006). Of the 63 illnesses, 61 resulted from agricultural applications and just two from non-agricultural applications. Five agriculturally-related and both of the non-agriculturally-related illnesses and injuries were attributed solely to endosulfan; the other 56 reports were associated with endosulfan in combination with other pesticides.

Of the seven illnesses and injuries attributed solely to endosulfan, one occurred as the result of exposure to field residues, three resulted from handling processes (mix/load, apply), two resulted from drift, and one followed a non-specified exposure. Of the 56 illnesses resulting from exposure to endosulfan in combination with other pesticides, 43 occurred as the result of exposure to field residues on treated crops, six occurred during the application process (mix/load, apply, flag), and seven occurred as the result of drift exposure.

Table 1 summarizes types of symptoms reported in association with endosulfan exposure. The majority of illnesses involved skin and eye effects, such as irritation and rashes. Several incidents involved more than one worker. None of the incidents resulting in multiple exposure involved endosulfan as the only pesticide. Of the 44 field worker illnesses and injuries, 31 (70%) harvesting cucurbits (melons, cucumbers), and seven (16%) occurred while working in grapes. The remaining six (14%) occurred in various other crops.

For illnesses where endosulfan was the sole pesticide involved, systemic effects were observed in four cases (two of which also had skin and eye involvement), while skin and eye effects occurred in three cases. In cases where endosulfan was used or encountered along with other pesticides, 27 people developed systemic symptoms (some also involved skin and eye effects), while 28 involved only skin and eye effects.

In the southeastern U.S., two incidents were reported in which mixer/loader/applicators (M/L/As) pouring endosulfan without proper protective equipment experienced serious illnesses (Brandt et al., 2001). In both cases, endosulfan splashed onto skin and clothing during mixing and loading; in the second case, drift during the application, enough that his clothes “appeared soaked,” was witnessed. Both individuals proceeded with the applications without washing skin or changing the contaminated clothing. Exposure durations were estimated at 4 - 5 hours. Evidence suggested that these exposures resulted in long-term neurological damage in one case, and in death in the other case.

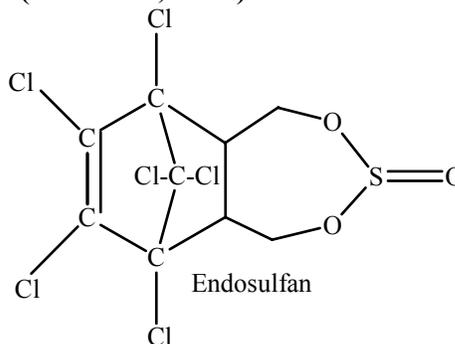
**Table 1. Illnesses Reported in California Associated with Endosulfan Exposure (1992-2003) <sup>a</sup>**

Systemic <sup>b</sup>	Skin	Eye	Systemic/ Skin	Systemic/ Eye	Skin/Eye	Systemic/ Skin/Eye	Total
<b>Endosulfan alone</b>							
2	1	0	0	0	2	2	7
<b>Endosulfan with other pesticides</b>							
14	23	2	7	1	4	5	56
<b>Total</b>							
16	24	2	7	1	6	7	63

a - This table summarizes types of symptoms reported, and includes illnesses possibly, probably or definitely associated with endosulfan exposure. “Definite” means that both physical and medical evidence document exposure and consequent health effects, “probable” means that circumstantial evidence supports a relationship to pesticide exposure, and “possible” means that evidence neither supports nor contradicts a relationship (Verder-Carlos, 2005).

b - Systemic illnesses include symptoms such as shortness of breath, nausea, dizziness, headache and numbness.

## F. PHYSICAL AND CHEMICAL PROPERTIES (Budavari, 2001)<sup>a</sup>



**Structure, common name and formula:** C<sub>9</sub>H<sub>6</sub>Cl<sub>6</sub>O<sub>3</sub>S

**Chemical Names:** 6,7,8,9,10,10-Hexachloro-1,5,5 $\alpha$  ",6,9,9 - $\alpha$ -hexahydro-6,9-methano-2,4,3-benz(o)-dioxathiepin-3-oxide; endosulfan technical; 5-norbornene-2,3-dimethanol -1,4,5, 6,7,7 -hexachlorocyclic sulfite

**Other Names:** Thiodan, Thionil, Thionate, etc. (see: Meister and Sine, 2006)

**CAS Registry #:** 115-29-7

**Molecular Weight:** 406.9

**Color:** Pure endosulfan is a colorless crystal. Technical grade endosulfan is brown in color.

**Physical State:** Crystalline solid

**Melting Point:** Endosulfan 106°C (pure); 70-100°C (technical)

**Density:** 20/4°C 1.735 g/ml (HSDB, 1999)

**Specific Gravity Vapor:** 14.0 (HCDB, 1986)

**Odor:** Terpene-like; Decomposition products and, similar to hexachlorocyclopentadiene, sometimes mixed with

## sulfur dioxide in odor.

**Solubility**<sup>b</sup>: a) Water 25°C: 60 to 100 ug/L (ppb) (Sittig, 1980; Sarafin, 1979a)  
20°C (pH 5.0): 0.33 mg/L ([http://em/docs/pubs/chem/allchems\\_pestchem.pdf](http://em/docs/pubs/chem/allchems_pestchem.pdf))

b) Organic Solvents at 20°C:

Insoluble	Dichloromethane, ethanol, ethyl acetate, hexane
Moderately Soluble	Methanol, acetone (at 20°C), kerosene, toluene
Very Soluble	Benzene, carbon tetrachloride, chloroform, xylene

**Partition Coefficients:**  $K_{ow} = 55,500$  and  $61,300$ ;  $\log K_{oc} = 3.5$  (Sarafin, 1979b)

**Vapor Pressure:** 20°C: 0.83 mPa (Sarafin, 1982)

**Henry's Law Constant (24.8 °C):**  $\alpha$ -endosulfan =  $4.9 \times 10^{-6} \text{ atm} \cdot \text{m}^3 \cdot \text{mol}^{-1}$ ;  $\beta$ -endosulfan =  $1.2 \times 10^{-6} \text{ atm} \cdot \text{m}^3 \cdot \text{mol}^{-1}$

lc

**Mixture of isomers:**  $1 \times 10^{-5} \text{ atm} \cdot \text{m}^3 / \text{mmol}$  (Suntio et al., 1988)  
 $1.01 \times 10^{-4} \text{ atm} \cdot \text{m}^3 / \text{mol}$  (Montgomery, 1993);

---

a - All references are for Budavari, 2001, unless otherwise referenced.

b - Coleman and Dolinger, 1982; HSDB, 1999; Maier-Bode, 1968

c - [http://www.cdpr.ca.gov/docs/emon/pubs/tac/tacpdfs/endosulfan/endosulfan\\_sum.pdf](http://www.cdpr.ca.gov/docs/emon/pubs/tac/tacpdfs/endosulfan/endosulfan_sum.pdf) -

## G. ENVIRONMENTAL FATE

### 1. Summary

Endosulfan hydrolysis increases with increased pH. Half-life due to hydrolysis is decreased from 150 days at pH 5.5 to 1 day at pH 8.0. Endosulfan is photolysed, with a half-life of approximately seven days, giving Endosulfan diol as the main product. Endosulfan sulfate is relatively stable to photolysis. Fungi and bacteria inhabiting the soil under aerobic conditions can degrade endosulfan. Fungi produce primarily endosulfan sulfate while the bacteria produce primarily endosulfan diol. Endosulfan metabolism under flooded (anaerobic) conditions yields primarily endosulfan diol (2-18%), endosulfan sulfate (3-8%) and endosulfan hydroxyether. Surface and well water have not been sampled since 1996, as endosulfan is not considered to be a potential drinking water contaminant. Endosulfan binds tightly to some soils and is not mobile. Air monitoring shows that endosulfan can volatilize from water, soil and plant surfaces for 1 to 11 days post application. Endosulfan is translocated to roots after application to leaves. It is also metabolized within the plant, so that the sulfate form is found in the roots. Translocation of endosulfan from leaves to roots is more rapid under warmer greenhouse conditions as compared to ambient outside temperatures. Bioaccumulation of the  $\alpha$ - isomer,  $\beta$ - isomer and sulfate metabolite occurs in both aquatic (mussels, fish, shrimp, algae) and terrestrial (mosquito, snail) wildlife. However, it rapidly decreases to undetectable levels after animals are transferred to clean water.

### 2. Hydrolysis

In laboratory experiments conducted by Peterson and Batley (1993),  $\alpha$ -endosulfan consistently degraded faster than  $\beta$ -endosulfan. Both isomers hydrolyzed faster in alkaline waters than in water close to pH 7.0. Half-lives in water at pH 8.5 (20°C) were 3.6 days for  $\alpha$ -endosulfan and 1.7 days for

$\beta$ -endosulfan. As  $\beta$ -endosulfan is less water-soluble than  $\alpha$ -endosulfan, it is also more likely to partition to sediment.

Endosulfan stability in aqueous solutions is dependent upon pH conditions (at 22°C: pH = 5.5 and 8.0, half-lives = 150 and 1 day, respectively) (Singh, et al., 1984, 1991). Endosulfan hydrolysis increased as pH increased (at 30°C distilled water: pH = 4.5, 7.0 and 9.5, half-lives = 87.7, 23.5 and 1 hour, respectively). Hydrolysis rates in seawater are similar to those in fresh water (20°C in seawater: pH 8.0, half-life of  $\alpha$ - isomer = 2.2 and  $\beta$ - isomer = 4.9 days) and under most conditions the  $\alpha$ - isomer hydrolyzes almost twice as fast as the  $\beta$ - isomer (Guerin and Kennedy, 1992). The hydrolysis product in surface and groundwater is endosulfan diol.

Losses of endosulfan, at 0.5 mg/L, from natural lake water and tap water were 89 and 69%, respectively (Ferrando et al., 1992). Natural lake water is more alkaline than the tap water and the half-life of endosulfan in tap water was 68 hours.

### 3. Photolysis

In general, chemical photolytic breakdown occurs only when there's an absorbing group (e.g. acetone in experimental conditions or humic acid in soil) stimulated by natural or artificial light. The UV-absorption spectra of the endosulfan isomers show significant adsorption only below 290 nm. As wavelengths below 290 nm were filtered out, endosulfan was photolytically stable (Stumph, 1987). In aqueous conditions, endosulfan was stable to photolytic breakdown (even with sensitizer) since endosulfan has low water solubility (0.3 mg/L). Results showed that photolytic half-lives (in distilled water) varied with the isomer. At 25°C and pH 5 (distilled water vehicle), the photolytic half-life for the  $\alpha$ -isomer was 382 days and for the  $\beta$ - isomer was 443 day. In sandy loam soil, under laboratory conditions, endosulfan achieved a half-life of 238 days (Gildemeister and Jordan, 1983).

Under environmental conditions, the photolytic half-life is approximately 7 days for endosulfan and the primary photolysis product is endosulfan diol (subsequently degraded to  $\alpha$ -hydroxyether). Endosulfan sulfate is stable to direct photolysis at wavelengths of less than 300 nm; however, it reacts with hydroxy radicals to give an estimated half-life of 1.23 hours (Stumph, 1987; HSDB, 1999). A study by Dureja & Mukerjee (1982) showed that when the  $\alpha$ - and  $\beta$ - isomers are in polar solvents or on plant leaves and receive irradiation or are exposed to sunlight, the  $\alpha$ - form isomerizes to the more stable  $\beta$ - isomer.

### 4. Microbial Degradation

#### a) Aerobic

An endosulfan soil degradation study, performed in silt loam and loamy sand, showed that after 60 days the primary metabolite was endosulfan sulfate (Gildemeister and Jordan, 1984). In studies performed by Martens (1976 and 1977) soil fungi, bacteria and actinomycetes were incubated *in vitro* with [<sup>14</sup>C]-endosulfan. Results showed that 16 of 28 soil fungi, 15 of 49 soil bacteria and 3 of 10 actinomycetes tested metabolized more than 30% of the applied endosulfan. Fungi produced primarily endosulfan sulfate while the bacteria produced primarily endosulfan diol (Martens, 1976; Martens, 1977). Both  $\alpha$ - and  $\beta$ - isomers are slowly oxidized in air and in microbial systems (White-Stevens, 1971). Endosulfan technical and emulsifiable concentrate (40%) at 10 ug/g soil decreased soil bacteria

and fungal populations initially, but after 3 weeks the levels were restored to normal (Tu, 1991).

Two strains of bacteria were co-cultured aerobically and were able to efficiently degrade endosulfan; however, the degradation of the soil-bound endosulfan was slower by 4-fold than was measured in culture media (50% of 50 ppm in soil degraded in 4 weeks) (Awasthi et al., 1997). In a field study, 6.7 kg/hectare of endosulfan incorporated into sandy loam soil was transformed to endosulfan sulfate (Stewart and Cairns, 1974). Half-lives for the  $\alpha$ - and  $\beta$ - isomers were reported to be 60 and 800 days, respectively. *Pseudomonas* microbes have been reported to isomerize and biodegrade endosulfan alcohol and endosulfan ether (Menzie, 1978). In a laboratory setting, *Pseudomonas* degraded endosulfan under aerobic conditions (pH 7, 20°C) with a half-life of 1 week (Greve and Wit, 1971).

Miles and Moy (1979) indicated that under certain soil conditions, an alkaline pH (induced by microorganisms) produced a different endosulfate metabolite profile. Endosulfan incubated in a mixed culture of microorganisms from a sandy loam soil or in sterile soil, was degraded at a significantly greater rate in the soil containing microorganisms (with the diol being the primary metabolite). Microbes increased the soil pH from 6.5 to 7.6 and, therefore, some degradation was probably due to non-specific hydrolysis. The microbes did not degrade endosulfan sulfate, formed in acidic organic soil (Miles and Harris, 1978).

Cotham and Bidleman (1989) found the  $\alpha$ - and  $\beta$  - endosulfan half-lives in seawater microcosms (pH 8) to be 5 and 2 days, respectively. When sterile seawater (pH 8) was used, the half-life for the  $\alpha$ - isomer was 2 to 3 days and for the  $\beta$ - isomer was 1 to 2 days. When a seawater/sediment microcosm was tested, the half-lives were 22 and 8.3 days for  $\alpha$ - and  $\beta$  - endosulfan, respectively, possibly due to the lower pH of this system (pH 7.3 - 7.7). Endosulfan diol was the primary metabolite identified in seawater only, as well as seawater/sediment microcosms.

In the study by Gildemeister and Jordan (1984), aerobic endosulfan degradation occurred with a half-life of 256 days (pH 6.4) in silty loam soil and 375 days (pH 4.7) in sandy loam soil.

#### b) Anaerobic

Endosulfan metabolism under flooded (anaerobic) conditions yields primarily endosulfan diol (2-18%), endosulfan sulfate (3-8%) and endosulfan hydroxyether (2-4%) (Martens, 1977). A mixed culture of microorganisms, isolated from sandy loam soil, was incubated in an aqueous nutrient medium at 20°C. Endosulfan was metabolized to endosulfan diol with half-lives of 1.1 to 2.2 weeks for the  $\alpha$ - and  $\beta$ - isomers, respectively (Miles and Moy, 1979).

In the study by Gildemeister et al. (1988), anaerobic endosulfan degradation occurred with a half-life of 143 days (pH 7.2) in sandy loam soil and 152 days (pH 6.4) in silty loam soil.

### **5. Mobility (water, soil, air, plants)**

#### a) Water Monitoring

In laboratory experiments conducted by Peterson and Batley (1993), consistently  $\beta$ -endosulfan degraded faster than  $\alpha$ -endosulfan; both isomers hydrolyzed faster in alkaline waters than in water

close to pH 7. Half-lives in pH 8.5 (water at 20°C) were 3.6 days for  $\alpha$ -endosulfan and 1.7 days for  $\beta$ -endosulfan. As  $\beta$ -endosulfan is less water-soluble than  $\alpha$ -endosulfan, it is more likely to partition to sediment as well.

Endosulfan has been monitored in both surface and ground water in California, and in tissues of fish and aquatic invertebrates. The monitoring data relevant to human exposure to endosulfan include surface waters where swimming or wading may occur (e.g., rivers or farm ponds), as well as surface and ground water sources of drinking water in California. Endosulfan residues occurring in drinking water could potentially result in exposure through swimming or bathing.

#### i. Surface Water

Historically, endosulfan has been detected numerous times in California surface waters. Guo and Spurlock (2000) summarized historical monitoring data, reported by nine different agencies between 1990 and July 2000, for pesticides in surface water in California. Monitoring for  $\alpha$ -endosulfan,  $\beta$ -endosulfan, and endosulfan sulfate was conducted between August 1990 and July 1996 (DPR, 2004). Table 2 summarizes these data and shows that endosulfan sulfate has been detected more frequently in surface water samples than  $\alpha$ - or  $\beta$ -endosulfan, and generally at higher concentrations.

**Table 2. Summary of Historical Surface Water Sampling Data for Endosulfan in California Between August 1990 and July 1996<sup>a</sup>**

Chemical	# Analyses	# Detections	Detection Frequency (%)	Concentration ( $\mu\text{g/L}$ ) <sup>b</sup> Percentiles		
				50 <sup>th</sup>	75 <sup>th</sup>	95 <sup>th</sup>
$\alpha$ -Endosulfan	764	40	5.2	0.024	0.049	0.105
$\beta$ -Endosulfan	764	41	5.4	0.023	0.038	0.066
Endosulfan Sulfate	661	114	17.2	0.025	0.066	0.141

a - Adapted from Guo and Spurlock (2000), which summarizes water sampling through July 2000. No monitoring for endosulfan has been reported since July 1996 (DPR, 2004), nor does the database differentiate between surface water systems that are sources of drinking water and those that are not (F. Spurlock, personal communication, June 7, 2005). The limits of quantitation ranged from 0.00005 - 0.10  $\mu\text{g/L}$ .

b - Values are to the closest percentile possible (as determined by Guo & Spurlock, 2000), and for detected concentrations only (non-detects omitted).

The highest total endosulfan concentrations reported in one sample were collected from the Alamo River in Imperial County on June 21, 1993. The concentrations reported to DPR were as follows:  $\alpha$ -endosulfan, 0.22  $\mu\text{g/L}$ ;  $\beta$ -endosulfan, 0.17  $\mu\text{g/L}$ ; and endosulfan sulfate, 0.58  $\mu\text{g/L}$  (DPR, 2004). The total endosulfan concentration, 0.97  $\mu\text{g/L}$ , was used in estimating short-term swimmer exposure. For long-term exposures, the median total endosulfan concentration of 0.072  $\mu\text{g/L}$  was calculated from the 50<sup>th</sup> percentile concentrations summarized in Table 2.

Endosulfan residues were detected in California surface waters in the Central Valley in 1991 through 1993, at concentrations up to 0.039  $\mu\text{g/L}$  (Ross et al., 1996; Ross et al., 1999; Ross et al., 2000); these detections are included in data summarized in Table 2. Water samples collected from two lakes in 1997 in the Sierra Nevada Mountains contained  $\alpha$ -endosulfan at concentrations ranging from 0.00030 - 0.0010  $\mu\text{g/L}$ ;  $\beta$ -endosulfan at concentrations ranging from 0.00017 - 0.0018  $\mu\text{g/L}$ ; and endosulfan sulfate at concentrations ranging from 0.00033 - 0.0029  $\mu\text{g/L}$  (Fellers et al., 2004). Movement of endosulfan into surface water via rainfall runoff and irrigation drainage was documented

in studies completed in the 1980s (Gonzalez et al., 1987; Fleck et al., 1991). Sampling of rainfall runoff from three treated fields in 1988 detected endosulfan in samples from all three fields, at concentrations ranging from 2.2 to 13 µg/L (Fleck et al., 1991). Irrigation drainage samples collected in October 1985 contained endosulfan at one of three sites (detection limit: 0.01 µg/L); the mean  $\pm$  standard deviation concentration at that site was  $0.014 \pm 0.005$  µg/L (Gonzalez et al., 1987).

In surface water systems, endosulfan residues have also been detected in sediment (Gonzalez et al., 1987; Fleck et al., 1991; Ganapathy et al., 1997; Weston et al., 2004); mussels (Singhasemanon, 1996; Ganapathy et al., 1997); amphibians (Sparling et al., 2001); and fish (Singhasemanon, 1995; Brodberg and Pollock, 1999).

The detection of endosulfan residues in surface water, sediment, and aquatic organisms, and concerns about endosulfan's toxicity, led DPR, in 1991, to begin requiring permit conditions to prevent use of endosulfan where it might be allowed to reach surface water (Okumura, 1992). California drinking water data (3 years) from between 2001-2003 were examined by the USDA-PDP (USDA, 2003, 2004, 2005). A total of 424 California water samples were analyzed with a limit of detection of 0.1 ppb or better. No endosulfan or endosulfan degradates were detected. The number of samples by year were: 2001, 144; 2002, 140; and 2003, 140. The samples were collected from municipal water processing facilities post-processing and ready to drink. These results suggest that drinking water systems in California are not likely to be a source of human exposure to endosulfan.

## ii. Ground Water

DPR has a well monitoring program that samples numerous wells each year to determine the presence and geographical distribution of agriculturally applied pesticides in groundwater. Troiano et al. (2001) describes the program that includes the criteria for selection of wells and sampling and analytical methods. Between 1986 and 2003, a total of 2,758 well water samples collected in 48 California counties (out of 58 counties total) were tested for the presence of endosulfan and endosulfan sulfate (Schuette et al., 2003). Endosulfan was detected in ten samples, at concentrations ranging from 0.01 - 34.7 µg/L. All ten detections were classified as "unverified," because follow-up sampling failed to detect endosulfan or endosulfan sulfate. These results, along with reported non-detection of endosulfan residues in monitoring of drinking water systems (USDA, 2003; 2004; 2005), suggest that drinking water systems in California drawing from ground water are not likely to be a source of human exposure to endosulfan.

## b) Soil

Numerous laboratory and greenhouse studies have shown that both  $\alpha$ - and  $\beta$ -endosulfan strongly adsorb to soil. Glass-column elution tests showed that both isomers adsorbed tightly to loamy sand, sandy loam, sandy clay loam and sandy clay soils (El Beit et al., 1981; Bowman et al., 1965). Model soil evaporation beds designed to test the feasibility of treating pesticide wastes showed that endosulfan did not move in loamy sand soil in tests for up to 54 weeks (Hodapp and Winterlin, 1989).

In California, the Environmental Monitoring and Pest Management Branch of DPR (EM & PM, 1991) estimated a  $K_{oc}$  value of 2000 for endosulfan, suggesting that soil mobility is expected to be slight.  $K_{oc}$  values for  $\alpha$ - and  $\beta$ -endosulfan in marine sediment were approximately 3,981 and 19,953, respectively (Peterson and Batley, 1993). Adsorption of the isomers to soil particulates is predicted

based on the relation between octanol/water partition coefficient ( $K_{ow}$ ) and  $K_{oc}$ , and have been estimated to be 2,887 and 1,958, respectively (USEPA, 2000a). Others demonstrated, in adsorption/desorption studies of  $\alpha$ - and  $\beta$ - isomers, that strong adsorption onto organic matter in soils, with no differences between the isomers, occurred (Rao and Murty, 1980; Gorlitz, 1987; Byers et al., 1965; Richardson and Epstein, 1971).

In California, the Environmental Monitoring and Pest Management Branch of DPR (EM & PM, 1991) determined soil adsorption coefficients for endosulfan to range for the  $\alpha$ - isomer, (pH 5.4) from 63 in silty loam soil to 678 (pH 5.8) in loamy sand. For the  $\beta$ -isomer, the range was 74 (pH 5.4) in silty loam soil to 445 (pH 5.8) in loamy sand.

In an agricultural area near Oxnard, CA, endosulfan was detected in concentrations of 20 - 30 ppm in 2 creek bed and drainage ditch sites at Point Mugu watershed (an agricultural area) (Leung et al., 1998). Other sites sampled from the same study had much lower concentrations.

Thiodan 3EC was applied on tomatoes or bareground in 5 weekly applications at 5 lb. a.i./acre total (Hacker, 1989). Soil sampling for  $\alpha$ - and  $\beta$ -, endosulfan diol and sulfate was performed before, immediately after and at designated intervals until 540 days after the last application. The half-life in soil of the  $\alpha$ - isomer was 46.1 days and for the  $\beta$ - isomer was 90.8 days. Endosulfan sulfate residues in soil decreased by a factor of 4 by day 539, from its peak concentration at 180 days post-treatment and diol residues were non-detectable 180 days after peak concentration. Data were similar whether Thiodan 3EC was applied on tomatoes or to bareground. Endosulfan and metabolites did not leach below 5 cm of soil under actual use conditions.

### c) Air

In California, endosulfan has been monitored and detected in 34/39 or 23/39 samples by 8 hours after application for the  $\alpha$ - and  $\beta$ -isomers, respectively (See Appendix A, Table 14 for a summary of endosulfan concentrations and locations of monitoring stations; Beauvais, 2007). Endosulfan can drift after aerial application and can be transported long distances before being removed by wet or dry deposition (NRCC, 1975). A model for environmental distribution of organic chemicals in air was characterized, in terms of persistence and spatial range, by Scheringer (1997). This model shows that endosulfan has a limited spatial range (15% of the earth's perimeter) and a persistence of less than 10 days. The spatial range will increase with increased sorption of the compound to particulate matter, a condition hypothesized to preclude a fast reaction of semivolatile compounds with OH radicals. This suggests that bystander populations (non-occupational) could potentially be exposed to endosulfan in ambient air. Therefore, endosulfan is under consideration for listing as a toxic air contaminant (California Food and Agricultural Code: 14021-14027).

The endosulfan air/water distribution also determines the fate of the material in the atmosphere. The air/water distribution of endosulfan was determined using a wetted wall column (Rice et al. 1997a). Pure  $\alpha$ - endosulfan equilibrated in the column and the ratio of  $\alpha$ - to  $\beta$ -endosulfan in the gas phase became 8 to 92 at 20°C, showing that the  $\alpha$ - isomer converts to the  $\beta$ - isomer (Rice et al., 1997b). Endosulfan can be volatilized from surface water, with a half-life for volatilization from surface waters of approximately 11 days to more than a year (Callahan et al., 1979). This low volatilization is also predicted by the vapor pressure and Henry's Law Constant values for endosulfan (see F. PHYSICAL AND CHEMICAL PROPERTIES, page 5). However, Cotham and Bidleman

(1989) reported substantial volatilization losses from aqueous surfaces in seawater/sediment microcosm tests.

Endosulfan is quickly volatilized from plant surfaces (Archer, 1973; Ware, 1967). Willis et al. (1987) showed that the limited loss of endosulfan in soybean field runoff was attributed to volatilization/degradation of the compound from plant surfaces. Air samples taken in a wind tunnel under defined conditions (20°C, 1 m/sec air velocity; relative humidity 40 - 60%) showed 60% of endosulfan was volatilized from French bean surfaces and 12% volatilized from a silty sand soil after 24 hours (Rudel, 1997).

#### d) Plants

Endosulfan mobility in plants under laboratory and greenhouse conditions, showed endosulfan (both  $\alpha$ - and  $\beta$ - isomers) and metabolites (diol, ether and sulfate) penetrated plant surfaces and were translocated from leaves to roots in bean and sugar beet plants (Beard and Ware, 1969). Under controlled laboratory conditions, the majority of residues were from extracts of the plant surface (clipped at soil level). The next higher endosulfan concentrations were found in extracts of the entire macerated plant and the lowest amounts were in roots. After 16 days, most of the residues were in the sulfate form, located on the plant surfaces. Under greenhouse conditions, a different pattern occurred. Translocation of  $\alpha$ - and  $\beta$ - isomers occurred to a far greater extent, facilitated by the higher greenhouse temperatures. By post-treatment day 4 the majority of the residues (primarily endosulfan sulfate) were found in roots of both beans and sugar beets.

### 6. Plant Residues/Metabolites

Endosulfan residues can be found on tobacco and in tobacco products ( $\alpha$ -,  $\beta$ -, sulfate and other metabolites) in the range of 0.12 - 0.83 mg/kg (Chopra, et al., 1978; USEPA, 1980b). Alpha-endosulfan comprised 13%, and  $\beta$ - endosulfan was 78%, where the sulfate was 29% of the total residue (Dorough, et al., 1978).

Endosulfan (Thiodan EC, 0.25 lb/acre) was applied to apples and alfalfa in 3 or 1 sprayings, respectively (Worthing, 1991; Maier-Bode, 1968). Results showed 50% of the  $\alpha$ - and  $\beta$ - residues were lost within 3-7 days. The primary metabolite was endosulfan sulfate, which in both cases was less than 0.2 ppm after 15 days. Similar results were obtained when various formulations of endosulfan (WP, Dust, EC) were used in California on pears, peaches, cherries, strawberries, grapes and watermelon (0.5 - 4 lb/acre, 1-3 sprayings) (Maier-Bode, 1968; Menzie, 1969). Vegetables and grasses treated with 0.21 lb/acre (1-9 applications of EC, WP or dust) showed 5-50% endosulfan residue after 3-7 days.

### 7. Bioaccumulation (aquatic, terrestrial)

Bioaccumulation of endosulfan is reported in marine animals (e.g. mussels) and in freshwater animals (e.g. crayfish) and terrestrial animals (Naqvi and Vaishnavi, 1993). In fresh water ecosystems, residues in fish peak within 7-14 days of continuous exposure. Maximum bioconcentration factors (BCFs) are less than 3,000 and residues are eliminated within 2 weeks after transfer to clean water. A peak BCF of 600 for  $\alpha$ -endosulfan occurred in mussel tissue after exposure in saltwater to 0.14-2.05 ug/l for 50 hours (Ernst, 1977; Reisch, et al., 1979). A similar study by Roberts (1972) showed that

mussels (*Mytilus edulis*) exposed to endosulfan (isomers not specified) at 100, 500 or 1000 ug/l for 14, 42, 70 or 112 days obtained peak bioconcentration at 70 days (BCF = 22.5). Tissue concentrations fell rapidly after transfer of animals to fresh seawater (depuration half-life = 34 hours) (Ernst, 1977). Bioaccumulation of  $\alpha$ -,  $\beta$ - and endosulfan sulfate was higher in the edible and whole-body of striped mullet (max BCF = 2,755) after a 28-day exposure to endosulfan in seawater, according to Schimmel, et al. (1977). These residues decreased to undetectable levels in 48 hours after transfer of the animals to uncontaminated seawater. A similar observation was made in treated zebra fish (Toledo and Jonsson, 1992).

In fresh water studies, mosquito fish, catfish, and freshwater eels were exposed to endosulfan in static tests (USEPA, 2000b). Maximum tissue concentrations in mosquito fish (933 ug/kg:  $\alpha$ - isomer) were found in fish exposed to 16 ug/L technical endosulfan for 24 hours. The maximum tissue concentrations in fish exposed to 2 ug/L for 7 days was 143 ug  $\alpha$ - isomer/kg. Mean endosulfan residues in catfish were 61.3 ug/kg ( $\alpha$ - isomer) following 7 days exposure to 0.7 ug/L technical endosulfan. After 43 hours exposure to 1 ug/L technical endosulfan, mean residues in freshwater eels were 0.145 ug/kg for the  $\alpha$ - isomer, 0.138 ug/kg for the  $\beta$ -isomer and 0.117 ug/kg for endosulfan sulfate (Novak and Ahmad, 1989).

Endosulfan is also bioconcentrated in 2 strains of fish (*Labeo rohita* & *Channa punctata*) that were treated with  $\alpha$ - and  $\beta$ -endosulfan at 0, 0.1414 and 0.2274 ug/l for one month (Ramaneswari and Rao, 2000). Tissue analyses showed that the isomers of endosulfan persisted in the fish. Both the  $\alpha$ - and  $\beta$ -isomers were persistent in both strains of fish, with  $\alpha$ - occurring at higher concentration. In *L. rohita*, the  $\alpha$ - form was bioconcentrated 5.2 times and had a bioconcentration factor (relative uptake of endosulfan from it's medium by the organisms) of 37.5. The  $\beta$ -form was bioconcentrated 7.7, with a bioconcentration factor of 55.4. In *C. punctata*, the  $\alpha$ - form bioconcentration was 1.8 times and had a bioconcentration factor of 13.2 and the  $\beta$ -form bioconcentration was 11.8, with a bioconcentration factor of 13.4. Endosulfan sulfate was found as a metabolite in *L. rohita* only (bioconcentration = 0.54; no bioconcentration factors were reported).

Bioaccumulation is different among terrestrial and aquatic organisms for the  $\alpha$ -,  $\beta$ - and sulfate compounds (Suntio et al., 1988). The  $\alpha$ -/ $\beta$ -/sulfate BCF ratios were 999/3863/1654 (algae), 5763/39457/29430 (snail), 831/1508/763 (mosquito) and 304/388/1741 (fish - species not stated).

A review of bioaccumulative potential and toxicity of endosulfan was published in 1993 (Naqvi and Vaishnavi, 1999).

### III. TOXICOLOGY PROFILE

**Overview:** The toxicology profile is designed to provide data on the biological fate (pharmacokinetics) and general toxicity of endosulfan. The pharmacokinetics section (A.) considers absorption through oral and dermal routes, distribution, biotransformation (metabolic fate) and excretion, as well as the half-life in the body. The toxicity section (B. through I.) is comprised of acute toxicity (B.) in humans or animals (single dose, technical and formulations), subchronic toxicity (C.) in animals (repetitive doses up to 90 days with or without a recovery period) and (D.) chronic toxicity in animals (up to 2 years). Subchronic and chronic studies provide data on intermediate and long-term effects of endosulfan on hematology, clinical chemistry, macro- and micropathological effects. Genetic toxicity (E.) examines the potential for endosulfan to cause direct gene mutations or aberrations. Endosulfan effects for 2 generations in rats was an in-depth examination of reproductive toxicity (F.) and developmental toxicity (G.) examines effects of endosulfan on fetuses exposed during organogenesis. Neurotoxicity (H.) examines both the potential for endosulfan to induce nerve pathological effects, as well as the potential to induce behavioral effects. The last section (I.) consists of supplemental studies that are related to the potential toxicity of endosulfan. For regulatory purposes under SB950 it is necessary for DPR to designate which studies are acceptable according to FIFRA Guidelines. Studies that are not acceptable, but contain useful information or are studies from the open literature are considered to be supplemental and will be so designated in the toxicity section.

#### A. PHARMACOKINETICS

**Summary:** The majority of endosulfan, regardless of exposure route, is excreted rapidly in feces, with virtually no retention in tissues, despite the lipophilicity of endosulfan and its primary metabolite, endosulfan sulfate. After a single endosulfan administration in the rat by oral gavage, elimination of both  $\alpha$ - and  $\beta$ -isomers is 75% in feces and 13% in urine by 120 hours. When the bile duct was cannulated, elimination of both isomers in feces was decreased by about two-thirds at 48 hours. Elimination in urine after bile duct cannulation however remained relatively unchanged. If the enterohepatic recirculation was a major path, then elimination in the urine would have increased and feces would have remained relatively unchanged. Therefore, enterohepatic circulation, conjugation and elimination in the urine is not a major route for endosulfan metabolism. At 48 hours, oral absorption (urine + bile) was approximately 59.7% for the  $\alpha$ - isomer and 39.3% for the  $\beta$ -isomer. It is not known how much radioactivity in the feces represented metabolites. It appears that as much as 13% of the  $\alpha$ - isomer radioactivity and 10.8% of the  $\beta$ -isomers radioactivity in feces represented metabolites. At 120 hours, 88% of  $\alpha$ - [ $^{14}\text{C}$ ]-endosulfan and 87% of  $\beta$ - [ $^{14}\text{C}$ ]-endosulfan had been eliminated. The majority of the radioactivity eliminated in feces was unmetabolized endosulfan. The default policy for DPR is that if oral absorption is 80% and greater, the absorption is assumed to be 100%. After female rats were treated dermally with endosulfan, 47.3% of administered dose was absorbed; however, the process took approximately 5 days. By this time, however, 95% of the absorbed material had been eliminated. The highest tissue concentrations after gavage administration in male rats were: kidney, seminal vesicle, epididymis and liver and in females, kidney, liver and visceral fat. After dermal administration, fatty tissues had the highest concentrations. Endosulfan also was located in the brain, a lipophilic tissue; however, this was a site of low concentrations. After oral treatment in rats, endosulfan metabolites rapidly accumulated in liver and kidney, suggesting these

organs are the primary sites of biotransformation. These organs show increased weights after endosulfan treatment along with increases in xenobiotic metabolizing enzymes such as P450s and glutathione-transferases.

## 1. Absorption

### a) Oral

Specific studies to quantify the oral absorption of endosulfan in animals have been performed and there is sufficient evidence to indicate that endosulfan is absorbed through the gastrointestinal tract in both humans and animals (Nath and Dikshith, 1979; WHO, 1984; Chugh et al., 1998).  $\alpha$ - and  $\beta$ -endosulfan, as well as the sulfate metabolite, have been detected in human autopsy samples (liver and kidney) following acute ingestion in an attempted suicide (Demeter et al., 1977; Demeter and Hendrickx, 1978). The isomers were primarily found in the stomach, small intestine (due to large ingested volume), liver and kidney and appeared at a ratio similar to the formulation. Endosulfan has also been located in the visceral fat, urine, brain, respired CO<sub>2</sub> and blood stream (indicating systemic absorption has occurred; Deema et al., 1966).

Balb/c mice were fed a single oral dose of 0 to 0.30 mg of endosulfan in 300 mg of diet (Deema et al., 1966). Results showed that 65% of the [<sup>14</sup>C]-endosulfan was recovered after 24 hours and the greatest concentration of endosulfan radioactivity in males was in feces. Below feces in descending order of endosulfan radioactivity in males was visceral fat, urine, liver, small intestine, kidney, brain, respiratory CO<sub>2</sub> and blood. In females the descending order of endosulfan radioactivity concentration was feces, small intestine, urine, visceral fat, liver, kidney, respired CO<sub>2</sub> and blood. The primary metabolite recovered from both sexes was endosulfan sulfate.

Male and female rats were gavaged with a single dose of [<sup>14</sup>C]  $\alpha$ - and  $\beta$ -endosulfan at 2 mg/kg (Dorough, 1978). In addition, a group of males had the bile duct cannulated. After 48 hours, the percentage of administered dose recovered in females was 61.6% in feces and 11.1% in urine for the  $\alpha$ -isomer and 55.1% in feces and 16.0% in urine for the  $\beta$ -isomer. Percentages of administered radioactivity recovered in cannulated males were 21.9% in feces, 47.2% in bile and 12.5% in urine for the  $\alpha$ -isomer and 15.2% in feces, 28.9% in bile and 10.4% in urine for the  $\beta$ -isomer by 48 hours. At 48 hours, oral absorption (urine + bile) was approximately 59.7% for the  $\alpha$ -isomer and 39.3% for the  $\beta$ -isomer. This was the only time frame tested by bile cannulation for entrance of endosulfan metabolites into the enterohepatic cycle. The enterohepatic circulation delays the elimination of xenobiotics. This occurs via continuous recycling of a compound from the bile duct into the intestinal lumen, which is then reabsorbed from the intestine and delivered to the liver via the portal blood. Enterohepatic recirculation facilitates further metabolism and conjugation until a compound is eliminated in the urine. It is not known how much radioactivity in the feces represented metabolites. It appears that as much as 13% of the  $\alpha$ -isomer radioactivity and 10.8% of the  $\beta$ -isomer radioactivity in feces represented metabolites. These data also indicate that the biliary endosulfan metabolites do not enter the enterohepatic cycle, since urinary elimination of radioactivity was not affected by bile cannulation. Endosulfan does not form the metabolites that usually favor the enterohepatic cycle, such as glucuronide, sulfate or glutathione conjugates. With conjugated metabolites, an enterohepatic cycle can result where biliary secretion and intestinal reabsorption continue until renal excretion eliminates the compound from the body. At 120 hours, 88% of  $\alpha$ - [<sup>14</sup>C]-endosulfan had been eliminated and 87% of  $\beta$ -[<sup>14</sup>C]endosulfan had been eliminated in the urine and feces (bile). It appears, that the majority of

the radioactivity in the feces was unmetabolized endosulfan. The default policy for DPR is that if oral absorption is 80% or greater, the absorption is assumed to be 100%. Unlike most other chlorinated hydrocarbons, there were no detectable residues in fat after 7 days.

In the same study by Dorough (1978), female rats fed 0.5 mg/kg/day of  $\alpha$ - or  $\beta$ -endosulfan for 14 days showed that the kidney had the greatest radioactivity, followed by liver, visceral fat and subcutaneous fat in descending order of concentration. Recovery of radioactivity after the 14 day treatment was similar to that observed for a single dose, regardless of the isomer. After 14 days of feeding, elimination was 61-65% of the consumed radioactivity. An additional 8% was eliminated during a 14-day period following treatment. The half time for residues in tissues was 7 days after treatment was discontinued and was 3 days in liver. There was no appreciable difference in percent recovered in rats, either as a function of the isomer administered or the quantity consumed.

A later study by Chan et al., (2005) used  $^{14}\text{C}$ -Endosulfan (5 mg/kg; >95% radiopurity) in a single dose by gavage to male Sprague-Dawley rats in 3 assays: 1) Blood time course and distribution: 18 treated males were sacrificed at 30 minutes and at 1, 2, 4 and 8 hours following treatment. 2) Excretion: The urine and feces of 3 treated rats were collected in metabolism cages at 24 and 96 hours. 3) Blood time course and distribution following up to 3 doses of 5 mg/kg  $^{14}\text{C}$ -Endosulfan: 5 groups (3/group): Group I: 1 animal terminated 1 hour; Group II: 1 treatment, sacrifice at 3 hours; Group III: 2 endosulfan treatments (3 h between doses), sacrificed 2 h after last dose; Group IV: 3 treatments (3 h intervals), sacrificed 2 h after last dose; Group V: 3 treatments a 3 h intervals, sacrifice at 25 h after last dose. Necropsies were performed after assay #1 and liver, kidney, fat, GI tract, muscle, brain, heart, lung, spleen, testis and thyroid gland were examined for  $^{14}\text{C}$  activity at 1, 2, 4 and 8 hours post-dose. For the assay #3, blood was collected at 2, 3, 5, 8 and 25 hours post-dose. Results showed that the 3 rats in assay #2 had radioactivity detected in all tissues at each time point. In assay 2, 106.8% +/- 26.2% of  $^{14}\text{C}$  was collected at 96 hours, with fecal (94%) being the major route of elimination and urine (12.4%) the lesser. In assay #1 the greatest amount of  $^{14}\text{C}$  was in the GI tract (20 mg “endosulfan equivalent”/L) and the least amount was in muscle (0.18 mg/L).  $^{14}\text{C}$ -endosulfan-derived radioactivity in blood had a distribution half-life of 31 minutes and a terminal elimination half-life of 193 hours. Blood concentration reached its maximum (Cmax) of 0.36 mg/L at 2 hours after dosing. Endosulfan was rapidly absorbed from the GI tract in rats, with an absorption rate constant (ka) of 3.07/hour. The absorption of endosulfan was considered to be 100% as was observed in the study by Dorough, 1978.

#### b) Dermal

Absorption, distribution and elimination were estimated in male rats (4/dose/timepoint) treated with a single dermal dose of [ $^{14}\text{C}$ ]-endosulfan in a water vehicle at 0.10, 0.83 or 10.13 mg/kg (Craine, 1986). Animals were sacrificed at 0.5, 1, 2, 4, 10 or 24 hours. Approximately 80% at each dose was bound to the skin and the bound amount was in proportion to the amount applied. Binding to skin occurred within 0.5 hour of application and did not increase with time of exposure up to 24 hours. The remaining 20% of chemical was washed off with soapy water. At 10 hours, 72% of the applied dose was bound to the skin and not absorbed, while 8% was systemically absorbed. By 24 hours, 25% of the bound endosulfan (20% of applied dose) was absorbed. Rates of absorption at 0.5 hour were: 2.8, 21.7 and 453.9  $\mu\text{g}/\text{cm}^2$  of skin/hour at the low, mid and high dose, respectively. At 24 hours, absorption rates were 0.1, 0.7 and 6.3  $\mu\text{g}/\text{cm}^2$  of skin/hour at the low, mid and high dose, respectively. Total dermal absorption was approximately 25% of bound material or 20% of total administered dose by 24 hours and by 48 hours. Distribution to blood and tissues was observed at 0.5 hours and

increased with time of exposure and dose. At 24 hours, endosulfan levels (ng/g of tissue) in kidney were 3 times higher than the comparable levels observed in liver and fat. Blood levels were 2-3 times higher than the brain concentrations. After 10 hours, approximately 10% of material bound to skin was excreted in feces at about 3 times the rate of excretion in urine. Excretion was rapid once endosulfan was absorbed.

A similar study was performed with female CD rats (4/dose/timepoint, Craine, 1988) with dermal administration of (<sup>14</sup>C)-endosulfan 3-EC in a water vehicle at 0.1, 1.0 and 10 mg/kg, or approximately 0.037, 0.37 and 3.7 mg/cm<sup>2</sup>. The site of application was washed at 10 hours post-dosing. Animals were sacrificed at 24, 48, 72 and 168 hours post-dosing. Absorption was measured by adding radioactivity in skin (after washing), feces, urine and carcass. After exposure, approximately 80% of the dose (across all doses) remained bound to the skin. At 24 hours, 41.1% of the applied dose was still bound to the skin but decreased to 23.8 and 7.0% at 48 and 72 hours, respectively. At 24 hours, total penetration (% of dose) was 22.1, 16.1 and 3.8% at 0.1, 1.0 and 10 mg/kg, respectively and concentrations peaked at 48 hours. At 168 hours (5 days) 95% of the absorbed dose had been excreted at all doses and at 168 hours the last portion of endosulfan remaining on skin was also absorbed. Elimination was rapid, once endosulfan was absorbed. A total of 47.3% (mean of the 2 lowest doses) of the applied dose was absorbed, and absorption approached saturation at 1.0 mg/kg. The half-life at 0.1 and 1.0 mg/kg was approximately 72 hours. The Worker Health and Safety Branch used 47.3% as the dermal absorption value in their occupational exposure assessment for endosulfan (see Beauvais, 2007 for complete explanation of the rationale).

## 2. Distribution

### a) Oral

Distribution studies were performed on male rats gavaged for 15 days at 5 or 10 mg/kg/day endosulfan in an  $\alpha$ - to  $\beta$ - ratio of 2 to 1 (Gupta, 1978). At day 16, the isomers in the cerebrum, cerebellum and brain stem showed  $\alpha$ - to  $\beta$ - ratio of 63 to 1, with the  $\beta$ - isomer undetected in the cerebellum. Plasma levels had a 5 to 1 ratio for  $\alpha$ - to  $\beta$ -isomers and only the sulfate metabolite was detected in plasma. At 15 days post-treatment, plasma concentrations had declined more rapidly than brain concentrations. The pattern of distribution followed the same trend at both treatment levels.

Wistar rats, gavaged for 30 days with endosulfan at 0.75, 2.5, 5.0 mg/kg/day for males or 0.25, 0.75, 1.5 mg/kg/day for females showed endosulfan levels in fatty tissues to be twice as high as those found in the liver and the kidney (Dikshith et al., 1984). However, when ITRC strain male rats were gavaged for 30 days at the much higher dose of 11 mg/kg/day endosulfan, the kidney had the highest endosulfan concentration (Nath and Dikshith, 1979).

Endosulfan distribution in male ITRC rat reproductive tissues was examined after exposure to  $\alpha$ - and  $\beta$ - endosulfan by gavage at a ratio of 2 to 1 at 2.5 or 7.5 mg/kg/day for 60 days (Ansari et al., 1984). Distribution patterns for the  $\alpha$ -isomer were, in descending order, kidney, epididymis, ventral prostate/spleen, testes, brain, and liver. Distribution for the  $\beta$ -isomer was, in descending order, seminal vesicle, epididymis, heart, ventral prostate, spleen, and liver. For the  $\alpha$ - and  $\beta$ -isomers combined, the distribution pattern for residues, in descending order was, kidney, seminal vesicle, epididymis, and liver. Female albino rats, fed 0.25 mg/kg/day of  $\alpha$ - or  $\beta$ - isomers for 14 days, showed a residue distribution pattern of kidney, liver, visceral fat and subcutaneous fat, in descending order of

concentration (Dorough et al., 1978).

b) Dermal

Endosulfan was dermally applied daily to rats for 30 days at 18.8, 37.5, or 62.5 mg/kg/day (males) and 9.8, 19.7 or 32.0 mg/kg/day (females) (Dikshith et al., 1988). Residue analyses at 30 days showed that the greatest concentrations in males were in fatty tissue, followed by kidney, blood, liver, and brain in descending order. In females the greatest concentrations of endosulfan residues were also found in fatty tissues, followed by liver, blood, kidney and brain in descending order. Females, however, had higher concentrations of endosulfan in fatty tissue than males.

When endosulfan was applied at highly toxic doses (263 mg/kg; 24 hours) to skin of rabbits, only slight erythema was observed (Industria Prodotti Chimici, 1975).

Crossbred calves (5 total) weighing 60 to 170 kg and ranging from 4 to 11 months were exposed to a 4% endosulfan dust formulation (Thiodan 4 Dust, Rigo Company) in an attempt to remove lice (Nicholson and Cooper, 1977). The actual dose was not specified in the report. Approximately 15 hours later, 1 calf was dead and the remaining 4 showed muscle tremors, twitching of the ears, snapping of the eyelids and violent body jerks that caused the calf to fall as if shot. Frenzied activity included running backward, somersaulting and aimless jumping as if to avoid imaginary objects. The calves were covered with mud and the ground in the small pasture where they were housed was crisscrossed with deep tracks and indications of violent convulsive activity. Subsequently a second calf died and 1 was in lateral recumbency with clonic-tonic convulsions and opisthotonos. The latter calf, a 4-month old Holstein-Friesian heifer weighing 70 kg, died moments later and was necropsied. There were no gross lesions and laboratory analyses showed endosulfan concentrations of 0.73 ppm in brain, 3.78 ppm in liver, and 0.10 ppm in rumen contents. The remaining 2 calves appeared normal, however, when sprayed with a water hose, 1 calf, the oldest and largest, became hyperexcitable and developed twitching of the ears, blepharospasm, muscle tremors, violent clonic-tonic convulsions and ataxia. The signs disappeared within 10 minutes after spraying was discontinued and the calf again appeared normal. Later in the day, however, this calf died. The remaining 60 kg, 4-month old female Hereford recovered without complications.

c) Intravenous

Cats received a single i.v. injection of 3 mg/kg endosulfan through a cannula in the femoral vein and were sacrificed at 15 min., 30 min., 1, 2, 4 or 6 hours (Khanna, et al., 1979). Two patterns of endosulfan distribution in the CNS occurred, depending on the lipid concentrations of the specific area. Uptake and release peaked within 15 min. in areas with low percentage of lipid, such as cerebral cortex and cerebellum. Lower uptake and slower release occurred in areas of higher lipid percentage, such as spinal cord and brain stem. The concentration of endosulfan in plasma and liver also peaked at 15 min. Endosulfan sulfate formation reached a peak in 30 min.

### 3. Biotransformation

#### a) *In Vivo* Studies

##### i. Oral

Endosulfan is readily metabolized in rats, mice and sheep following oral exposure (Deema et al., 1966; Dorough et al., 1978; Gorbach et al., 1968). Endosulfan in its two stable isomeric forms can be biotransformed into endosulfan sulfate and endosulfan diol (WHO, 1984). These compounds can be further metabolized to endosulfan lactone, hydroxyether and ether. Figure 1 shows the pathway for the biotransformation of endosulfan in rat and sheep based on Dorough et al. (1978) and Gorbach et al. (1968). The study by Dorough et al. (1978) showed the major portion of residues in the excreta and/or tissues of rats were unidentified polar metabolites that could not be extracted from the substrate, whereas the nonpolar metabolites, including sulfate, diol,  $\alpha$ -hydroxyether, lactone and ether derivatives of endosulfan, were found in minor amounts. Metabolites in feces, including sulfates and glutathione conjugates, do not appear to enter the enterohepatic cycle and are voided in the feces (Dorough et al., 1978).

When mice were treated with a single dose of  $^{14}\text{C}$ -endosulfan, high concentrations of endosulfan sulfate were found primarily in liver, intestine and visceral fat after 24 hours (Deema et al., 1966). Five days after a single oral administration of  $^{14}\text{C}$ -endosulfan to rats, the diol, sulfate, lactone and ether metabolites were detected in feces (Dorough et al., 1978). In sheep, endosulfan sulfate was detected in feces and endosulfan alcohol and  $\alpha$ -hydroxyether were detected in urine (Gorbach et al., 1979). All studies showed a large quantity of the parent compound in the tissues and excreta. Gupta and Ehrnebo (1979) observed that half the parent endosulfan was excreted unchanged in rabbits after intravenous injection. The metabolites (sulfate, diol) were detected in tissues and excreta following longer exposures to endosulfan (Deema et al., 1966; Dorough et al., 1978). Since endosulfan sulfate appears rapidly in the liver following intravenous administration of endosulfan in cats, it may be concluded that the liver is a site of high metabolic conversion of endosulfan isomers to endosulfan sulfate (Khanna et al., 1979).

Male rats fed 34 or 68 mg/kg/day endosulfan for 30 days showed endosulfan accumulation and storage in the kidney (Hoechst, 1987). Histological examination of the kidney showed granular, yellowish pigmentation and an increase in the number and size of lysosomes in the proximal convoluted tubules that diminished during the 30-day recovery period. The lysosomal changes may have been due to intracellular storage of endosulfan. The changes decreased significantly after a subsequent 30-day recovery period, suggesting that endosulfan is metabolized in the kidneys (Hoechst, 1987).

## ii. Inhalation

Endosulfan (65%  $\alpha$ - and 30%  $\beta$ -isomers; 99.9% pure) was administered as an aerosol (whole body) to “young” (225-250 g) Sprague-Dawley rats (6 males/dose) at 0, 2, 4, 8, 16 and 24 mg/kg body weight for 3 hours per day, 5 days per week for 30 days (Bebe and Panemangalore, 2003). At the end of treatment, rats were terminated and livers, lungs and erythrocytes were assayed for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and concentrations of glutathione (GSH). Endosulfan decreased erythrocyte SOD (21% in all groups), liver SOD (12-20%), and lung SOD (21% and 51% at 16 and 24 mg/kg, respectively). Erythrocyte GPX was increased at the lower doses but was decreased at 16 and 24 mg/kg (26 and 19%, respectively). Liver GPX was increased at 24 mg/kg and lung GPX was decreased (20-38%) across all groups. GSH in liver and lung was decreased by 30% at the lower doses and by 41-70% at the high doses. Endosulfan exposure can modify antioxidant enzymes such that it contributes to oxidative stress in some tissues.

### b) *In Vitro* Studies

An *in vitro* study by Dehn et al. (2005) showed that HepG2 cells (human hepatocytes) treated with endosulfan (95% pure; both *trans*- and *cis*-isomers) at 10 mM had an increase in cytochrome P450 (CYP) 1A (n=18) and CYP2B (n=56) and a decrease in reduced glutathione (GSH; n=29) after a 24-hour exposure.

Endosulfan was used on *Saccharomyces cerevisiae* and human cell lines (HepG2 and HeLa) in order to determine the toxic mechanism (Sohn, et al., 2004). Results showed that the IC<sub>50</sub> for cytotoxicity was 49  $\mu$ M and 86  $\mu$ M for HepG2 and HeLa cells, respectively. Treatment of *S. cerevisiae* resulted in oxidative damage as shown by thiobarbituric acid-reactive substance (TBARS) production, in a dose-dependent manner and the growth inhibition was recovered by treatment with lipid-soluble antioxidants ( $\alpha$ -tocopherol;  $\beta$ -carotene) suggesting that endosulfan toxicity may be induced by reactive oxygen species (ROS) generation. Inhibition of cellular respiration by endosulfan and recovery of respiration activity by antioxidants confirmed (according to the authors) that endosulfan treatment resulted in oxidative stress and inhibits respiration via ROS generation.

Both *trans*- and *cis*-endosulfan (HPLC grade), each added to “single donor” human liver microsomes (HLM) plus an NADPH-generating system, resulted in the formation of the primary metabolite (considered to be the toxic metabolite) endosulfan sulfate (Lee et al., 2006). Production of the sulfate metabolite ( $V_{max}$  pmol/min/pmol P450) for the *trans*-isomer was 1.48 versus 4.40 for the *cis*-isomer. This resulted in an intrinsic clearance ( $Cl_{int}$  ul/min/pmol P450) of 3.5 times higher for the *cis*-isomer (to sulfate metabolite) than for the *trans*-isomer, indicating that the *cis*-isomer is cleared more rapidly than the *trans*-isomer. The correlation between P450s involved with endosulfan metabolism and formation of endosulfan sulfate showed that the *trans*-metabolism was correlated with CYP2B6 and CYP3A and *cis*-endosulfan metabolism was correlated with CYP3A activity. Stereoselective endosulfan sulfate formation from human recombinant P450s (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5) showed that *trans*-endosulfan is mediated by CYP2B6, CYP3A4 and CYP3A5 and *cis*-isomer was mediated by CYP3A4 and CYP3A5.

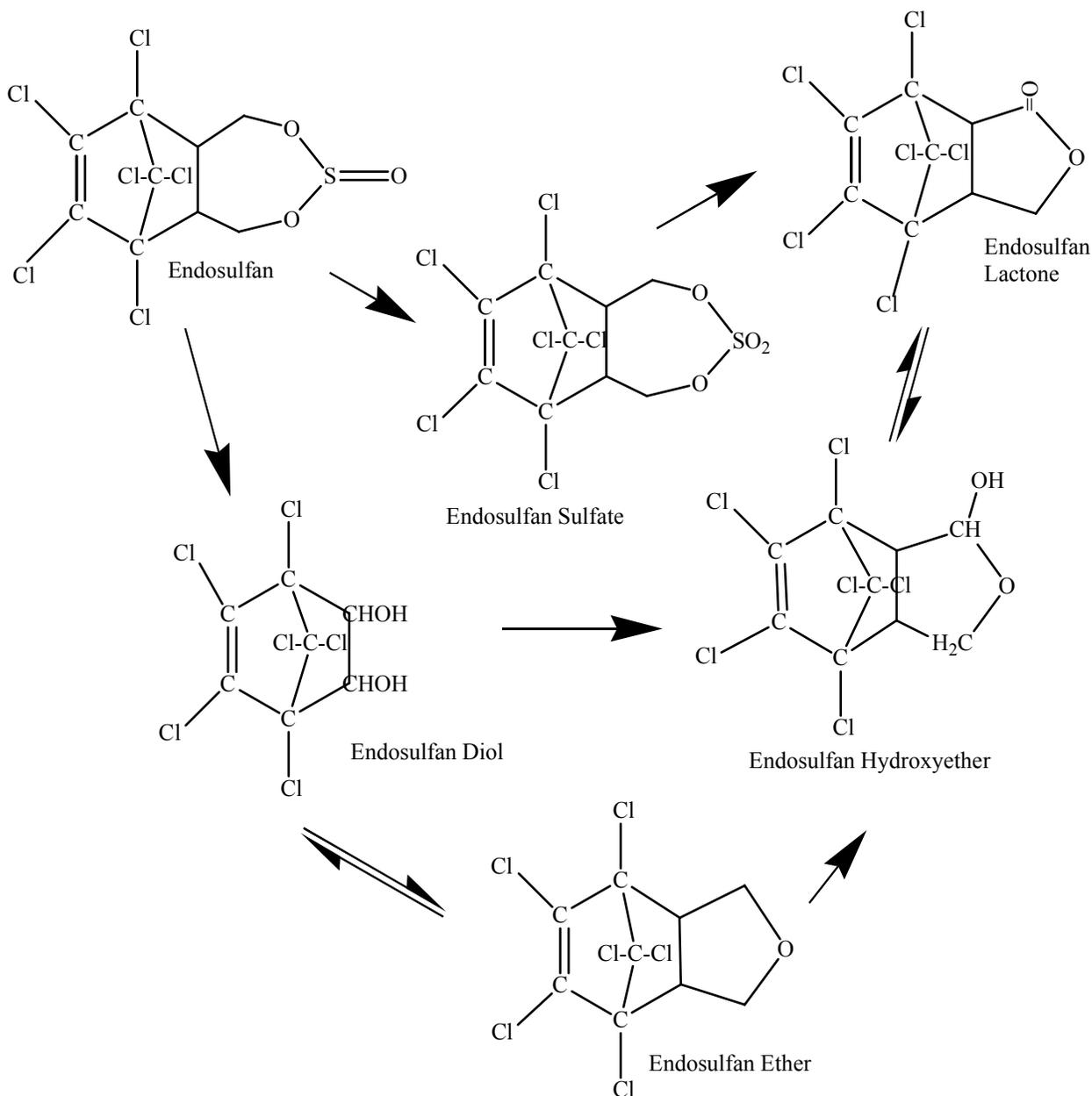
Technical endosulfan (*trans*- and *cis*-isomers) was used on Chinese Hamster Ovary (CHO-K1) cells at 0 (DMSO), 6.25, 12.5 and 25  $\mu$ g/ml (50  $\mu$ g/ml used for cytotoxicity assay only) to assay for glutathione (GSSG), glutathione peroxidase (GPX), reductase (GTR) and S-transferase (GST) activities (Bayoumi et al., 2001). GSSG and GPX were statistically significantly increased at all doses, GTR was decreased at 25  $\mu$ g/ml and GST was decreased at all doses. Lactate dehydrogenase (LDH) was increased by endosulfan (indicating increased membrane permeability), but only at very high doses (100  $\mu$ g/ml and greater).

### 4. Excretion

Endosulfan excretion in human urine was assessed after an agriculture worker was exposed

under greenhouse conditions (Arrebola, et al., 1999). In Almeria, Spain, a 34-year old man, wearing a Tivek Pro-Tech protective overall, gloves and a breathing mask sprayed 300 liters of endosulfan (0.7 g/l) on cucumbers and peppers within a 1000-m<sup>2</sup> flat roof experimental greenhouse of polyethylene. A semi-stationary high volume two-stroke sprayer (flow rate = 3.7 l/min) was used for spraying from ground level upwards to a height of 2 m for 25 min. Ten urine samples were taken over 3 days post-exposure (control = urine from a non-occupationally exposed man). Urine samples were extracted to obtain  $\alpha$ - and  $\beta$ - endosulfan and metabolites, which were then analyzed by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS). The highest concentration of endosulfan in urine (5368 pg/ml) was reached at approximately 0.2 days after application. A decline in  $\alpha$ - and  $\beta$ - endosulfan concentration occurred for 1.5 days until a constant value (2239-2535 pg/ml), similar to the non-occupationally exposed man (2416 pg/ml), was reached. Metabolites were not found above detection limits. The  $\alpha$ -isomer was excreted faster than the  $\beta$ -isomer by first order kinetics, with half-lives of 0.738 d<sup>-1</sup> ( $\alpha$ -) and 0.60 d<sup>-1</sup> ( $\beta$ -), or approximately 1.35 and 1.67 days, respectively.

**Figure 1. Proposed Metabolic Pathway in Rat and Sheep for Endosulfan (Dorough, et al., 1978; Gorbach et al., 1968; Bebe and Panemangatore, 2003; Lee et al., 2006)** Phase I reactions on endosulfan are performed with P450s: CYP2B6, CYP3A4 & CYP3A5; Phase II reaction is with GST; Other enzymes involved with endosulfan metabolism are antioxidants: SOD, GPX and CAT



Limited information on excretion of endosulfan and metabolites by exposed workers was obtained from urinary samples analyzed by gas chromatography-tandem mass spectrometry (Vidal *et al.*, 1998). To validate the analytical method, urine samples were collected from nine pest control operators (PCOs) in Spain. Four of the PCOs had applied pesticides the previous day, and 5 the previous week, with all applications lasting 2-5 hours. Self-reported working conditions indicated lack of protective overalls, breathing masks, or gloves. Endosulfan and metabolites (endosulfan ether and endosulfan lactone) were detected in urine from all four PCOs who applied pesticides the previous day. In these 4 samples,  $\alpha$ -endosulfan concentrations ranged 787-894 pg/ml, and  $\beta$ -endosulfan concentrations ranged 801-896 pg/ml. Endosulfan and metabolites (endosulfan lactone and endosulfan sulfate) were detected in urine from 4 of the 5 PCOs who applied pesticides the previous week. Concentrations were lower than in workers applying pesticides the previous day;  $\alpha$ -endosulfan

concentrations ranged 84 -123 pg/ml, and  $\beta$ -endosulfan concentrations ranged from below the detection limit of 18 pg/ml -169 pg/ml. No information was provided about endosulfan formulations or amounts applied, thus, no relationships can be determined between these results and exposures. Additionally, the intermediate metabolic products, endosulfan diol and  $\alpha$ -hydroxy ether, were not included in the assay (From Beauvais, 2001).

Lactating sheep were administered a single oral dose of [ $^{14}$ C]-endosulfan (65%  $\alpha$ - and 35%  $\beta$ -endosulfan) at 0, 0.30 or 0.26 mg/kg (Gorbach et al., 1968). Twenty-two days after dosing, elimination was 50% in feces and 40% in urine. Peak levels of elimination were reached at 2-3 days and comprised 73-74% of the total dose administered. This is similar to the elimination levels achieved in the rat after 14 days of treatment in diet. A total of 0.9-1.8% of radioactivity was eliminated in the milk after 17 days, with peak elimination at 1-2 days. The diol and  $\alpha$ -hydroxyether metabolites were detected in urine, and endosulfan sulfate was detected in milk. No major metabolites were retained in the fat or in the organs at 40 days.

Goats fed endosulfan for 28 days showed rapid elimination (Indraningsih et al., 1993). Elimination half-lives were between 1.1 and 3.1 days for endosulfan residues in organs and tissues.

Endosulfan was administered in a single i.v. dose to rabbits at 2 mg/kg in a 7 to 3 ratio of  $\alpha$ - to  $\beta$ -, showing lower plasma clearance for the  $\alpha$ - isomer (Gupta and Ehrnebe, 1979). At 5 days post-dosing, 37% and 11% appeared in urine and 2.7% and 0.4% appeared in feces as the unmetabolized  $\alpha$ - and  $\beta$ -isomers, respectively.

## 5. Other Studies

### a) Protein Intake and Endosulfan Distribution

Female Wistar rats were fed endosulfan in low (5%) or high (24%) protein diets at 0.05 or 10 mg/kg/day (Das and Garg, 1981). Rats were treated for 9 and 18 weeks on the 5% diet, and for 18 weeks on the 24% diet. Results showed a dose-related increase in  $\alpha$ -,  $\beta$ -, and sulfate residues in perirenal adipose from the 5% protein diet after 9 and 18 weeks.  $\alpha$ -,  $\beta$ -, and sulfate residues were lower in perirenal fat of rats on the 24% protein diet compared to the 5% protein diet.

### b) Enzyme Induction

Endosulfan was administered by gavage to male Wistar rats at 7.5 or 10 mg/kg/day for 15 or 30 days to assess induction of kidney enzymes (Singh and Pandey, 1989a). Microsomal and cytosolic proteins in kidney were increased after 30 days and induction of alcohol dehydrogenase and cytosolic GST occurred. P450 was inhibited and non-enzymatic lipid peroxidation occurred resulting in microsomal membrane damage. Kidney microsomal and cytosolic proteins were increased by 36% and 20%, respectively, after 30 days. There was a decrease in reduced GST and an increase in NAD/NADH, potentially leading to overall renal toxicity. Using the same protocol, Singh and Pandey (1989c) assessed the effects of endosulfan on selected liver enzymes. P450, alcohol dehydrogenase, glutathione S-transferase, NADPH cytochrome c-reductase, and NADH oxidase activities were significantly increased while cytochrome b5 and NADH cytochrome b5 reductase were decreased. At 30 days, testosterone, androstenedione, aromatase and 3 $\beta$ - and 17 $\beta$ -hydroxysteroid dehydrogenase were significantly decreased at 30 days, while dihydrotestosterone and androstanediol were increased.

Both Wistar Utrecht and SPF Wistar male rats showed significant induction of liver microsomal enzymes (24% above control), aniline hydroxylase (23% above control) and aminopyrine demethylase (91% above control) at 20 mg/kg/day for 2 weeks (Den Tonkelaar and Van Esch, 1974). There were no effects at 5 mg/kg/day.

Liver microsomal P-450 was induced in both sexes of rat after single and multiple administrations of endosulfan (Fabacher, et al., 1980; Siddiqui et al., 1987; Tyagi et al., 1984). Aminopyrine-N-demethylase, aniline hydroxylase and glutathione S-transferase activities were induced in hepatic and extrahepatic tissues in the rat (Agarwal et al., 1978; Narayan, et al., 1984, 1990a and 1990b; Den Tonkelaar, et al., 1974; Robacker, et al., 1981; Singh and Pandey, 1989c).

### c) Tumor Promotion

The  $\alpha$ - and  $\beta$ -endosulfan isomers were each tested for their ability to promote tumors in the two-stage altered hepatic foci bioassay in male rats (Fransson-Steen et al., 1992). The stronger was  $\alpha$ -endosulfan. Since the phenobarbital and methylcholanthrene-inducible forms of hepatic microsomal P-450s were only marginally induced by technical,  $\alpha$ - and  $\beta$ -endosulfan, it was concluded that the induction of microsomal P-450 as the agent of tumor promotion was unlikely. Instead, the authors suggested that endosulfan functions by inducing clonal expansion of initiated cells.

Fransson-Steen and Warngard (1992) showed that one possible mechanism of tumor promotion by endosulfan is the inhibition of gap junctional intercellular communication. In primary rat hepatocytes,  $\beta$ -endosulfan is a more potent inhibitor of intercellular communication than  $\alpha$ -endosulfan. However, the two isomers had similar inhibitory potency in WB-Fischer 344 rat liver epithelial cells. Although the mechanism of inhibition of intercellular communication by endosulfan is not known, results from studies in IAR 20 rat liver epithelial cells have suggested an effect on connexin 43, the main gap junction protein in this cell line (Kenne et al., 1994). Phosphorylation of connexins is one post-translational alteration involved in regulation of gap junctional communication (Musil et al., 1990). In the IAR20 cell line, endosulfan was found to increase phosphorylation of connexin 43 initially. Longer exposures, however, led to hypophosphorylation. The biological significance of gap junctional intercellular communication in tumor promotion in *in vivo* studies is not known.

Flodstrom, et al. (1988) used endosulfan and the metabolites 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*, but *in vitro* tests indicated endosulfan might be a promotor.

### d) Effects *In Vitro* on Cellular Respiration

Human T-cell leukemic line (Jurkat cells) was used *in vitro* to assess the cytotoxic and apoptogenic potential of endosulfan and to provide evidence of the involvement of mitochondria in endosulfan-mediated apoptosis (Kannan, et al., 2000). Endosulfan technical (70:30  $\alpha$ - and  $\beta$ - isomers) at 10 - 200  $\mu$ M was used on Jurkat cells for 0 - 48 hours that were subsequently analyzed for biochemical and molecular characteristics of T-cell apoptosis. Endosulfan decreased cell viability and inhibited cell growth in a concentration and time-dependent manner. Apoptosis was statistically significantly increased at 10-200  $\mu$ M (90% at 48 hr, 50 mM). Mitochondrial transmembrane potential

in treated cells was significantly decreased in a concentration and time-dependent manner (within 30 min) and was correlated with other parameters of apoptosis. The mechanism for T-cell apoptosis was proposed to be: uncoupling of oxidative phosphorylation, leading to excess R-OH, leading to GSH depletion, leading to oxidative stress, the disruption of mitochondrial trans-membrane potential, then release of cytochrome C and other apoptosis-related proteins to cytosol and finally to apoptosis. This study shows endosulfan may contribute to T-cell and thymocyte loss and have a possible role of mitochondrial dysfunction and oxidative stress in endosulfan toxicity.

Increased oxygen consumption, induced by endosulfan, was demonstrated *in vitro* in T- and B-lymphocytes from male albino rats (Kannan, et al., 1982; abstract). Thymocytes, splenocytes and bone marrow cells treated at  $10^{-4}$  to  $10^{-7}$ M endosulfan showed several-fold-stimulation of cellular respiration, which was dose-dependent. Bone marrow cells were not affected by endosulfan treatment.

Rat liver mitochondria were treated with endosulfan at 5, 10, 25, 33, 50 & 100 ug/ml, with endosulfan sulfate at 10, 25 and 50 ug/ml, with endosulfan diol at 10, 25 and 50 ug/ml and with endosulfan lactone at 10, 25, 50 and 100 ug/ml. This *in vitro* study was performed to test the effects on respiration and mitochondrial enzyme activities (Dubey, et al., 1984). Results showed that endosulfan stimulated state-4 respiration ( $\beta$ -hydroxybutyrate + ADP) at lower concentrations (25 & 33 ug/ml) but inhibited it at higher ones. The sulfate and diol metabolites had similar effects but at 50 ug/ml. State-3 respiration (no ADP added) was inhibited at all the concentrations of endosulfan and metabolites used (5-100 ug/ml). The lactone metabolite showed no effects. Endosulfan activated  $Mg^{+2}$ -ATPase 25 fold at 33 ug/ml, while the sulfate and diol activations were not quite doubled at each dose. The lactone metabolite had no effect. Activities of the respiratory chain-linked enzymes (succinate dehydrogenase, succinate-cytochrome c-reductase, NADH-dehydrogenase, cytochrome c-oxidases,  $Mg^{+2}$ -ATPase, monoamine oxidase) were inhibited in a dose-related manner by endosulfan (but not metabolites). Both the respiratory control ratio and the ADP:oxygen ratio decreased sharply at endosulfan concentrations above 10 ug/ml. This study indicates that endosulfan and metabolites both uncouple oxidative phosphorylation and inhibit the electron transport chain in mitochondria. Thus, the *in vivo* cytotoxic/insecticidal properties attributed to endosulfan and metabolites might be due to impairment of mitochondrial bioenergetics.

#### e) *In Vitro* Cellular Communication (liver)

Hepatocyte gap junctional intercellular communication was inhibited by endosulfan, as well as by the sulfate, lactone and ether metabolites (Ruch et al., 1990). Gap junctional intercellular communication was also inhibited by both  $\alpha$ - and  $\beta$  - isomers in primary Sprague-Dawley rat hepatocytes, as well as WB-F344 rat liver cell lines (Fransson-Steen & Warngard, 1992).

## B. ACUTE TOXICITY

### 1. Summary:

#### a) Technical Material

There were no acceptable oral LD<sub>50</sub> studies for endosulfan; however, the lowest LD<sub>50</sub> was 7.38 mg/kg in the male mouse (Gupta et al., 1981) and 9.58 in the female rat (Scholz and Weigand, 1971b), with clinical signs of neurotoxicity. The dermal LD<sub>50</sub> ranged from 74 mg/kg in female rats to 1000

mg/kg in guinea pigs. An  $LC_{50}$  was obtained from an acceptable acute inhalation study in the rat. The  $LC_{50}$  for females was 0.0126 mg/L and for males was 0.0345 mg/L and there were clinical signs of irregular respiration, passivity, disequilibrium, trembling, tremors, tono-clonic convulsions and reduced excitability. A NOEL for this study was not achieved. Ocular irritation in rabbits was slight and dermal irritation tests showed mild erythema in rabbits after a single 24-hour exposure. Endosulfan technical was not a dermal irritant in the guinea pig.

## b) Endosulfan Formulations

Values for each test type ranged broadly depending upon the formulation, species and gender; however, all rat oral  $LD_{50}$  values were higher than those observed for technical material. The clinical signs, however, were similar. The largest contrast between genders was 194.4 versus 18.8 mg/kg for males and females, respectively, with Thiodan 2 CO/EC. On the other hand, Thionex  $LD_{50}$  was 125 mg/kg for males and females. The dermal  $LD_{50}$  in the rat with Thionex 35EC was 3.5 times higher than that for the technical material and in rabbit, the values were 1.4 to 14 times higher. Rabbit dermal  $LD_{50}$  was 2000 mg/kg with 50% WP and 1076 and 930 mg/kg for males and females, respectively for Thiodan 2 CO/EC. For the rat the dermal  $LD_{50}$  was 256 mg/kg for both genders for Thionex 35 EC. Although there was great variation among formulations and technical  $LC_{50}$ 's, they were all within the same range except Thiodan 2 CO/EC (4 times greater). Ocular and dermal irritation values were similar between technical and formula, and Thiodan 2 CO/EC was not sensitizing. All studies with formulated products were performed according to FIFRA Guidelines.

## 2. Studies Performed with Technical Material

### a) Rat

#### i. Gavage

Endosulfan was administered by gavage in a single dose to Sherman rats (5/sex/dose) at 0 (ground-nut oil), 31.6, 46.4, 68.1, 100, 147 and 215 mg/kg (males) and at 0, 6.3, 8.0, 10 and 12.5 mg/kg in females (Scholz and Weigand, 1971a & b). Beginning at 1 hour post-dosing, males showed depression, lacrimation, exophthalmia, labored respiration and ataxia. Several animals (number not specified) also showed salivation, tremors, clonic- and tonic convulsions and depressed righting, placement and pain reflexes. Males at 147 and 215 mg/kg showed salivation, rapid and labored respiration and depressed or absent righting, placement and pain reflexes. Death was preceded by bloody discharge from the eyes, gasping, tonic and clonic convulsions and coma. Surviving animals at 100 mg/kg and below showed ataxia and diarrhea, rapid and/or labored respiration and depression for 24 hours post dosing. They were essentially normal by 48 hours. Males that died showed hyperemic or hemorrhagic lungs, irritation of the pyloric portion of the stomach and the small intestine and congested kidneys and adrenals. Males that survived to autopsy showed congested areas of consolidation in the lungs and congestion in the adrenals. The  $LD_{50}$  for males was 48 mg/kg bodyweight. The results of the study performed in females were presented only as a summary (Scholz and Weigand, 1971a, b). Females dying within 2 to 24 hours showed disequilibrium, muscular tremors and extension spasms. Macroscopic post-mortem examinations showed reddening of shorter portions of the small intestine. The  $LD_{50}$  for females was 10 mg/kg. **Supplemental.**

Male albino Wistar rats were gavaged with endosulfan at 30 mg/kg (single dose, 4 rats) or at 0

(corn oil), 10 and 15 mg/kg/day (5 days, 6 rats per dose) in order to examine oxidant stress-inducing effects in liver and cerebral tissues (Hincal, et al., 1995). Glutathione redox ratios ( $[\text{GSSG}/(\text{GSH} + \text{GSSG})] \times 100$ ), a measure of lipid peroxidation, were increased in a dose-related manner in brain and liver after both single and multiple-dose treatments. Selenium, as a measure of selenium-containing glutathione peroxidase (repairs oxidative damage), was decreased by 11% in liver and brain tissue at 15 mg/kg (only dose tested). Oxidant stress was more pronounced in cerebral tissue, where endosulfan serves as a GABA-antagonist. **Supplemental.**

Endosulfan technical was administered by gavage to male albino rats (5/dose) at 10, 21.5, 46.4, 100, 215, and 464 mg/kg (Elsea, 1957). The acute  $\text{LD}_{50}$  was 110 mg/kg, with confidence limits from 55 to 220 mg/kg. At 10 and 21.5 mg/kg, animals showed signs of depression, while at 46.4 mg/kg and greater, there was increased preening, salivation, excessive masticatory movements, lacrimation, exophthalmia, and rapid, labored respiration. Prior to death, bloody nasal discharge, ataxia, sprawling of the limbs, tremors, depressed or absent righting, placement and pain reflexes and Straub-like tails were noted. The severe clinical signs of phonation, tonic and clonic convulsions, gasping and coma, immediately preceded death. The surviving animals at 10 and 21.5 mg/kg appeared normal within 24 hours. Survivors at 46.4 and 100 mg/kg showed slight depression, nasal discharge and labored respiration through the 48-hour period following dosing. Subsequently, these animals exhibited normal behavior. The 1 survivor at 215 mg/kg was depressed and showed bloody nasal discharge and rapid and labored respiration through 48 hours following dosing and nasal discharge during the remainder of the observation period. Gross autopsies on animals that died showed hyperemic or hemorrhagic lungs, irritation of the small intestine and congested kidneys and adrenals. Other surviving animals showed no gross pathological findings. There were no data presented, beyond the number of deaths. **Supplemental.**

#### ii. Dietary

Boyd and Dobos (1969) tested the effects of a protein-deficient diet on endosulfan tolerance in albino rats. Results would have application to the choice of endosulfan as a pesticide in countries where the diet is low in protein. Weanling albino rats were fed for 28 days with diets of laboratory chow, 3.5% casein (low protein) or 26% casein (normal protein prepared diet). After 4 weeks on these diets, rats were treated with endosulfan at 0, 50, 75, 100, 125, 140, 150, 160, 175, 200 and 225 mg/kg/day (laboratory chow); 0, 10, 20, 25, 30, 50 and 60 mg/kg (low protein) or 0, 50, 75, 90, 110, 120, 125, 140 and 160 mg/kg (normal protein). There were 15-16 rats per dose per group. Rats fed a protein-deficient diet were more susceptible to the acute toxicity of endosulfan. The  $\text{LD}_{50}$  for laboratory chow was 121 +/- 16 mg/kg, normal protein diet was 102 +/- 16 mg/kg and low protein diet showed 24 +/- 10 mg/kg. At the  $\text{LD}_{50}$  for all diet groups, rats showed initial stimulation, then CNS depression, epistaxis, soft stools, oligodipsia, decreased body weight, hypothermia, diuresis, glycosuria and local GI inflammation. Effects were observed at all doses within each group; however, they disappeared by 2 - 4 weeks in surviving rats. Other studies were performed with the same results (Boyd, 1972; Boyd et al., 1970). **Supplemental.**

#### iii. Inhalation

An acute, nose-only, inhalation study was performed with endosulfan (97.2% pure) in Wistar rats (5/sex/dose) at 0 (polyethylene glycol + ethanol), 0.0036, 0.0123, 0.0288, 0.0401 and 0.0658 mg/L (approximately equivalent to 0.567, 1.197, 4.61, 6.42 and 7.57 mg/kg) in aerosol form for 4 hours

(Hollander and Weigand, 1983). Observation time periods were at 11, 149, 179, 240, 314 and 434 minutes and 1, 7 and 14 days post-dosing. The LC<sub>50</sub> values were calculated by a method described in the report. The LC<sub>50</sub> for males was 0.0345mg/L air (5.52 mg/kg) and for females was 0.0126 mg/L air (2.02 mg/kg). Clinical observations were made in all dose groups; however, they increased in incidence and severity in a dose-related and time (post dosing) related manner. At 0.0036 ug/L, there was dyspnea, trembling, passivity and disturbed equilibrium (discontinued after 449 minutes), with all animals surviving, and no pathological effects. At 0.0123, 0.0288 and 0.0401 ug/L, there were tremors, tonic-clonic convulsions, decreased corneal reflex, decreased pupillary light reflex, decreased righting reflex, decreased startle reflex, decreased paw reflex and decreased cutaneous reflex. These effects increased with time and dose. At 0.0123 ug/L there was no pathological effects and no deaths. At 0.0288 ug/L, 4/5 animals died on study, with 2/4 showing little dark-red spots in the lung. At 0.0401 ug/L, all animals died, with 2/3 showing pathological findings (little dark-red spots in the lung). At the high dose of 0.0658 mg/L animals showed dyspnea, disturbed equilibrium, trembling, tremors, tonic-clonic and convulsions, before all animals died (Table 3). There was a concentration-related reduction of bodyweight gains observed in males until 3 days after the exposures and in females until 14 days after the exposure. A NOEL was not achieved (LOEL = 0.567 mg/kg). **This study was, however, acceptable.**

**Table 3. Clinical Signs of Neurotoxicity Observed in a Rat LC<sub>50</sub> Study (Hollander & Weigand, 1983)**

Effects Observed	# Affected at Each Dose of Endosulfan—mg/kg (Time of onset)				
	0.567	1.197	4.61	6.42	7.57
<b>Males (5/dose treated)</b>					
# Died (over time span)	--	0	0	5 (101-243")	--
Dyspnea	--	5 (28")	5 (11")	5 (6")	--
Passivity	--	5 (240")	0	0	--
Disturbed equilibrium	--	5 (240")	5 (179")	5 (41")	--
Trembling	--	0	5 (149")	5 (61")	--
Tremors	--	0	0	5 (81")	--
Tonic/clonic convulsions	--	0	5 (179")	5 (91")	--

Corneal reflex decreased	--	0	5 (240'')	2/2 (240'')	--
Pupillary light reflex decreased	--	0	5 (240'')	2/2 (240'')	--
Righting reflex decreased	--	0	5 (240'')	2/2 (240'')	--
Startle reflex decreased	--	0	5 (240'')	2/2 (240'')	--
Paw reflex decreased	--	0	5 (240'')	2/2 (240'')	--
Cutaneous reflex decreased	--	0	5 (240'')	2/2 (240'')	--
<b>Females (5/dose treated)</b>					
# Died (over time span)	0	3 (218-323'')	4 (224-314'')	5 (89-161'')	5 (64-120'')
Dyspnea	5 (34'')	5 (28'')	5 (11'')	5 (6'')	5 (5'')
Trembling	5 (164'')	5 (67'')	5 (59'')	5 (36'')	5 (35'')
Disturbed equilibrium	5 (219'')	5 (123'')	5 (130'')	5 (31'')	5 (20'')
Tremors	0	5 (123'')	0	5 (41'')	5 (40'')
Tonic/clonic convulsions	0	4 (240'')	5 (130'')	5 (51'')	5 (50'')
Corneal reflex decreased	0	4 (240'')	4 (224'')	0	0
Pupillary light reflex decreased	0	4 (240'')	4 (224'')	0	0
Righting reflex decreased	0	4 (240'')	4 (224'')	0	0
Startle reflex decreased	0	4 (240'')	4 (224'')	0	0
Paw reflex decreased	0	4 (240'')	4 (224'')	0	0
Cutaneous reflex decreased	0	4 (240'')	4 (224'')	0	0

-- = Males or females not dosed at these concentrations.

“ = minutes postdosing for the effect to occur (14 day post-dosing observation period).

#### iv. Dermal

A single endosulfan treatment was administered dermally, without occlusion, to Sherman rats (60 males and 70 females) at unspecified dose levels (Gaines, 1969). Results provided LD<sub>50</sub> values of 130 mg/kg for males and 74 mg/kg for females. Dermal effects were erythema and slight desquamation. The study lacked body weights, actual doses tested and clinical effects. **Supplemental.**

#### b) Dog - Oral

A single oral dose of endosulfan in a gelatin capsule was administered at 0, 34, 39.5, 50, 65, 84.5, 109.8, 142.7 or 185.5 mg/kg to 5 mixed breed dogs per sex per dose (Nogami, 1970). Dogs that died had clonic and tonic convulsions, followed by respiratory paralysis. Necropsy of these dogs showed congestion of the blood in lungs and livers as well as congested blood in the stomach and small intestines. The LD<sub>50</sub> was 76.7 mg/kg. There were no deaths at 34 and 39.5 mg/kg; however, clinical observations were not described in the study unless death occurred. It is not known if the surviving animals had signs of toxicity at 34 or 39.5 mg/kg. There was no differentiation between effects in males versus females. **This was only a summary and is supplemental.**

#### c) Rabbit, Guinea Pig - Dermal Irritation

In a summary of a study by Industria Prodotti Chimici (1975), results showed slight erythema after a single 24-hour exposure of the skin of rabbits to 263 mg/kg endosulfan technical. A study performed in guinea pigs showed no skin irritation at 48 hours after a 6-hour exposure to 40% endosulfan technical in polyethylene glycol (Jung and Weigand, 1983). The study did not specify the actual amount of endosulfan administered. **This study was supplemental.**

d) Rabbit

i. Dermal

Endosulfan technical, dissolved in chloroform, was painted on the skin of female albino rabbits (4/dose) at 125 to 225 mg/kg (Gupta and Chandra, 1975). The use of an occlusive bandage was not indicated. Animals were observed for 7 days and subsequent post-mortem and histopathological exams were performed. Only data for animals at 225 mg/kg were included in the report. The onset of effects appeared at 0-3 hours, with 3/4 showing hyperresponsiveness to sudden sound and tactile stimuli and 1/4 showing moderate tremors. In addition, unspecified numbers of animals at 225 mg/kg were stated to have exhibited hyperexcitability, dyspnea, decreased respiration, and discharge from eyes. At 225 mg/kg, these effects progressed to tonic and clonic convulsions and subsequent death in all animals by 24 hours. Liver, kidney and adrenal toxicity was observed at all doses, however the extent and incidence at each dose was not stated. Liver changes were degeneration of hepatocytes with foamy cytoplasm and bile duct proliferation. Proximal convoluted tubules in kidneys were necrotic and desquamated and adrenal cortex showed swollen foamy cytoplasm, with eccentric nuclei.

**Supplemental.**

Endosulfan was administered dermally to albino rabbits (4/dose, unspecified gender) at 0 (cotton seed oil), 46.4, 100, 215, 464 and 1000 mg/kg (Elsea, 1957). The treated area of closely clipped skin was occluded under a rubber dental dam and wrapped with gauze for the entire 24-hour exposure period. Gross necropsies were performed on all animals and daily observations were performed for dermal irritation and systemic toxicity for 7 days. Clinical signs were not described. A slight erythema, observed in all treated animals, disappeared within 1 to 4 days. It was stated that the treated skin of surviving animals at the higher doses (doses not specified) showed slight atonia and/or slight desquamation during the final 3 to 4 days of observation. Gross necropsies performed on animals that died on study showed congested lungs containing hemorrhagic areas, granular-appearing livers, irritation of the large intestine and congested kidneys. Surviving animals necropsied at termination appeared normal. There were only data for time of death provided in this study. The LD<sub>50</sub> for dermal exposure was 359 mg/kg. **Supplemental.**

ii. Ocular Irritation

In a study by Gupta and Chandra (1975), endosulfan technical was administered to Industrial Toxicology Research Centre bred female albino rabbits (6/dose). Aqueous suspensions were 5, 10 and 20% with an unspecified amount of endosulfan per concentration. At the highest dose, there was no irritation or congestion. Authors speculated that this lack of effect is possibly due to rapid removal of instilled suspension by lacrimal fluid. **Supplemental.**

Albino rabbits (3 total) were treated with a single application of 3.0 mg in the conjunctival sac of the left eye of each rabbit (Elsea, 1957). The treated eye was held closed for 30 seconds, after which an immediate reading was made. Observations for gross signs of eye irritation were continued at 1, 4 and 24 hours post dosing and daily thereafter for 6 days. Immediately following application, the eyes showed a very mild irritation, characterized by a very slight erythema and vascularization of the sclera and nictitating membrane (accompanied by lacrimation). Subsequently, the signs of eye irritation gradually subsided and at 24 hours post dosing and daily thereafter; the eyes of each rabbit appeared grossly normal. Throughout the observation period of 7 days, each animal exhibited normal

appearance and behavior, including a normal increase in body weight. This report was an abstract only. No data were included. Supplemental.

e) Mice -- Oral

Endosulfan was administered by gavage to mice at 13 mg/kg/day for 5 days to investigate the effect on kidney pathology and on biochemistry (G6PD, CAT, SOD, GSH and MDA) in the supernatant of kidney homogenate (Caglar, et al., 2003). Mitochondrial degeneration in the cytoplasm, lipofuscin granules and membranous structures were observed in proximal convoluted tubule cells. In some glomeruli, there were ultrastructural changes such as fusion in the pedicels (foot-like process) and focal thickening at the glomerular basal membrane. Cytoplasmic bulges were also observed in some of the distal convoluted tubule cells. All enzymes were affected in kidney tissue supernatant. The kidney degeneration, observed mainly in the proximal convoluted tubule cells, was hypothesized to be responsible for membrane alterations leading to kidney pathology. Supplemental.

### 3. Studies Performed With Endosulfan Formulations

a) Rat

i. Gavage

Studies with formulations in rats showed that LD<sub>50</sub> for endosulfan 50 WP (50% endosulfan) was 82 mg/kg for males and 30 mg/kg for females. For Thionex 35 EC (32.42% endosulfan), the LD<sub>50</sub> was 25 mg/kg for both genders and for Thiodan 2 CO/EC (23.1% endosulfan) the LD<sub>50</sub> was 194.4 mg/kg for males and 18.8 mg/kg for females (Intox Labs, 1985a; Freeman, 1989a; Lightowler, 1978). Clinical signs were generally similar among formulations: convulsions, tremors, decreased motor activity, piloerection and hunching. Deaths occurred generally between 30 minutes and 6 hours of dosing. The shortest interval (30 min.) was for Thiodex 35 EC, where the intervals for the other formulations were similar. Necropsy revealed that endosulfan 50 WP induced meningeal hemorrhage, reddening of the small intestines and brain and raised white nodules in the lung with hepatic and pulmonary congestion. Thionex 35 EC treatment was associated with meningeal hemorrhage, hepatic and pulmonary congestion and clear oral discharge. There were no lesions observed at necropsy with Thiodan 2 CO/EC. Acceptable study.

Endosulfan 33.7% EC was administered by oral gavage to Wistar rats (5/sex/dose) at 0, 35, 53 and 80 mg/kg (Senshaiah, 1997a). Mortality: 35 (M:0/5, F: 3/5), 53 (M:2/5, F:5/5), 80 (M:3/5, F:5/5). Clinical Signs (lethargy, tremors, tonic clonic convulsions, exophthalmos, and piloerection) were observed. Necropsy revealed that the animals dying on study had congestion of the lungs, mild pallor of the kidney (one animal) and focal hemorrhage of the thymus (one animal). The survivors showed no treatment-related lesions at termination. The LD<sub>50</sub> for both sexes was 44.6 mg/kg (Toxicity Category I). This study was acceptable.

ii. Dermal

Thionex 35 EC was applied dermally to Charles River CD rats at 0, 0.457, 0.64, 0.897, 1.254 or 1.754 ml/kg for 24 hours with occlusion (Lightowler and Gardner, 1978). Clinical observations showed vocalization, decreased motor activity, snout pigmentation, skin exfoliation and tremors at 0.9

ml/kg. Death occurred at all dosages within 28 hours of testing. Necropsies revealed pulmonary congestion with clear or bloody areas. The LD<sub>50</sub> was 25 mg/kg of formulated product (13B49 mg/kg, Category II), estimated by probit analysis). This study was acceptable.

iii. Inhalation

Endosulfan (37% EC) was administered to Wistar rats (5/sex/dose) in air at 0.16, 0.5 and 5.6 mg/L (nominal concentrations of 0.3, 2.5 and 15.9 mg/L for a 4 hour exposure; Seshaiyah, 1997). Mortalities in males (0/5, 3/5, 5/5) and in females (0/5, 5/5, 5/5) were observed along with clinical signs of chromorhinorrhea, sluggishness, and ataxia. At necropsy, lungs (congestion and emphysema) and livers (mottled in females) were affected. The LC<sub>50</sub> in both sexes was greater than 0.16 mg/L but less than 0.5 mg/L (Category II). This study was acceptable.

Sprague-Dawley rats were exposed for 4 hours to Endosulfan 50 WP in air at 0, 0.3, 2.5 or 15.9 mg/L (Rosenfeld, 1985). The actual concentrations in the breathing zone (measured gravimetrically) were 0.16, 0.5 and 5.6 (maximum attainable concentration) mg/L, respectively. Clinical signs were chromorhinorrhea, sluggishness, ataxia, oral discharge and perineal staining. Target organs were lungs (congestion and emphysema) and livers (mottled in females), as above. The NOEL was 0.3 mg/L (0.16 mg/L measured). This study was acceptable.

Wistar rats were exposed to Thionex 35 EC in air for 4 hours at 0, 0.027, 0.052, 0.124, 0.262 or 0.393 mg/L (Buch and Gardner, 1983). Clinical signs were decreased motor activity, hunched posture, muscle spasticity and tremor, piloerection and pigmented staining of the snout (observed at all doses). A NOEL was not established in this study. There were no deaths in males at 0.124 mg/L and below and no deaths in females at 0.027 mg/L. This study was acceptable.

Sprague-Dawley rats were exposed to Thiodan 2 CO/EC in air for 4 hours at 0, 1.02, 1.48 or 1.96 mg/L (males) and 0.28, 0.39 or 1.96 mg/L (females) (Freeman, 1989c). Clinical signs of neurotoxicity were observed at all doses in both sexes. There were no deaths in males at 1.02 mg/L and no deaths in females at 0.28 mg/L. This study was acceptable.

b) Rabbit

i. Dermal

New Zealand rabbits were treated dermally with Thiodan 2 CO/EC at 0, 500, 1000 or 2000 mg/kg for 24 hours with occlusion and exhibited clonic convulsions, loss of muscle control, tremors, hypersensitivity to touch, oral discharge and grinding of the teeth (Freeman, 1989b). The NOEL was 500 mg/kg (both sexes). This study was acceptable.

Endosulfan 50 WP was applied dermally on New Zealand rabbits at 0 or 2000 mg/kg for 24 hours with occlusion (Intox Labs, 1985b). Clinical observations showed soft stools, diarrhea and nasal discharge. Necropsy showed no gross lesions in surviving animals. There were no deaths in males and 1/5 females died. This study was acceptable.

Endosulfan (33.7% EC) was administered to the skin of New Zealand White rabbits (5/sex/dose) at 0, 100, 320 or 400 mg/kg for 24 hours under an occlusive wrap (Bakili, 1997a).

Mortality at 100 (M: 2/5, F: 1/5), 320 (M/F: 4/5) and 400 (M/F: 5/5) mg/kg was observed. Clinical signs of hyperactivity, gasping, tremors, paralysis of the hind limbs and erythema at the site of application were observed. Necropsy revealed lung congestion in conjunction with emphysema, dark red patches on the surface of the kidney, congestion of the kidney and liver, focal hemorrhages on the thymus, frothy exudate and hemorrhagic streaks in the trachea, paleness and atrophy of the spleen. The LD<sub>50</sub> (95% confidence limits) was 131 (55 to 311) mg/kg in males, and 178 (107 to 295) mg/kg in females (Toxicity Category I). This study was acceptable.

ii. Primary Eye Irritation

Endosulfan 50 WP was administered to New Zealand White rabbits (6) at 0.45 mg/eye (weight equivalent of 0.1 ml of the test article)/eye (Intox, 1985c). Results showed corneal opacity grade 1 (1/6 at 48 hrs after exposure, clearing by day 6), conjunctival irritation (grade 2 in 1/6, grade 1 in 2/6 at 24 hrs after exposure, persisting in 1/6 (grade 1) at day 6, clearing at day 17). There was no iritis (Category III). This study was acceptable.

Thiodan 2 CO/EC was administered to New Zealand White rabbits (9) at 0.1 ml/eye (Freeman, 1989d). Three animals had their eyes washed with tap water 20 to 30 seconds post-dose and 6 rabbit eyes were unwashed post-dose. No animals died. At day 1, grade 1 corneal opacity (2/9) and grade 1 B 2 conjunctival irritation (5/9) occurred and at day 2 there was corneal opacity (2/9). By day 3 there was no eye irritation (Toxicity Category III). This study was acceptable.

A primary eye irritation study was performed with endosulfan (33.7% EC; 0.1 ml/eye) on New Zealand rabbits (6 females) by ocular instillation with 0.1 ml/eye (Bakili, 1997b). Four of the animals exhibited hindlimb paralysis (3 Bday 3, 1 Bday 8) and 1 died on day 6. Corneal opacity was evident in 5 animals at 48 hours (4/6 = grade 1; 1/6 = grade 2). Opacity cleared by day 7 in 3 animals, however the 4<sup>th</sup> died and the 5<sup>th</sup> had grade 3 opacity throughout the 21-day observation period. No iritis was evident. Conjunctival redness, grades 3 (1/6), 2 (4/6) and 1 (1/6), was noted at 24 hours. By 7 days, grade 1 was evident in one animal only. Chemosis, grades 3 (1/6) and 2 (5/6), was apparent at 24 hours, diminishing to grades 2 (1/5) and 1 (1/5) at 7 days and clearing by day 21. Discharge, grades 2 (5/6) and 1 (1/6) and grades 2 (1/5) and 1 (2/5) were noted at 24 hours and 7 days, respectively, clearing by day 21 (Toxicity Category I). This study was acceptable.

iii. Dermal Irritation

Studies performed on New Zealand White rabbits (6) with Endosulfan 33.7% EC at 0.5 ml/site, one site/animal for 4 hours under a semi-occlusive wrap (Bakili, 1997c). Two of the animals died within 48 hours of dosing. Three of the remaining 4 animals suffered hindlimb paralysis. Erythema grade 1 (5/5) at 1 hour post-exposure, grade 1 (3/4) at 24 hours, grade 1 (4/4) at 48 hours and grade 1 (2/4) at 72 hours was observed (cleared by day 7). Edema, not evident at 1 hour post-exposure occurred at grade 1 (1/4) at 24 hours through day 7 (clear by day 14) (Toxicity Category IV). This study was acceptable.

Older studies performed with Endosulfan 50 WP (Intox, 1985c) and Thiodan 50 WP (FMC, 1983) used New Zealand White rabbits (6/study) to test for dermal irritation at 0.5 g/site with occlusion for 4 hours. Thiodan 50 WP was used on both abraded and unabraded skin. There was no irritation in either study (Toxicity Category IV). This study was acceptable.

Thiodan 2 CO/EC was used on New Zealand White rabbits (6) to test for dermal irritation at 0.5 ml/site for 4 hours with occlusion (Freeman, 1989e). At 4.5 hours post dose, grade 1 erythema (2/6), at day 1 grade 1 erythema (1/6), at day 2 grade 1 B 3 erythema (6/6), however by day 14 post-dose, desquamation occurred (4/6). In another study Thionex 35 EC was used on New Zealand white rabbits (4) at 0.5 ml/site for 4 hours with occlusion (Crown, 1982). Results showed that a mild to moderate erythema was induced at 24 and 48 hours. Both studies were Toxicity Category III and were acceptable according to FIFRA Guidelines.

c) Guinea Pig -- Dermal Sensitization

Hartley guinea pigs were tested with Drexel Endosulfan 3EC (33.7% EC; 10/sex/dose), Thiodan 2 CO/EC (25% w/v solution in tap water; 10/sex/dose) and Endosulfan 50 WP (5% w/v; 10 females treated, 5 control) at 0 (physiological saline) or 0.1 ml for 4 hours in a dermal sensitization assay (Sundar, 1997, Freeman, 1989f, and Intox, 1985e). Positive controls were used with Thiodan 2 CO/EC (1-chloro-2, 4-dinitrobenzene, DNCB) and Drexel Endosulfan 3EC (mercaptobenzothiazole) and they functioned as expected. Thiodan 2 CO/EC and Endosulfan 50 WP were non-sensitizing. For Drexel Endosulfan 3EC challenge (24 hours post-exposure) one animal each exhibited a grade 2 and a grade 1 erythema response. At 48 and 72 hours, the score for both these animals was grade 1. The test material is a moderate dermal sensitizer in this assay based upon the 10% response rate. All studies were acceptable.

d) Calf - Dermal

Calves dusted with a 4% dust formulation of endosulfan showed neurological signs (tremors, twitching, convulsions) and death within 24 hours of exposure (Nicholson and Cooper, 1977). The actual exposure dose and length of treatment were not stated in the report. **Supplemental.**

#### 4. Comparisons of Effects and LD<sub>50</sub>s in Technical versus Formulations

Endosulfan technical and formulated products, in general, had higher LD<sub>50</sub> values for males than for females. Oral exposure had comparable male and female LD<sub>50</sub> values for both technical and formulated products. Dermal LD<sub>50</sub> values were comparable for both sexes; however, formulated product values were approximately 2 - 20 times higher than those of technical material. Inhalation exposure showed comparable LD<sub>50</sub> values between technical and formulations; however, male values were approximately 3 times higher than those of females. In general, oral toxicity was highest and dermal was the lowest for both technical and formulated endosulfan.

### C. SUBCHRONIC TOXICITY

**Summary:** There were several studies that were acceptable according to FIFRA Guidelines **and many others in the open literature with useful information (supplemental).** Rats were the major species studied and effects occurred, in general, at greater than 1.0 mg/kg/day in both dietary and gavage studies. The primary effects were observed in the hematology and liver metabolism assays. **A decrease in plasma (serum, 31%) and RBC (12%) ChE was observed in female rats (subchronic, dietary), but only at toxic doses (27.17 mg/kg/day).** In addition, kidney and liver pathological effects were observed. Gavage studies, as well as dermal, inhalation and intraperitoneal rat studies, showed tonic/clonic convulsions and behavioral (memory) effects in addition to some of the effects also

observed in the dietary studies. In the rat dietary studies, NOELs ranged from 1.0 to 5.0 mg/kg/day (1 mouse study = 2.3 mg/kg/day), gavage studies ranged from 0.625 to 3.0 mg/kg/day (1 mouse study = 3.0 mg/kg/day), dermal studies ranged from 1.0 to 54 mg/kg/day, inhalation was 1.0 ug/L (0.194 mg/kg/day) and intraperitoneal were 0.5 - 3.0 mg/kg/day. There was some variation between sexes, with each sex being more and less sensitive even within study types. In many of the **open literature** studies, primary effects were neurotoxicity and testicular toxicity; however, effects to the immune system were also denoted by a decrease in cell-mediated immunity in rats at 2.0 mg/kg/day or greater.

## 1. Rat

### a) Dietary

The most sensitive indicator of subchronic toxic effects occurred in a rat subchronic dietary study, which included a 4-week, post-treatment recovery. CD rats (25/sex/dose treated, 5/sex/dose recovery) were fed endosulfan in the diet for 13 weeks at 0, 10, 30, 60, or 360 ppm (Barnard, et al., 1985). Achieved doses in males were 0, 0.64, 1.92, 3.85 or 23.41 mg/kg/day and in females were 0.75, 2.26, 4.59 or 27.17 mg/kg/day. Results showed an increased incidence in clinical signs (hair loss in dorsal/scapular/cervical regions in early part of the study) among females at 4.59 mg/kg/day and greater and in males there were enlarged kidneys at 3.85 mg/kg/day and greater (these effects in M/F were reversed during recovery). Microscopically, livers showed granular brown pigment in males and centrilobular enlargement of hepatocytes at 23.41 mg/kg/day for males and 27.17 mg/kg/day for females. In kidneys, discoloration (pigmentation) was increased primarily at 3.85 mg/kg/day and greater in males and for females, 4.59 mg/kg/day and greater but it was reduced to trace amounts or was completely reversed after the 4-week recovery. Granular/clumped pigment remained in males after recovery. Both the discoloration and the granular/clumped pigments continued after treatment, but did not seem to have any toxicological effect. Packed cell volume was statistically significantly decreased in males throughout treatment and recovery at 23.41 mg/kg/day. RBCs were statistically significantly decreased in males ( $\geq 1.92$  mg/kg/day, week 6;  $\geq 3.85$  mg/kg/day, week 13 and at 23.41 mg/kg/day week 17 recovery). In females RBCs were statistically significantly decreased ( $\geq 4.59$  mg/kg/day, week 6; 27.17 mg/kg/day, week 13, reversed at week 17 recovery). In males hemoglobin (Hb) was statistically significantly decreased ( $\geq 1.92$  mg/kg/day week 6; 23.41 mg/kg/day week 13;  $\geq 3.85$  mg/kg/day at recovery). In females Hb was decreased ( $\geq 4.59$  mg/kg/day, week 6;  $\geq 0.75$  mg/kg/day—not dose related, week 13; reversed at recovery). Mean corpuscular hemoglobin concentration was decreased at 23.41 mg/kg/day in males and 27.17 mg/kg/day in females, throughout treatment and recovery. Mean cell volume was increased in males early in treatment and after recovery at 23.41 mg/kg/day and at 4.59 mg/kg/day and greater, throughout treatment and recovery in females. Females at 27.17 mg/kg/day had lower plasma cholinesterase (ChE, 59% of control) and RBC ChE (88% of control) activities and dark urine with increased ketones. Brain ChE, on the other hand, was increased at 4.59 mg/kg/day (119% of control) and at 27.17 mg/kg/day (120% of control) in females at termination. At 0.75 mg/kg/day, brain ChE was increased 104% of control and at 2.26 mg/kg/day it was increased 115% of control. This effect was likely incidental because it was not observed in males and the standard deviations in all dosed groups were almost twice that of control. In addition, the effects were not dose-related and were not observed in any other subchronic or chronic study reviewed by DPR. Increased absolute liver weights were reported in both sexes at 23.41 mg/kg/day (males) and 27.17 mg/kg/day. Males had significantly increased epididymal weights at 23.41 mg/kg/day, which were 9% greater than control. Absolute kidney weights were statistically significantly increased at 3.85 mg/kg/day and greater in males (9% and 24%, respectively) and in

females at 27.17 mg/kg/day (9%), compared to control. Water consumption and the water-to-food intake was statistically significantly decreased in males at week 5 at 1.92 mg/kg/day or greater, but was reversed by week 12. Females had decreased water consumption week 5 (also later reversed) at 27.17 mg/kg/day.) The NOEL was established at 30 ppm (1.92 mg/kg/day (M) and 2.26 mg/kg/day (F)), based on the occurrence of kidney pathological effects, hematology effects, decreased plasma and RBC cholinesterase, decreased water consumption and increased absolute kidney and liver weights. These NOELs will be used to calculate the margin of exposure for potential subchronic, seasonal human exposures to endosulfan. This study is acceptable. See Tables 4 and 5 for the major effects.

**Table 4. Endosulfan Effects in a 13-Week Rat Subchronic Diet Study and 4 Week Recovery (Barnard et al. 1985)**

Effects (Mean values)	Endosulfan Dose (mg/kg/day)--Males					Endosulfan Dose (mg/kg/day)--Females				
	0	0.64	1.92	3.85	23.41	0	0.75	2.26	4.59	27.17
Food Intake g/rat, wk 1-2 <sup>a</sup>	342	353	352	349	339	260	261	258	250	229**
Body Wt Gain (g) Wk 1-2	106	107	112	109	103	50	51	49	46	34**
Water to Food Ratio	1.5	1.4	1.3*	1.3*	1.3*	1.6	1.6	1.6	1.6	1.5
Water Consumed (g) Wk 5	272	256	247**	241**	245**	216	215	214	216	190*
<b>HEMATOLOGY</b>										
Packed Cell Vol (%) wk 6	51	52	51	51	49*	48	49	49	48	48
Week 13	52	53	52	51	49**	48	49	49	50	48
Week 17	52	52	53	52	49**	49	49	49	44	47

Hemoglobin (g/dl) wk 6	14.6	14.9	14.1*	13.8**	13.4**	13.7	13.9	13.4	12.8**	12.4**
Week 13	16.1	16.5	16.1	15.9	15**	15	14.5*	14.4*	14.5*	14.3**
Week 17	16.6	16	16	15.7*	15**	14.8	15.2	15.9	13.7	14.5
RBC (x10 <sup>6</sup> /cmm) wk 6	7.9	7.9	7.5*	7.2**	7**	6.8	6.9	6.7	6.4*	6.1**
Week 13	9.4	9.4	9.2	8.8*	8.6**	7.9	7.5	7.7	7.7	7.3**
Week 17	8.8	8.4	8.4	8.2	7.8**	7.1	7.4	7.6	6.6	7.0
MCHC <sup>b</sup> (%) Week 6	28.8	28.9	27.7**	27.3**	27.3**	28.6	28.6	27.3*	26.9**	26.2**
Week 13	30.9	31.4	30.7	31	30.6	31	30.4	29.8**	29.2**	29.8**
Week 17	31.9	30.6*	30.2**	30.3**	30.3**	30.3	30.9	32.4*	30.8*	30.9*
Mean Cell Vol (fl) wk 6	65	66	68**	70**	70**	71	70	73	74**	78**
Week 13	56	56	57	58	57	61	64	63	65**	66**
Week 17	59	63	63	63	64*	69	66*	64*	68*	67*
<b>CHOLINESTEREASE ACTIVITY</b>										
Plasma (umol/ml/min) Wk 5	0.42	0.43	0.44	0.42	0.42	1.21	1.20	1.11	1.13	0.79**
Week 12	0.47	0.46	0.50	0.45	0.47	1.61	1.55	1.57	1.70	0.95**
Week 16	--	--	--	--	--	1.42	1.63	1.39	1.42	1.44
RBC ChE-mol/ml/min Wk 5	1.68	1.63	1.51	1.63	1.57	1.65	1.66	1.57	1.57	1.64
Week 12	1.75	1.87	1.71	1.87	1.78	1.89	1.65	1.79	1.77	1.66*
Week 16	--	--	--	--	--	2.41	2.21	2.07	2.23	2.28
Br ChE (umol/g/min) Term	7.06	7.66	5.88	6.32	7.0	5.31	5.51	6.08	6.30*	6.35*
Week 16	--	--	--	--	--	4.92	4.84	5.04	4.54	4.74
Plasma ChE -%cont Wk 5	--	102	105	100	100	--	99	91	93	65**
Week 12	--	98	106	97	100	--	96	96	106	59**
Week 16	--	--	--	--	--	--	115	98	100	101
RBC ChE -% cont-Wk 5	--	97	89	97	93	--	100	95	95	99
Week 12	--	107	98	107	102	--	87	95	94	88*
Week 16	--	--	--	--	--	--	92	86	93	95
Brain ChE -% cont Termin	--	109	83	90	99	--	104	115	119*	120
Week 16	--	--	--	--	--	--	98	102	92	96

a - Selected weeks were time periods where parameters showed statistical significance in at least 1 sex.

b - MCHC = Mean Corpuscular Hemoglobin Concentration

\*, \*\* - Significant at p < 0.05 & 0.01, respectively.

NOTE: Blood tests were on 10/sex/dose. Body weights, food consumption, water consumption and organ weights were for 20/sex/dose at termination and 5/sex/dose at recovery. ChE assays were on 10/sex/dose.

**Table 5. Pathological Effects in a 13-Week Rat Endosulfan Diet Study, With 4-Week Recovery (Barnard et al., 1985)**

Effects Observed (mean values)	Endosulfan Dose (mg/kg/d)--Males					Endosulfan Dose (mg/kg/d)--Females				
	0	0.64	1.92	3.85	23.41	0	0.75	2.26	4.59	27.17
<b>MACROSCOPIC PATHOLOGICAL EFFECTS<sup>a</sup>: Organ Weights (grams)<sup>a</sup></b>										
Brain – Termination	2.04	2.08	2.10	2.07	2.07	1.84	1.84	1.86	1.93**	1.96**
Brain—Recovery	1.97	2.0	2.03	2.08*	2.08*	1.92	1.88	1.88	1.88	1.94
Week 16	--	--	--	--	--	1.42	1.63	1.39	1.42	1.44
Liver – Terminal	20.3	21.7	21.0	21.7	24.8**	12.0	11.6	11.4	12.0	15.03**
Recovery	22.4	22.0	22.2	20.4	22.9	12.6	13.4	12.1	11.9	13.8
Kidneys – Termination	4.10	4.14	4.18	4.49**	5.10**	2.56	2.53	2.50	2.57	2.82**
Recovery	4.01	3.96	4.09	4.15	4.74**	2.48	2.75	2.56	2.59	2.63

Epididymides -- Term	1.21	1.27	1.29	1.27	1.33*	--	--	--	--	--
Recovery	1.28	1.11	1.25	1.35	1.41	--	--	--	--	--
<b>MACROSCOPIC PATHOLOGY<sup>b</sup></b>										
Enlarged Kidneys	0/0	0/0	0/0	2/0	11/1	0/0	0/0	0/0	0/0	0/0
<b>MICROSCOPIC PATHOLOGICAL EFFECTS<sup>b</sup></b>										
<u>Liver</u> : Granular Brown Pigment in Hepatocytes	0/0	0/0	0/0	0/0	5/0	0/0	0/0	0/0	0/0	0/0
<u>Liver</u> : Centrilobular Enlargement of Hepatocytes	0/0	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/0	5/0
<u>Kidney</u> : Yellow Discolored Cells (Prox CT) <sup>c</sup> -- Trace	0/0	14/3	17/1	7/0	0/4	0/0	0/0	1/0	8/0	19/0
Minimal	0/0	0/0	2/0	9/5	7/0	0/0	0/0	0/0	0/0	0/0
Moderate	0/0	0/0	0/0	3/0	13/0	0/0	0/0	0/0	0/0	0/0
Granular/Clumped Pigment in St/Prox. CT <sup>d</sup> -Trace	0/0	0/0	0/2	3/5	0/0	1/0	0/0	0/0	0/2	17/5
Minimal	0/0	0/0	0/2	4/0	7/1	0/0	0/0	0/0	0/0	2/0
Moderate	0/0	0/0	0/0	0/0	13/4	0/0	0/0	0/0	0/0	0/0
Yellowish Material in Lumen (Prox. CT) <sup>c</sup> -Minimal	0/0	0/0	0/0	0/0	13/0	0/0	0/0	0/0	0/0	0/0
Intracytopl Eosinophilic Drops (Prox. CT) <sup>c</sup> -Minimal	0/0	0/0	0/0	0/0	6/0	0/0	0/0	0/0	0/0	0/0

a – Organ weights were for 20/sex/dose at termination

b – The number of observations in the main study (of 20 rats)/# observations in the recovery study (of 5 rats).

c – Prox CT = proximal convoluted tubuled.

d– St/Prox. CT = Straight portions and/or occasional proximal convoluted tubules.

\*, \*\* - Significant at p < 0.05 & 0.01, respectively.

Wistar male rats were fed endosulfan in their diet for 4 weeks at 0, 360 or 720 ppm (equivalent dose of 34 or 68 mg/kg/day) (Leist and Kramer, 1985). Of the 20 treated rats per group, 10 were terminated after 4 weeks and 10 were kept for a 4-week recovery period. Pigmented kidney cells were observed in male Wistar rats after 4 weeks at both doses. The LOEL was 34 mg/kg/day. A NOEL was not achieved in this study. This study was acceptable.

Wistar rats (80/sex/dose) were fed endosulfan in the diet for 30 days at 0, 3.0 and 6.0 mg/kg/day (Paul, et al., 1995). Results showed a sex-related difference in neurobehavioral and hepatic effects. Mortality (30%, 24/80) occurred in females at 6.0 mg/kg/day, but no males died. Absolute liver weights were increased significantly in a dose-related manner in both sexes, though hepatomegaly was greater in females. Serum and liver transaminases and liver alkaline phosphatase levels were significantly increased, primarily in females at 6.0 mg/kg/day. Motor activity was more markedly stimulated in males; however, increases were significant in both sexes at 6.0 mg/kg/day. Learning and memory were significantly affected in both sexes at 3.0 mg/kg/day (only dose used for neurobehavioral group). The LOEL was 3.0 mg/kg/day, based on mortality, systemic and neurobehavioral effects. **Supplemental.**

#### b) Gavage

Albino rats (6/sex/dose) were gavaged with endosulfan technical at 0 (peanut oil), 0.75, 2.5 and 5.0 mg/kg/day (males) and 0.25, 0.75 and 1.5 mg/kg/day (females) for 30 days (Dikshith et al., 1984).

Results at all doses showed hyperexcitability, tremor, dyspnea and salivation that disappeared after 3-4 days. These effects were considered transitional and therefore were not used to establish a LOEL. Relative liver (17%), kidney (9%) and testes (55%) weights were significantly increased at 5.0 mg/kg/day. Females had statistically significantly decreased kidney weights at 1.5 mg/kg/day. Some blood parameters were significantly affected (liver alkaline phosphatase, RBC, neutrophils) in both sexes of treated rats at the high doses. Levels of total endosulfan were statistically significantly increased in blood serum, fatty tissue, and liver of both sexes and in female kidneys at the high dose. The NOEL for males was 2.5 mg/kg/day and for females was 0.75 mg/kg/day. **Supplemental.**

IIRC female rats (15/dose) were gavaged with endosulfan technical at 0 (corn oil), 1.0, 2.5 and 5.0 mg/kg/day for 7 or 15 days (Gupta and Gupta, 1977). Pentobarbital was administered (50 mg/kg/day) i.p. 24 hours after the final dose to evaluate whether the hepatic microsomal enzymes induced by endosulfan affected the sleeping time. The NOEL was 1.0 mg/kg/day based on increased liver weights and sleeping time. **Supplemental.**

IIRC male rats (8/dose) were treated by gavage at 0 (peanut oil), 5.0 and 10 mg/kg/day for 15 days (Gupta and Chandra, 1977). Absolute organ weights were significantly decreased (kidney, lungs and testes) and 3/8 died at 10 mg/kg/day. Although no individual data for pathological effects were reported, it was stated that at both doses, livers showed focal necrosis, Kupffer cell hyperplasia and bile duct proliferation. At 10 mg/kg/day, kidneys showed congestion and focal tubular degeneration. Testes at 10 mg/kg/day showed seminiferous tubule degeneration and interstitial edema. One-third of the tubules in a section were devoid of spermatogenic elements and were lined by a single layer of cells consisting of Sertoli cells and some spermatogonia. The study was limited by the fact that there was high mortality at 10 mg/kg/day. A NOEL was not established. **Supplemental.**

Adult male Wistar rats (6/dose/treatment) received doses of endosulfan (E) by gavage at 0 (groundnut oil), and 7.5 mg/kg/day, ethanol (EtOH in saline) at 1.5 mg/kg/day or both at 7.5 mg/kg/day (E) + 1.5 mg/kg/day (EtOH) for 30 days (Singh and Pandey, 1991). Subsequently, livers were weighed, cytosolic and microsomal mixed function oxidases (MFO), NADPH-ICDH, 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase (G<sub>6</sub>PDH) were assessed. Results showed that endosulfan induced liver MFOs (cytochrome P450, NADPH cytochrome c reductase), cytosolic GSH-s-transferase (conjugation) and non-enzymatic microsomal lipid peroxidation and decreased NADH cytochrome b<sub>5</sub> reductase. Along with enzyme increases, protein content and thus liver wet weights were also increased. When endosulfan treatment was combined with EtOH, as has been described in human illness reports associated with endosulfan, there was enhanced hepatotoxicity. Effects observed with endosulfan alone also occurred with endosulfan plus EtOH; however, the response was greatly enhanced. In addition, there was an increase in the cytosolic reducing equivalent (NADPH) generating enzyme (NADP-isocitrate dehydrogenase). It is hypothesized that enzymes in the microsomal EtOH oxidizing system induced from chronic EtOH ingestion “spills over” to other drug metabolizing systems and when endosulfan exposure also occurs, hepatotoxicity is potentiated. **Supplemental.**

Ethanol (0.2 g/kg) and endosulfan (2 mg/kg) treatment of Wistar rats for 35 days resulted in hepatomegaly and decreased body weight (Paul et al., 1992). Effects were greater in males, suggesting that males are more susceptible than females to the metabolic stress induced by their interaction. Chronic endosulfan exposure resulted in increased EtOH sleeping time in females (not males). The authors concluded that female rats do not metabolize EtOH as readily since they have a greater

susceptibility to the hepatotoxicity of endosulfan. **Supplemental.**

Endosulfan was administered via gavage to adult male Wistar rats at 0 (corn oil; n=10) and 2.0 mg/kg/day (n=60) for 6 weeks to test for effects on B cells (islets of Langerhans) in pancreas (Kalender et al., 2004). Results showed that blood glucose levels were significantly increased at the end of the 3<sup>rd</sup> and 4<sup>th</sup> weeks ( $p < 0.05$ ) and the 5<sup>th</sup> and 6<sup>th</sup> weeks ( $p < 0.01$ ) after administration of endosulfan to rats compared with the control group. Electron microscopy showed swelling of mitochondria (end of 2<sup>nd</sup> and 3<sup>rd</sup> weeks), vacuoles in cytoplasm (end of week 4), dissolution of mitochondrial matrix (end of 5<sup>th</sup> week) and picnotic nuclei in B cells in islets of Langerhans (end of 6<sup>th</sup> week). **Supplemental.**

### c) Dermal

Wistar rats (6/sex/dose) received daily dermal endosulfan treatments for 30 days at 0, 18.75, 37.5 or 62.5 mg/kg/day (males) and 9.83, 19.66 or 32.0 mg/kg/day (females) (Dikshith, et al., 1988). Endosulfan was dissolved in acetone and was painted on clipped and cleaned lateral abdominal skin. The treatment area was not occluded. Hyperexcitability, tremors, dyspnea and salivation occurred at all doses. The report did not indicate time of onset of clinical signs but they were gone by 1-week post-dosing. There were no deaths. Males had significantly increased relative testes weights of 278% and 215% at 37.5 and 62.5 mg/kg/day, respectively. Plasma glutamic oxaloacetic transaminase (GOT) was increased at 32 mg/kg/day in females and glutamic pyruvic transaminase (GPT) activities were significantly decreased at all doses in both sexes. An explanation for the decreased GPT was not provided in the report. There were no effects on weight or histopathological effects for liver or kidney. Animals receiving the highest dose of endosulfan showed residues in their fatty tissue. A NOEL was not achieved in this study. **Supplemental.**

Wistar rats (6/sex/dose) were treated dermally at 0 (sesame oil), 1, 3, 9 and 27 mg/kg/day for both sexes plus 81 mg/kg/day for males only, for 30 days (6 hr/d, 5 d/wk, with occlusion) (Ebert et al., 1985a). Mortality was increased in males with 2/6 dying at 9 mg/kg/day and 3/6 at 81 mg/kg/day and in females with 4/6 dying at 27 mg/kg/day. Males at 81 mg/kg/day and females at 27 mg/kg/day showed acute lung congestion (dilation of alveolar vessels), blood vessel congestion, cardiac ventricles filled with blood, acute heart and circulatory failure. Males had convulsions and diffuse brain edema at 81 mg/kg/day. Clinical signs in animals that died were increased salivation, blood-encrusted nose, passivity, dyspnea, tono-clonic convulsions and increased respiratory rate. The systemic NOEL was 3 mg/kg/day based on an increase in mortality, lung and cardiovascular effects. The ChE NOEL was less than 1 mg/kg/day, based on a significant decrease in serum ChE activity in males (M: 72 - 79% in males at 9 mg/kg/day or greater; F: 19 - 38% at 9 mg/kg/day or greater—not statistically significant). Brain ChE activity was decreased in both sexes at all doses (statistically significant in M: 6 - 28% at  $\geq 3$  mg/kg/day and in F: 14 - 18% at  $\geq 1$  mg/kg/day). This was not acceptable according to FIFRA Guidelines since it was reported that the endosulfan administration method caused some of the deaths at all doses, dosing material was not characterized and a complete histopathological examination was not performed. The subsequent study from the same laboratory was performed with revised treatment methods (see below, Ebert et al., 1985b).

Endosulfan technical was administered dermally to SPF Wistar rats (6/sex/dose--Main group & 5/sex/dose--recovery group) for 21 treatments in 30 days. Treatments were Monday through Friday for 6 hours per day at 0 (sesame oil), 12, 48, 96 and 192 mg/kg/day for males and 3, 6, 12 and 48

mg/kg/day females (Ebert et al., 1985b). The recovery group was observed for 14 days after the final dermal treatment. The systemic NOEL for males was 48 mg/kg/day and 6 mg/kg/day for females. At doses of 12 mg/kg/day and greater, the females showed pilo-erection, increased salivation and lacrimation. At 48 mg/kg/day, females also showed blood-encrusted nares and dacryohemorrhage and 4/6 females died between days 2 and 22 following tonic-clonic convulsions. Males at 192 mg/kg/day died (2/6 by day 9). Liver and kidney pathological effects were observed in males at 192 mg/kg/day and in females at 48 mg/kg/day. ChE NOEL was 48 mg/kg/day, based on a significant decrease in serum ChE activity at 192 mg/kg/day and brain ChE activity at 96 mg/kg/day in males immediately after treatment. Values were comparable to controls after the recovery period. This was not an acceptable FIFRA Guideline study since dosing material was not characterized and a complete histopathological examination was not performed. Note that both of the above studies (Ebert et al., 1985a & b) were performed in the same laboratory and the Ebert et al., 1985b was designed to be a repeat of 1985a, only with corrections to the dosing methods and differences in dose levels. However the dosing material was not characterized in either experiment, and there was incomplete histopathology. Therefore, these studies are considered to be supplemental.

Endosulfan water-dispersible powder (49.5% a.i.) was administered dermally 21 times over 30 days (Mon - Fri) to SPF Wistar rats (6/sex/dose--Main group & 5/sex/dose--recovery group) at 0 (physiological saline), 40, 160, and 640 mg/kg/day (males) or 40, 80 and 160 mg/kg/day (females) to the shaved nape skin (Ebert, 1987). Exposure was for 6 hours under an occlusive bandage. The recovery group (all doses except 40 mg/kg/day) was observed for 22 days after the final dermal treatment. Results showed clinical signs and increased deaths in females at 80 mg/kg/day and greater. A transitory skin irritation was observed in both sexes, but it was reversed by week 3. Males had statistically significantly decreased body weight gains at 640 mg/kg/day. Some hematological parameters were affected at the high dose of both sexes. Macro- and microscopically, there was an increase in yellow coloration and in sperm granulomas in the epididymides at 160 mg/kg/day and greater and at 640 in the recovery group. The systemic NOEL was 40 mg/kg/day, for both sexes. Males had statistically significantly decreased serum ChE at 640 mg/kg/day (-13%) and in females it was decreased at 80 mg/kg/day (-28%) and 160 mg/kg/day (-46%) when measured one day following the last dosing. Brain ChE in males was decreased 15% at 640 mg/kg/day. No ChE effects were observed in males at recovery. Females showed statistically significant decreases in serum ChE at 80 mg/kg/day (-24%) after recovery and at 160 mg/kg/day (-23%) when tested 23 days after the last dose. Statistics were not performed on the 3 surviving recovery females at 160 mg/kg/day; however, serum ChE was decreased by 23% at recovery day 23 in this group. This study was acceptable.

Endosulfan emulsifiable concentrate (33.3% a.i.) was administered dermally (semi-occlusive bandage) to Wistar rats (10/sex/dose terminated after 4 weeks + 5/sex/dose 4 week recovery) at 0 (vehicle = 4% carboxymethylcellulose), 0 (formulation base: HOE 002671 0I EC00 A302 administered in the vehicle), 27, 54 or 81 mg/kg/day (males) and 9, 12, 18 or 36 mg/kg/day (females) for 6 hours per day for 4 weeks (21-22 applications), followed by a 4 week recovery period (Thevenaz, et al., 1988). Results showed treatment-related deaths in females at 12 mg/kg/day (1/15), 18 mg/kg/day (1/15) and 36 mg/kg/day (4/15) weeks 1, 2 and 4. Minimal-moderate erythema and slight edema were observed in endosulfan-treated animals (reversed in the recovery group). Clinical signs indicated neurotoxicity (tremor, Straub-tail, trismus, saltatory spasms, extension spasms, tetanoid spasms), with onset at 1 hour post-dosing and lasting 30 minutes. Females (1/15) at 36 mg/kg/day died following a spasm attack. These effects were observed in males at 81 mg/kg/day and in females at  $\geq$  12 mg/kg/day (no data included). Clinical biochemistry effects were increased in both sexes at 81

mg/kg/day (M) and 36 mg/kg/day (F). Systemic NOEL = 27 mg/kg/day (M) and 9 mg/kg/day (F). In addition, serum cholinesterase (ChE) activity was statistically significantly decreased in males (-13% at 27 mg/kg/day, -17% at 54 mg/kg/day, -22% at 81 mg/kg/day) and females (-22% at 12 mg/kg/day, -29% at 18 mg/kg/day, -32% at 36 mg/kg/day). This is an acceptable FIFRA Guideline study

#### d) Inhalation

A preliminary subchronic rangefinding study (based on the acute inhalation LC<sub>50</sub> study; Hollander and Weigand, 1983) was performed with Wistar rats (5/sex/dose) that were treated 7 times (6 hr/day) over 9 days at 0.0024 mg/L and 0.0065 mg/L to determine the doses for the definitive study (Hollander et al., 1984). Results showed that clinical signs of neurotoxicity (disequilibrium, tremors, trembling and sporadic tono-clonic convulsions) occurred both during and after dosing. Two females died either during or after the 6<sup>th</sup> treatment (0.0065 mg/L; 1.11 mg/kg/d) and females had decreased body weights at 0.0024 mg/L and greater. Since effects were observed at 0.0024 mg/L, it was decided that 0.002 mg/L (0.387 mg/kg/d) would be the highest concentration for the subchronic main study. An intensification of the signs of intoxication was expected to occur following longer dosing. Supplemental.

Endosulfan technical was administered by inhalation (aerosol, nose only) 21 times over 29 days (Mon - Fri, 6 hours/day) to Wistar rats (15/sex/dose) at 0 (air only), 0 (ethanol - polyethylene 400 (1:1)), 0.0005, 0.0010 and 0.0020 mg/L air (approximately equivalent to 0.097, 0.194 and 0.387 mg/kg/day) (Hollander et al., 1984). Following the 29-day treatment period, 10 per sex per dose were terminated and the remaining 5 per sex per dose were observed over a 29-day recovery period. The NOEL was 0.0010 mg/L (0.194 mg/kg/day). At 0.0020 mg/L (0.387 mg/kg/day), 1/10 males showed signs of emaciation, pale skin, squatting position and high-legged position. Bodyweight gains were decreased in males at 0.0020 mg/L (0.387 mg/kg/day) from day 20 of treatment (not significant). On day 20 of treatment, males showed a significant decrease in food consumption at 0.0020 mg/L (0.387 mg/kg/day). Relative water consumption was slightly increased from days 9 to 24 in males at 0 (vehicle control) and at all treatment levels, when compared to air only control. Clinical chemistry for females showed significant (but reversible) effects in chloride, calcium, creatinine and SGOT at 0.0020 mg/L (0.387 mg/kg/day). Cholinesterase activity was not measured. While many effects were not statistically significant, the doses selected in this study were based on a rangefinding study performed by the same laboratory. In the rangefinding study (based on the acute inhalation LC<sub>50</sub> study; Hollander and Weigand, 1983) 5 rats per sex per dose were treated 7 times (6 hr/day) over 9 days at (0.0024 mg/L) and (0.0065 mg/L). Results showed that clinical signs of neurotoxicity (disequilibrium, tremors, trembling and sporadic tono-clonic convulsions) occurred both during and after dosing. Two females died either during or after the 6<sup>th</sup> treatment (0.0024 mg/L) and females had decreased body weights at 0.0024 mg/L and greater. Since effects were observed at 0.0024 mg/L it was decided that 0.002 mg/L would be the highest concentration for the subchronic main study. An intensification of the signs of intoxication was expected to occur following longer doses. Acceptable.

#### e) Immunotoxicity

Male Wistar rats (10-12/dose/sacrifice time) were fed endosulfan technical in the diet at nominal doses of 0 (ground nut oil), 5, 10 or 20 ppm (equivalent achieved doses of: 0, 0.5, 1.0 or 2.0 mg/kg/day) for 8, 12, 18 and 22 weeks to evaluate subchronic treatment on humoral and cell-mediated immune responses in albino rats (Banerjee and Hussain, 1986). Rats were immunized with tetanus

toxoid (TT- stimulated group) in Freund's complete adjuvant subcutaneously 20 days before terminating the exposure with an equal number of animals (NI - unstimulated group) not immunized (10-12 rats/dose/sacrifice time). The humoral immune response was characterized by serum globulin (SG) level, immunoglobulin (IgM & IgG) concentration and antibody titre against tetanus toxoid. The cell-mediated immune (CMI) response was measured by lymphocyte migration inhibition (LMI) and macrophage migration inhibition (MMI) factors. At 22 weeks, spleen/body weight ratio was statistically significantly decreased at 2.0 mg/kg/day in TT groups. Albumin/ globulin ratio was statistically significantly increased weeks 12-22 at 2.0 mg/kg/day and at 22 weeks at 1.0 mg/kg/day in TT groups. Antigen-induced increases (TT) in SG (8-22 weeks), IgG (12-22 weeks), LMI (8-22 weeks) and MMI (8-22 weeks) were observed at 2.0 mg/kg/day. It was concluded that endosulfan exerts a marked suppression of the humoral and CMI responses in rats. Both IgG and CMI were decreased in a dose-time related manner. It was concluded that endosulfan treatment disrupts the immune system in male rats. Clinical effects were not described in this study. Therefore, it is unknown whether animals experienced neurotoxicity after treatment. **Supplemental.**

Male Wistar rats (16/dose) were fed endosulfan technical in the diet at nominal doses of 0, 10, 30 or 50 ppm (equivalent achieved doses of: 0, 1.0, 3.0 or 5.0 mg/kg/day, respectively) for 6 weeks (Banerjee and Hussain, 1987). The study was designed to evaluate the effects of subchronic doses of endosulfan on humoral and cell-mediated immune responses. After 25 days of exposure, the animals were immunized subcutaneously with tetanus toxoid. Serum antibodies to the toxin, IgG, IgM, LMI (lymphocyte migration inhibition) and MMI were measured. There were no "overt signs of toxicity", however, the schedules for and extent of observations was not described. At termination, relative liver weights were significantly increased by 15% at 5 mg/kg/day. The immune system showed signs of suppression, compared to the control, by a dose-related decrease in serum antibody to tetanus toxoid. Serum IgG (28%) and IgM (25%) and  $\gamma$ -globulin (33%) were significantly decreased at 5.0 mg/kg/day, compared to the control. Group hemagglutination was significantly decreased by 14% at 3.0 and 43% at 5.0 mg/kg/day. Cell mediated immunity was decreased in a dose-related manner as indicated by the suppression of LMI by 24% and 40% at 3.0 and 5.0 mg/kg/day, respectively. MMI was significantly decreased by 20% and 44% at 3.0 and 5.0 mg/kg/day, respectively. The NOEL was 1.0 mg/kg/day based on increased relative liver weights and a decreased serum antibody response to tetanus toxoid. **Supplemental.**

## 2. Mouse - Dietary

Endosulfan in the diet was fed to CD-1 mice (20/sex/dose) at 0, 2, 6, 18 and 54 ppm (calculated as: 0, 0.26, 0.76, 2.3 or 6.9 mg/kg/day) for 13 weeks (Barnard, et al., 1984). At 6.9 mg/kg/day, 1 male and 1 female showed clinical signs (convulsions and salivation), while 12 males and 10 females died during treatment without any clinical signs. Mortality occurred during the first half of the treatment period. Primarily males showed a decrease in food consumption and body weight gain at 6.9 mg/kg/day. The NOEL was 2.3 mg/kg/day, based on mortality. No data were presented in the report (summary only).

Endosulfan was fed in the diet to Hoe NMRKf (SPF71) mice at 0 and 18 mg/kg/day (equivalent to 3.7 mg/kg/day for males and 4.6 mg/kg/day for females) for 6 weeks (Donaubauer, et al., 1985). An increase in absolute and relative liver weights was reported in both sexes. The study did not include hematology, clinical chemistry or pathological/histopathological effects. A NOEL was not achieved. There were no data presented (summary only).

## D. CHRONIC TOXICITY and ONCOGENICITY

**Summary:** For evaluation of chronic toxicity and oncogenicity of endosulfan, there were 3 rat, 2 mouse and 1 dog dietary study, in addition to 1 dog study performed with endosulfan in capsules. One rat combined (chronic and oncogenicity), 1 mouse oncogenicity and 1 chronic dog study (all dietary) were acceptable based on FIFRA Guidelines. The primary effects in the rat studies were to the vascular system, and the kidney, along with a decrease in body weight gain. The mouse oncogenicity study showed mortality as the primary effect. In the mouse studies, a target organ was not identified. The primary effects observed in the chronic, dog-dietary study were mortality (premature termination) and neurotoxicity. The lowest NOEL for chronic studies was 0.57 mg/kg/day obtained in the chronic dog study (M: 0.57 mg/kg/day; F: 0.65 mg/kg/day) based on increased mortality, and neurotoxicity. Results for oncogenicity studies performed in the rat and the mouse showed no tumor incidence that was treatment-related, dose-related or otherwise different in incidence across dose groups. Endosulfan is categorized as “A4” (not classifiable as a human carcinogen) by the American Conference of Governmental Industrial Hygienists (Substances and Physical Agents and Biological Exposure Indices, Cincinnati, OH, 2005).

### 1. Rat - Dietary

CrI:CD (SD) BR rats (70/sex/dose) were fed endosulfan in the diet for 104 weeks at 0, 3.0, 7.5, 15 or 75 ppm (Ruckman et al., 1989). This main group was intended primarily for tumorigenic evaluation. Also treated for 104 weeks was a satellite group of 20 rats/sex/dose, intended for blood sampling at intervals and for sacrifice after 104 weeks of treatment. There were no interim sacrifices in this study. The intakes of endosulfan were 0.1, 0.3, 0.6 or 2.9 mg/kg/day in males and 0.1, 0.4, 0.7 or 3.8 mg/kg/day in females, based on food consumption. Bodyweight gain was decreased 8% to 18%, compared to controls in both sexes at 2.9 mg/kg/day in males and 3.8 mg/kg/day in females (statistically significant in both sexes). Kidney enlargement occurred in females at 3.8 mg/kg/day. Progressive glomerulonephrosis was increased in both sexes at the high dose (statistically significant in females at 3.8 mg/kg/day) and was stated in the report to be a common, age-related, spontaneously occurring renal disease associated with proteinuria (especially in males). There was a non-dose related increase in glomerulonephritis in males at 0.3 mg/kg/day and greater. The chronic NOEL was 0.6 mg/kg/day in males, based on 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. There was a slight increase in the incidence of pituitary adenomas in males at 75 ppm but there was no dose-related trend. Incidences were, control through high dose (n = 50), 23 (control), 18, 16, 21 and 27 for males and 31, 31, 39, 34 and 32 for females. In females, the incidences for mammary fibroadenomas were 34, 34, 36, 29 and 31. Incidences of adenoma, fibroadenomas with atypia and adenocarcinomas also showed no trend with dose. The conclusion is the study did not identify any tumor types with exposure to endosulfan. In females the NOEL was 0.7 mg/kg/day, primarily based on the increased incidence in enlarged kidneys and progressive glomerulonephritis at 3.8 mg/kg/day. The study was acceptable. See Table 6, below for observations.

**Table 6. Non-neoplastic Pathological effects in a 104-Week Dietary Rat Oncogenicity Study<sup>a</sup>**

Observations <sup>b</sup>	Males - Doses (mg/kg/day)					Females - Doses (mg/kg/day)				
	0	0.1	0.3	0.6	2.9	0	0.1	0.4	0.7	3.8
<b>Kidneys</b>										
Enlargement	38	32	39	34	39	10	18	19	17	26**
Percent of animals with enlargement	54	45	55	48	55	14	26	27	24	37
Marked Progressive Glomerulonephrosis <sup>c</sup>	20	18	22	24	30	1	6	6	5	8**
Percent with glomerulonephrosis	29	26	31	34	43	1	8	8	7	11
Number with glomerulonephrosis/total	20/70	18/70	22/70	24/70	30/70	--	--	--	--	--
<b>Blood Vessels<sup>d</sup></b>										
Aneurysms <sup>e</sup>	10	6	14	10	19*	0	1	1	0	0
Percent of animals with aneurysms	14	8	20	14	27	0	1	1	0	0

a - Ruckman, et al., 1989

b - The incidence = # of lesion bearing animals per animals at risk (70/sex/group). This includes the satellite animals.

c - *Marked* progressive glomerulonephrosis Historical Controls from 6 studies that were performed in male Sprague-Dawley rats (50/study: Incidence = 11, 19, 8, 13, 5 and 14; mean = 11.6). No historical control data were presented for females. Glomerulonephrosis was considered a direct cause of death and treatment-related. It was not observed in satellite animals that were terminated at one year.

d - Includes main and satellite rats found dead, killed *in extremis* or at scheduled sacrifice. Aneurysms were not observed in rats that died on study at 1 year and later. Aneurysms, that affected pancreas, mesentery and/or liver, were observed after week 80. The effects were considered to be treatment-related.

e - Aneurysm Historical Controls (6 studies) were performed in male Sprague-Dawley rats (50/study: Incidence = 6, 9, 2, 4, 7, and 2; 50, 50, 45, 55, 50 and 50 kidneys examined, respectively; mean = 5). No historical control data were presented for females.

\*, \*\* - P ≤ 0.05 and 0.01, respectively (1- tailed test) by Fisher Exact Test.

Endosulfan was fed in the diet to male Osborne-Mendel rats at 0, 281 or 562 ppm during weeks 1 to 4; at 350 or 700 ppm week 4 through 11; at 450 or 900 ppm during weeks 11 through 21; and at 600 or 1200 ppm during weeks 21 through 44 (Powers et al., 1978). At 1200 ppm 13/50 male rats died. At week 44, treatment was discontinued for 1 week and then resumed for 4 weeks. In week 54 the doses were reduced to 450 and 900 ppm. Ten weeks later, dosing was discontinued. Week 74, males formerly at 900 ppm were sacrificed and males at 450 ppm were again dosed, before sacrifice at week 82. Females were treated with endosulfan at 0, 89 or 178 ppm from weeks 1 through 4; at 150 or 300 ppm weeks 4 through 11; at 225 or 450 ppm weeks 11 through 21, and at 300 or 600 ppm at weeks 21 through 44. At week 44, treatment was discontinued for 1 week, followed by 4 weeks of dosing. Week 54, doses were reduced to 225 or 450 ppm. Treatment continued to week 78, then animals were observed for 32 weeks prior to sacrifice. Nominal time weighted average doses were 0, 4.08 and 9.52 mg/kg/day in males and 0, 2.23 and 4.45 mg/kg/day in females for 73 - 81 weeks. High mortality was reported in males, apparently the result of nephrotoxicity (chronic inflammation, toxic nephropathy, calcium deposition). At the high dose 52% of the males died by week 54 and 92% died by the end of the study. High mortality was observed for males from the low-dose group, since 94% died. Toxic nephropathy was also reported in females (58% in the high dose, 54% in the low dose); however, the nephrotoxicity did not result in early deaths in females. Testicular atrophy was reported in males (24/47, 51% at high dose, 18/47, 38% at low dose, 3/19, 16% in controls) as well as parathyroid hyperplasia (18/47, 38% at high dose, 19/48, 38% at low dose, 0/19, control). No oncogenic effects were reported. A NOEL was not established in this study. **The study, due to high mortality in males and inconsistent dosing levels for both sexes, was considered to be supplemental.**

At the Joint Meeting on Pesticide Residues (JMPR) a report was presented where rats were fed endosulfan at 0, 10, 30, and 100 mg/kg/day for 104 weeks (WHO, 1984). No individual animal data were available for evaluation (summary only). Survival decreased at 10 and 30 mg/kg/day. At 100

mg/kg/day survival was significantly reduced at 26 weeks. Decreased body weight gain and effects in hematological parameters were observed. At 100 mg/kg/day, males had enlarged kidneys, and histological evidence of renal tubular damage with interstitial nephritis. Hydropic changes were evident in the liver cells. There was no treatment-related increase, or in fact any increased incidence in tumors at any dose, compared to controls. The NOEL was 30 mg/kg/day, based on increased mortality, and enlarged kidneys, renal and liver pathological effects. **Supplemental.**

## 2. Mouse - Dietary

Endosulfan technical was fed in the diet to NMRI Hoe:NMRKf (SPF71) mice (80/sex/dose) at 0, 2, 6 or 18 ppm (Males: 0.28, 0.84 or 2.48 mg/kg/day; Females: 0.32, 0.98 or 2.8 mg/kg/day) for 24 months (Donaubauer, 1988; Hack et al., 1995). Interim sacrifices of 10/sex/group were performed at 12 and 18 months. Males at 2.48 mg/kg/day showed a 17% decrease in body weights. Results in females showed that mortality was increased at 2.8 mg/kg/day (43/60, 72%) when compared with controls (33/60, 55%). Deaths began to occur in males at 45 weeks and in females at 15 weeks. Mortality occurred primarily between weeks 27 and 52 at 2.48 mg/kg/day for males and 2.8 mg/kg/day in females. From weeks 79 through 104, there was no difference among groups for mortality. There was no specific target organ toxicity. There were no clinical signs of neurotoxicity. Bodyweight gain was statistically significantly decreased in males at 2.48 mg/kg/day, however the reduction was only 5% and therefore not considered to be a noteworthy effect. At termination (104 weeks), there was no treatment-related oncogenicity. The most common neoplasm was multicentric lymphosarcoma in both sexes. With an n of 60, that included all those that died or were terminated in the main group, the incidences were 11, 13, 18, and 16 for males and 22, 25, 21 and 15 for females, control group through high dose. In animals that died sporadically in the main group, the incidence of multicentric lymphosarcomas that were the final contributors to death (FCTD), compared to the overall incidence of these tumors was, 11/11, 8/13, 14/18 and 11/16 for males and 11/22, 18/25, 15/21 and 12/15 for females, control group through high dose. Therefore, these tumors did not appear to be associated with treatment throughout the study. Multicentric lymphosarcomas occurred initially at 12 months (FCTD) at 2/10 and 2/10 in control and low dose males and in no other groups for either gender. At 18 months, these tumors occurred at 0, 1, 0, 0, of 10 males and 1, 1, 1, and 0 of 10 females in the controls through high dose groups. No tumor type showed a positive trend with increasing dose in either sex. Therefore, endosulfan was not considered to induce tumors in mice after 104 weeks of dietary treatment. This interpretation was supported by the USEPA review of the same study (USEPA, 2001b), which stated that there were no increases in incidence of any neoplastic lesion that was observed in either sex at any dose. These results were later published in the open literature (Hack et al., 1995). The chronic NOEL was 0.84 (males) and 0.98 (females) mg/kg/day, based on increased mortality in the main group of females at 2.8 mg/kg/day. This study was acceptable.

## 3. Dog - Dietary

Endosulfan was administered orally in capsules to mongrel dogs at 0, 0.075, 0.25 and 0.75 mg/kg/day for 1 year, dosed 6 days a week (Malloy, 1959). The 0.075 mg/kg/day group received 2.5 mg/kg/day for the first 3 days. There were no effects observed at any dose. **Supplemental.**

Endosulfan was fed to Beagle dogs (6/sex/dose) at 0, 3, 10, 30 or 30/45/60 ppm or measured

dosages of 0, 0.22, 0.57, 2.09, and 2.2/3.08/3.7 mg/kg/day for males and 0.19, 0.65, 1.98, and 1.95/2.78/3.57 mg/kg/day for females for 1 year (Brunk, 1989). In the high dose group, dogs were treated for 54 days at 2.2 mg/kg/day in males and 1.95 mg/kg/day in females; for 52 days at 3.08 mg/kg/day in males and 2.78 mg/kg/day in females and 19 - 40 days at 3.7 mg/kg/day in males and 3.57 mg/kg/day in females. One male at 2.09 mg/kg/day was killed *in extremis* on day 126, after 125 treatments. All high dose dogs were sacrificed on days 146 to 147, due to an onset of extreme sensitivity to noise, frightened reactions to optical stimuli and jerky or tonic contractions of the muscles in the chaps (temporal muscles), extremities and face, after the dose was increased to 3.7 mg/kg/day in males and 3.57 mg/kg/day for females. 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 (see Table 7 for major effects). Both sexes showed neurotoxicity (impairment of the reflex excitability and postural reactions), which developed with increasing doses at the high dose level. On the morning of the 136th day, after 135 applications, one female, at the high dose, was found with its fur wet and smeared with excrement. Since the clinical reactions occurred during the time between 3 p.m. and 7 a.m., the dogs in all groups were subsequently treated on a number of days at an earlier hour. It was then possible to observe at various intervals a sudden and violent contraction of the abdominal muscles with contraction of the upper abdomen, and also convulsive movement of the chaps, though not followed by vomiting. These reactions occurred starting 2.5 to 6 hours after treatment. Neurological symptoms, having to do with reflexes, were noted only at termination. Decreased body weights were observed (not significant) in males at 2.09 mg/kg/day (-5%) and 3.7 mg/kg/day (-7%), beginning at week 44 (44th weighing). Both sexes showed a temporary decrease in percent of food consumed at 2.09 mg/kg/day (and greater) for males, and 1.99 mg/kg/day (and greater) for females. The decreases were not statistically significant when compared to controls. The NOEL was 0.57 mg/kg/day for males and 0.65 mg/kg/day for females, based on violent contractions of the upper abdomen and convulsive movement in males at 2.09 mg/kg/day and greater, beginning at 2.5 to 6 hours post-feeding. Body weights for males and food consumption for both genders were decreased at doses of 1.98 mg/kg/day or greater. This study was acceptable.

**Table 7. Major Effects Observed in Dogs After 1 Year of Endosulfan Treatment<sup>a</sup>**

Observations <sup>b</sup>	Males - Doses (mg/kg/day)					Females - Doses (mg/kg/day)				
	0	0.22	0.57	2.09	2.2/3.08/3.7	0	0.19	0.65	1.98	1.95/2.78/3.57
Behavior <sup>c</sup>	0	0	0	0	6**	0	0	0	0	6**
Abdominal Contract. <sup>d</sup>	0	0	0	3	6**	0	0	0	2	6**
Premature Termination <sup>e</sup>	0	0	0	1	6**	0	0	0	0	6** <sup>d</sup>
Decreased Reflexes <sup>f</sup>	0	0	0	1	2	0	0	0	0	4*

a - Brunk, 1989

b - There were 6 dogs/sex/dose.

c - Extreme sensitivity to noise, frightened reactions to optical stimuli and jerky (tonic) contractions of the muscles in the extremities, face and chaps. Some reactions were violent and some animals were unable to drink. Therefore, all animals in this group were terminated early to prevent needless suffering. The effects were initiated when animals were dosed at 3.7 (males) or 3.57 (females) mg/kg/day (day 107).

d - Animals displayed sudden and violent contractions of the abdominal muscles with contraction of the upper abdomen, and convulsive movements of the chaps, not followed by vomiting.

e - Sacrifice took place on days 126 (1 male), 146 (6 females) and 147 (5 males) - male 4298 *in extremis*, all others due to marked and increasing nervous symptoms.

f - Neurological tests were performed at 0, 6 weeks, 3, 6, 9 months and at termination. Reflex reactions were: Cranial Nerve Reflexes (pupillary, blink and corneal reflexes); Segmental Reflexes: (Flexor, patellar, anal and cutaneous reflexes); Postural Reactions (extensor postural thrust reaction, placing reaction (visual & tactile) and righting reactions.) One male showed absent patellar reflex and absent postural reactions (exterior postural thrust reaction, placing reactions (visual and tactile) and righting reaction) at 2.2 (males) or 1.95 (females) mg/kg/day. Two

males

and four females at 3.7 (males) or 3.57 (females) mg/kg/day showed diminished visual and tactile placing reactions at termination.

\* - Statistically different from control at  $p < 0.05$  (Fisher's Exact Test).

\*\* - Statistically different from control at  $p < 0.01$  (Fisher's Exact Test).

## E. GENOTOXICITY

**Summary:** There were inconclusive findings from contradictory results of genotoxicity induced by endosulfan (technical), as measured by the gene mutation, chromosomal aberration and other genotoxic effects tests submitted to DPR. Studies evaluated by DPR in 1986 were evaluated under TSCA Guidelines (TSCA, 1984 & revised Guidelines, 1985). Since then, TSCA Guidelines were incorporated into revised FIFRA Guidelines. Therefore, the acceptability of the studies described below will be denoted as “according to current FIFRA Guidelines” for regulatory purposes under SB950, while studies that are not FIFRA Guideline acceptable or are open literature studies are denoted as “supplemental” (USEPA, 1998). The overall assessment of genotoxic potential of endosulfan shows that tests are both positive and negative in bacterial systems, in micronucleus, rat hepatocyte and Chinese hamster ovary tests. However, mouse bone marrow tests were all positive, as were *in vivo* Syrian hamster and human RBC tests. Other studies performed in human and mouse tissues were equivocal or positive. An *in vitro* test with rat and human fetal liver cells showed that endosulfan formed DNA adducts. Therefore, while endosulfan is mutagenic and clastogenic and it induces effects on cell cycle kinetics in two different mammalian species (rat and mouse), in other systems tests are negative, even within the same species. USEPA considers that some of these test data may be suspect because some of the formulations of endosulfan may have contained epichlorohydrin, a known genotoxic chemical, as a stabilizer (Hoechst, 1990). Tables 14 and 15.

Although there are numerous gene toxicity studies in the published literature, not all were described below. Only the studies that were thorough, and competently reported including acceptable studies and those submitted by the registrant, were selected.

### 1. Gene Mutation

Endosulfan technical (99% pure) was assayed with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 and with *Escherichia coli* WP2 *hcr* strain (no S9) at 0 (DMSO), 5, 10, 50, 100, 500, 1000 or 5000 ug/plate (with and without enzymatic activation--S9), in duplicate (single trial) (Shirasu, et al., 1978). There were no treatment-related increases in gene reversion or mutagenicity with any strain of bacteria. In addition, *Bacillus subtilis* strains M45 (rec -) and H17 (rec+) were tested with endosulfan at 0, 20, 100, 200, 500, 1000 and 2000 ug/disk (no S9) in the rec assay. Endosulfan showed only slight inhibitory zones at 100 ug/disk and higher, with only a slight difference in the lengths in the inhibitory zones of the 2 strains, indicating the result was negative. Supplemental.

Endosulfan technical (97.2% pure) was assayed with *Schizosaccharomyces pombe* at 0 (DMSO), 62.5, 125, 250 or 500 ug/ml for four hours both with and without enzymatic activation (from Aroclor-induced male Sprague-Dawley rat livers). There was no effect in mutation frequency (Mellano, 1984). Supplemental.

Mouse lymphoma L5178Y TK+/- cells were assayed with endosulfan (97.2% pure) at 0 (vehicle = DMSO), 6.25, 12.5, 18.8, 25.0, 37.5, 50, 75 or 100 ug/ml for four hours (with and without enzymatic activation from Aroclor-induced male  $\delta$  rat livers). There was no increase in mutation

frequency (Cifone, 1984a). A forward mutation assay was performed with mouse lymphoma L5178Y TK<sup>+</sup>/- cells, in a study by McGregor, et al. (1988). Endosulfan technical was used (no S9 metabolic activation) at 0, 3.125, 6.25, 12.5, 25 and 50 ug/ml and, in a repeat trial at 9.3, 14, 18.6, and 23.2 and 28 ug/ml (no S9). Both tests were positive at doses of 18.6 ug/ml and higher. **Supplemental.** Differential ampicillin sensitivity to endosulfan (35% pure) was examined in an *Escherichia coli* (*E. coli*) K12 prophage  $\lambda$  induction assay with *E. coli* WP2s ( $\lambda$ ) (Chaudhuri, et al., 1999). The strains used were: repair deficient: AB1886*BuvrA* 6; AB2494*BlexA* 1; AB2463*BrecA* 13 and repair-proficient AB1157. A weak dose-related increase in ampicillin resistance (gene mutation) was observed when endosulfan was used with ampicillin-sensitive *E. coli* AB1157 at 0, 10, 30 and 50 ug/ml. A weak peak prophage  $\lambda$  induction was observed at 200 ug/ml endosulfan in *E. coli* WP2s ( $\lambda$ ) cells after endosulfan treatment at 0, 200, 400 and 600 ug/ml. In this study, induction of *umu* gene expression in *Salmonella typhimurium* TA1535/pSK1002 cells was also examined after treatment with endosulfan at 0, 30, 50, 100, 150 and 500 ug/ml. Gene expression of *umu* was weakly induced in a dose-related manner up to 100 ug/ml. Responses in all assays were positive but weak. **Supplemental.**

## 2. Structural Chromosomal Aberrations

Endosulfan technical was assayed with human lymphocytes from a male volunteer to assess chromosomal aberrations *in vitro* (Milone and Hirsch, 1986). The cells were stimulated with phytohemagglutinin before exposure for 4 hours to 0, 1, 10, 100 and 200 ug/ml endosulfan, both with and without rat liver metabolic activation. Subsequently, after an additional 23 hours of incubation, lymphocytes were scored for chromosomal aberrations and mitotic indices. At 200 ug/ml, endosulfan was toxic to cells. There were no indications of treatment-related chromosomal aberrations. This study was acceptable.

Endosulfan technical was administered by gavage to NMRI mice (5/sex/dose) at 0, 0.2, 1.0 and 5.0 mg/kg (Cifone, 1983). Subsequently, mouse bone marrow was removed after 6 hours and assessed for the induction of micronuclei. There were no increases in micronuclei or changes in polychromatic erythrocyte to normochromatic erythrocyte ratios at any dose. This study was not acceptable under current FIFRA Guidelines. In another micronucleus test, endosulfan technical, at 43.3 mg/kg, was administered in 2 doses (24 hours apart), by gavage, to 4 male Swiss albino mice (Usha Rani, et al., 1980). Results of this study also showed no treatment-related increase in micronuclei. **Supplemental.**

Sheep peripheral lymphocytes from 2 donors were treated *in vitro* with endosulfan at 0 (DMSO),  $4 \times 10^{-7}$ ,  $4 \times 10^{-5}$ ,  $4 \times 10^{-4}$  and  $4 \times 10^{-3}$  M for 48 hours (Kovalkovicova, et al., 2001). Results showed that there was a significant increase in chromosomal aberration frequency (1.5%, 9%, 11%, 11%; at 0,  $4 \times 10^{-5}$ ,  $4 \times 10^{-4}$  and  $4 \times 10^{-3}$ , respectively;  $p < 0.05$ ), and the mitotic index was decreased at  $4 \times 10^{-5}$ ,  $4 \times 10^{-4}$  and  $4 \times 10^{-3}$  ( $p < 0.05$  or 0.01). Authors stated that mutagenicity was induced at higher concentrations than would be “attainable” under agricultural conditions. **Supplemental.**

Cytogenetic tests were performed to assess effects of endosulfan in mouse and rat spermatogonia and spermatocytes (Dikshith and Datta, 1977; Dikshith, et al., 1978). Male rats were gavaged with 0 (peanut oil), 11, 22, 36.6 and 55 mg/kg for 5 days (Dikshith and Datta, 1977). There was no increase in incidence of chromosomal aberrations or mitotic indices in spermatogonial cells or in bone marrow cells. Subsequently, male albino rats were gavaged with endosulfan at 0 (peanut oil) or 11.6 mg/kg/day for 30 days (Dikshith et al., 1978). Results showed after endosulfan treatment, the number of chromosome breaks was less in bone marrow and was absent in spermatogonial cells, compared to

controls (% comparison). Metaphases in both bone marrow cells (11.88 at 11.6 mg/kg/day, 25.45 for control;  $p < 0.001$ ) and spermatogonial cells (8.75 at 11.6 mg/kg/day, 11.81 for control;  $p < 0.05$ ) were significantly decreased. **Supplemental.**

Swiss male mice (8/dose) were gavaged with endosulfan (purity not stated) at 0 (distilled water), 22, 32 and 42 mg/kg/day for 5 days to examine the effect on chromosomal breakage in germ cells (Usha Rani and Reddy, 1986). Then, 60 days post-treatment, the mice were terminated and the testes were dissected out. One hundred spermatocytes were examined per mouse for structural and numerical chromosomal abnormalities at the diakinesis first metaphase stage of meiosis. To assess the significance of differences in the frequency of chromosomal abnormalities between control and treated groups the data were subjected to the Chi-squared test. Administration of endosulfan resulted in increased frequency of chromosomal aberrations and abnormal metaphases in spermatocytes (presumed to have been spermatogonia at the time of treatment) at all doses (Table 8). This effect was not observed in previous studies performed in rats (Dikshith and Datta, 1978). **Supplemental.**

**Table 8. Chromosome aberrations in Mice Induced by Endosulfan**

Effect Observed	Dose of Endosulfan (mg/kg/day)			
	0	22	32	42
# Metaphases Scored	800	800	800	800
# Abnormal Metaphases <sup>a</sup>	96 (12)	106 (13.2)	148 (18.5)	172 (21.5)
# Polyploids	24 (3.0)	30 (3.8)	37 (4.6)*	52 (6.5)**
# Aneuploids(19 II) <sup>b</sup>	3 (0.4)	6 (0.8)*	10 (1.3)*	7 (2.1)**
# Autosomal Equivalents (19 II 1 + 1)	30 (3.8)	31 (3.9)	44 (5.5)*	46 (5.8)*
# Univalents (19 II x+y)	39 (4.9)	36 (4.5)	56 (6.8)**	51 (6.5)**
Translocations	--	3 (0.4)*	--	5 (0.6)*

a - Numbers in parenthesis indicate percentage.

b - II = Bivalents.

\*, \*\* -  $p < 0.05$  and  $0.01$ , respectively.

### 3. Other Genotoxic Effects

Endosulfan technical was assayed with primary hepatocytes from male Fischer 344 rats in an autoradiographic unscheduled DNA synthesis assay at 0 (DMSO), 0.102, 0.255, 0.510, 1.02, 5.10, 10.2, 25.5 or 51.0 ug/ml (3 cultures/dose and 50 cells/culture were analyzed). Toxicity was observed at 51.0 ug/ml. No detectable increase in net grains per nucleus was observed at any concentration (Cifone, 1984b). This study was acceptable.

Human blood lymphocytes were examined from a population of endosulfan sprayers (floriculturists) in order to evaluate sister-chromatid exchanges (SCE) and chromosomal aberrations (Dulout et al, 1985). Blood samples were taken from 36 persons, and 21 individuals exhibited at least 1 symptom of chronic or acute intoxication, such as fatigue, numbness in higher and lower limbs, muscle weakness in legs and arms, pain in higher and lower limbs, leg cramps and abdominal pain. The incidence of SCE's increased with signs of intoxication, whereas the frequencies of chromosomal aberrations did not increase with the observed toxicity. **Supplemental.**

Human lymphocytes from healthy donors were treated with endosulfan at 0.7, 0.8, 0.9 and 1.0 mg/ml, in triplicate, for 2 hours (Jamil et al., 2004). Subsequently determinations of LC<sub>50</sub> using trypan

blue exclusion, and determinations of DNA damage using the comet assay (single cell gel electrophoresis: SCGE) were performed. Results showed an  $LC_{50}$  of 0.73 +/- 0.01 mg/ml. Single cell breaks in DNA were observed in a dose-related manner by SCGE. This study was not acceptable under current FIFRA Guidelines. In a related study by Sobti et al. (1983) human lymphoid cells of the LAZ-007 cell line were incubated with  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$ M endosulfan technical (0.41, 4.1 and 41 ug/ml, respectively), both with and without S9 metabolic activation, to test for cell proliferation, sister chromatid exchange (SCE) and cell cycle traverse inhibition. Cell proliferation decreased with increasing dose from 97 to 66%. SCE was increased in treated cells both with and without S9 at all concentrations. **Supplemental.**

Human liver blastoma cells (HepG2), fetal quail hepatocytes and fetal rat hepatocytes were treated with endosulfan *in vitro* (Dubois et al., 1996). Endosulfan induced the formation of DNA adducts in both fetal rat hepatocytes and HepG2 cells. This activity strongly correlated with high induction of CYP3A gene expression that is linked with induction of mRNA for the formation of P450. These results demonstrate that endosulfan has a genotoxic effect on these cell types, under the test conditions. In contrast, fetal quail hepatocytes had no CYP3A expression and there were no endosulfan-DNA adducts. **Supplemental.**

Analytical grade  $\alpha$ - and  $\beta$ -endosulfan isomers were used on HepG2 cells at concentrations of 0 (DMSO),  $10^{-12}$  to  $10^{-3}$  M (30 cells per dose; 2 experiments), in order to test for genotoxicity via sister chromatid exchange (SCE), micronuclei (MN) and DNA strand breaks (Lu et al., 2000). After 48 hours,  $\beta$ -endosulfan showed increased SCE at  $10^{-7}$  to  $10^{-5}$  M and increased MN at  $5 \times 10^{-5}$  to  $10^{-3}$  M.  $\alpha$ -endosulfan did not induce SCE or MN at these doses. After treating HepG2 cells for 1 hour at the same doses stated above, DNA strand breaks, as assessed by single-cell gel electrophoresis (SGE), were increased for  $\alpha$ -endosulfan at  $2 \times 10^{-4}$  to  $10^{-3}$  M and for  $\beta$ -endosulfan at  $10^{-3}$ M. The results indicate that both isomers of endosulfan are genotoxic under the conditions of this study. **Supplemental.**

To assess genetic damage produced by endosulfan in germ cells of eukaryotic organisms, induction of sex-linked recessive lethals (SLRL) and sex-chromosome loss (SCL) by endosulfan was tested in *Drosophila melanogaster* (Velazquez et al., 1984). Endosulfan (50% a.i./50% kaolin in dispersing + wetting agents), dissolved in DMSO and diluted with 5% sucrose solution, was fed to first instar *Berlin-K* wild type male larvae at 0, 50 and 100 ppm until the flies had grown to adults. For adult treatment, 2-3 day old males were starved for 4 hours then fed the test solution in glass filter feeding units for 48 hours at 0, 150 and 200 ppm. The SLRL Test: 4-5 day old *Berlin-k* males treated as larvae (0, 50 and 100 ppm) and as adults (0, 150 and 200 ppm) were crossed individually with three 3-4 day old *Basc* virgin females for 3 days. The sensitivity of the germ cell stages of the males treated as adults was determined using a 3-2-2 mating scheme (broods), followed by transferring the males to fresh virgin females. The progeny of individual P males were identified so that clusters of lethals could be detected. The SCL test: 3-4 day old *Ring-X* males (treated for 24 hours at 0, 50, 100 and 200 ppm) were mass-mated in bottles to 3-4 day old *y sp* virgin females in a ratio of 2 females per male for 3 days followed by two 2-day successive broods. The F1 offspring were scored and the exceptional phenotypes were noted. Results showed a statistically significant increase in percent lethals (SLRL) in the offspring of males treated at 100 ppm as larvae (# lethals/# chromosomes tested at 0 = 7/4527; 0.15% lethals and at 100 ppm = 10/1270; 0.79%;  $p < 0.05$ ; Kastenbaum and Bowman test). SLRL results in male germ cells exposed to endosulfan for 48 hours showed the number of lethals/number of chromosomes tested (%) were statistically significantly increased ( $p < 0.05$ ; Kastenbaum and Bowman

test) at 200 ppm, in Brood 1 (3 days; 12/1034 (1.16%)), Brood 2 (14/974 (1.44%)), Brood 3 (11/946 (1.16)) and in the total of all broods (37/2954 (1.25%)). SCL results with *Ring-X* adult males, treated at 0, 50, 100 and 200 ppm showed a statistical increase in F1 offspring scored for exceptional phenotypes, or SCL. For the pooled data (3 broods), the chi-square test showed that all doses yielded a similar and significant increase of entire SCL (# XO males at 0 = 26/4416, 0.59%; 50 = 243/23142, 1.05%; 100 = 212/23536, 0.09% and 200 = 50/5858, 0.92%). Partial Y chromosome losses were not detected. There was no dose-related effect. The results suggest a more pronounced clastogenic effect in sperm, since the increase in frequency of XO exceptional offspring was significant in brood 1 at all 3 concentrations tested. Endosulfan was considered in the report to be an efficient mutagen in *Drosophila*. **Supplemental.**

*Bacillus stearothermophilus* was treated *in vitro* with  $\alpha$ -endosulfan ( $\alpha$ -E) at 0, 5, 10 and 15  $\mu$ M or  $\beta$ -endosulfan ( $\beta$ -E) at 2.5, 5, 7.5, 10 and 15  $\mu$ M for 18 - 20 hours to test for growth inhibition due to effects on cell membranes as a possible mechanism of toxicity (Martins et al., 2003). In a second group  $\text{Ca}^{+2}$  (2.5mM) (membrane stabilizer) was added to the same doses of  $\alpha$ -E and  $\beta$ -E to test if endosulfan was acting directly on the membranes. Biophysical studies were also performed with the fluorescent fluidity probe 1,6-diphenyl-1, 3, 5-hexatriene (DPH) to assess the  $\alpha$ -E and  $\beta$ -E at 50 and 100  $\mu$ M on membrane fluidity in *B. stearothermophilus* liposomes. Results showed growth inhibition (measured by lag phase, specific growth rate, cell density in stationary phase) occurred in a dose-related manner at 15  $\mu$ M  $\alpha$ -E and in a dose-related manner at all concentrations of  $\beta$ -E at the stationary phase (none statistically significant). The DPH fluorescence polarization (monitors membrane organization) showed a  $\alpha$ -E and  $\beta$ -E, dose-related decrease in bacterial polar lipid dispersions. All observations in cells or liposomes were decreased or removed when  $\text{Ca}^{+2}$  was added. **Supplemental.**

L'vova (1984) performed a cytogenetics study with endosulfan formulation (Thiodan, purity not stated) by testing it on mouse bone marrow (*in vivo*) at 0.2, 1.0 and 5.0 mg/kg. Bone marrow was analyzed and frequency of aberrant chromosomal frequency (%) was increased ( $p < 0.05$ ) at 1.0 mg/kg (3.16%) and 5.0 mg/kg (2.33%) compared to control (0.83%). The effect, however, was not dose-related. In addition, cultured human peripheral blood lymphocytes were treated with endosulfan at 5.0 and 100  $\mu$ g/ml (100 metaphases investigated). The frequency of aberrant metaphases (%) was not increased in these cells. Subsequently, *Saccharomyces cerevisiae* strains T1 (PG-154) and T2 (PG155) were each treated with endosulfan at 10 and 100  $\mu$ g/ml. There was a significant increase ( $p < 0.05$ ) in mutagenic activity only in T2 (100  $\mu$ g/ml) in the form of percent increase in segregants (2.08%) and crossovers (0.141%) compared to solvent controls (0.24% & 0.039%, respectively). **Supplemental.**

Endosulfan technical was assayed on *Saccharomyces cerevisiae* D4 (diploid strain) in order to screen potential gene conversion at the "Ade 2" and "Trp 5" loci on different D4 chromosomes (Milone and Hirsch, 1984). D4 strain was treated for 4 hours with endosulfan at concentrations of 0 (dimethylsulfoxide), 100, 500, 1000 and 5000  $\mu$ g/ml, with or without rat liver metabolic activation. There was no treatment-related increase in gene conversion, when compared to controls. This study was acceptable. In a related study, *S. cerevisiae* D7 (diploid strain) was treated with technical endosulfan at 0 and 1%  $\mu$ g/ml to test for induction of mitotic crossing-over, mitotic gene conversion and reverse mutation (Yadav et al., 1982). Cultures were assessed at 10, 20 and 30 minutes after treatment. Endosulfan increased the frequencies of gene convertants and revertants at all time points. **Supplemental.**

An isomeric mixture of  $\alpha$ - and  $\beta$ -endosulfan, and endosulfan metabolites (sulfate, lactone, ether, hydroxyether, diol derivatives) at doses of 0.25 to 10  $\mu$ M were assayed for their potential to induce DNA damage in Chinese hamster ovary (CHO) cells and human lymphocytes using the Comet assay (Bajpayee et al., 2006). They were also assayed with *Salmonella typhimurium* strains TA98, TA97a, TA102, TA104 and TA100 (+/- S9 metabolic activation) for mutagenic potential at 1 – 20  $\mu$ g/plate. Results showed all compounds induced statistically significant ( $p < 0.01$ ) dose-related increases in DNA damage in both CHO cells and in human lymphocytes. Endosulfan lactone induced the most DNA damage in CHO cells while endosulfan caused the greatest damage in human lymphocytes. The tested compounds also were mutagenic with the *S. typhimurium* strains ( $p < 0.05$ ), primarily TA98. The diol and the hydroxyether metabolites induced the greatest gene mutation activity. Supplemental.

## F. REPRODUCTIVE TOXICITY

**Summary:** There were no reproductive effects related to treatment observed in the acceptable FIFRA Guideline study performed in rat. However, several studies in the open literature examined the effects of endosulfan on neonatal reproductive tract development, as well as effects on mature male reproductive tract. In all studies examined, there were few effects observed in female rats *in vivo*, even though an *in vitro* study showed that endosulfan has estrogenic potential. Supplementary studies were available in the open literature where the effects of endosulfan on prepubertal and neonatal rats and adults were examined. Results showed that there were effects in testes and they occurred at lower doses in prepubertal and neonatal rats than in adults. Preliminary epidemiology data suggest that endosulfan delays sexual development in pubertal males. Gavage studies performed in rodents also showed decreased sperm counts, altered spermatogenesis, decreased testis metabolism and steroidogenesis after endosulfan treatment. Many studies obtained from the open literature showed direct effects on the male reproductive tract, although these effects did not alter reproductive performance. Effects in the acceptable reproduction study were systemic (liver and kidney) and there were no effects in the reproductive parameters for either sex.

### 1. Rat

#### a) Dietary

Endosulfan was fed in the diet to CrI:COBS(CD)BR rats at 0, 3, 15 or 75 ppm endosulfan for two generations (Edwards, et al., 1984). There were 32 rats/sex/dose for the F0 generation and 28/sex/dose for the F1 generation. Each parental generation was mated twice. The F1a and F2a offspring were terminated of day 21- post partum and for 1 per sex per litter specified organs were weighed and tissues preserved against contingency of histopathological examination. Approximately 10 days after the weaning of the F1a and F2a pups, the F0 and F1b parental generations were remated to produce the F1b and F2b offspring. At 21 days post-partum for the F1b pups, 28 per sex per dose were selected to form the basis of the F1b generation. Based on actual food consumption, treatment was equivalent to 0.20, 1.0, 4.99 mg/kg/day for F0 males, 0.24, 1.23, 6.18 mg/kg/day for F0 females and 0.23, 1.18, 5.72 mg/kg/day for F1b males and 0.26, 1.32, 6.92 mg/kg/day for F1b females, as reported in the study. The NOELs are expressed as mean dosages of F0 and F1b generations for males and females. Developmental endpoints included measurements of neonatal body weights, sex ratio, pup mortality, total litter loss and litter size. There were no developmental markers measured for crown-rump length, skeletal stains, vaginal opening, preputial separation, etc. assessed in this study.

There were no treatment related mortalities or clinical signs of neurotoxicity. The reproductive LOEL was 5.40 mg/kg/day (F0 males) and 6.55 mg/kg/day (F0 females), based on a slight decrease in mean litter weight (1<sup>st</sup> mating) on day 12 (7%) and day 21 (9.7%) and at day 12 of the second mating (12%). The systemic parental NOEL was 1.1 mg/kg/day (males) and 1.30 mg/kg/day (females), based on an increase in relative heart, liver and kidney weights at the high dose, and a decrease in food consumption and body weight gain. Note that while weanlings in the first mating of the F0 generation showed a significant increase in relative pituitary weights in females (21%) at the high dose, the pup pituitary weights from the second F0 mating were unaffected in either sex at any dose. In addition, the uterine weights were significantly increased in the F1b weanlings from the first mating (6.92 mg/kg/day--17%) but it was not observed at the F1b second mating. A dose response was not observed for pituitary or uterine weight effects. The study was acceptable.

### c) Gavage

Adult male Wistar rats (12/dose) were administered endosulfan by gavage for 7 or 15 days at 0 (groundnut oil), 2.5, 5.0, 7.5 or 10 mg/kg/day to investigate gonadal toxicity (Singh and Pandey, 1989b). After 7 or 15 days of treatment, 6/dose were terminated. Testicular cytosolic and microsomal protein (induction of xenobiotic-metabolizing enzymes) were increased 81% and 101%, respectively at 10 mg/kg/day at 7 days, but returned to control levels by day 15. Glutathione S-transferase (GST) was significantly decreased at all doses at 7 days, but then increased in a dose-related manner up to 7.5 mg/kg/day by day 15. At 10 mg/kg/day, GST activity was 79% of control on day 15. The percent 17-hydroxysteroid dehydrogenase (17-HSD) activity was comparable to control at 7 days but was decreased significantly at 10 mg/kg/day by 15 days. The percent 3-HSD activity was not affected in a dose-related manner, although it was statistically significantly increased at 7 days (5 mg/kg/day) and at 15 days (7.5 mg/kg/day). However, this did not appear to be toxicologically relevant. The percent testosterone (T) in the serum was increased at  $\geq 7.5$  mg/kg/day at 7 days and at 2.5 and 10 mg/kg/day by 15 days. Percent T in testis was statistically significantly decreased at 5 and 10 mg/kg/day at 7 days and was increased at 10 mg/kg/day by 15 days. A NOEL was not achieved in this study and it was considered to be supplemental.

Adult male Wistar rats received endosulfan by gavage at 0 (ground nut oil), 7.5 and 10 mg/kg/day (Group A = 15 days; Group B = 30 days; 6/dose/time point) or Group C at 0 and 10 mg/kg/day (6/dose) for 30 days followed by 7 days on normal diet (Singh and Pandey, 1990). Results showed no changes in body weights, testicular wet weights or cytosolic and microsomal protein contents of testis in treated rats. Groups A and B had statistically significantly decreased LH, FSH, NADPH-cytochrome-c reductase at greater than or equal to 7.5 mg/kg/day, but this was reversed in Group C at 10 mg/kg/day. Testosterone, testicular testosterone, 3-hydroxysteroid-dehydrogenase and 17-hydroxysteroid-dehydrogenase were statistically significantly decreased in Group A at 10 mg/kg/day and in Group B at greater than or equal to 7.5 mg/kg/day. Testicular testosterone remained statistically significantly decreased at 10 mg/kg/day in Group C after recovery. Although initially increased in Groups A and B at greater than or equal to 7.5 mg/kg/day, by 30 days cytochrome b<sub>5</sub>, NADH-cytochrome b<sub>5</sub> reductase and glutathione-S-transferase were statistically significantly decreased at greater than or equal to 7.5 mg/kg/day. Endosulfan, therefore, is shown to affect the testis and critical endocrine function of the biosynthesis and secretion of testosterone, the primary androgen **but only at doses that are very high**. A NOEL was not established in this study. **Supplemental**.

## 2. Endocrine Effects in the Rat

## a) Neonatal/Prepubertal Reproductive Organs and Sex Hormones (Gavage)

A study was performed by Sinha et al. (1997) to examine the effect of endosulfan on testicular maturation. Weanling male Druckrey rats (prepubertal sexual maturity at 3 weeks old, 5/dose) were treated at 0 (peanut oil), 2.5, 5.0 or 10.0 mg/kg/day for 90 days (5 days/week) by gavage to investigate the possibility of permanent damage to the gonads. Results showed statistically significantly decreased sperm counts (cauda epididymis), increased sperm abnormality, decreased spermatid counts and decreased daily sperm production, as well as increased LDH, G6PDH and GGT, and decreased SDH, at all doses ( $\geq 2.5$  mg/kg/day). The effects observed in the mature rats (Sinha et al., 1995) were similar to those observed in weanlings; however, in mature rats most occurred at greater than or equal to 5 mg/kg/day, rather than at greater than or equal to 2.5 mg/kg/day as seen in weanlings. In addition, the effects observed in weanlings were dose-related, where they were not in the mature rats. The authors concluded that endosulfan exposure during testicular maturation might result in disturbed spermatogenesis at sexual maturity. The LOEL for weanling rats was 2.5 mg/kg/day. This study was not performed according to FIFRA Guidelines.

In a followup study by Sinha et al. (2001a) mated female Druckrey rats (3/dose) were treated by oral gavage with endosulfan (95.32% pure) at 0 (peanut oil), 1.0 and 2.0 mg/kg/day during gestation days 12 (time when fetal gonad begins to differentiate) through parturition (day 21). Neonates were weighed 5-6 hours after birth and males were weighed and fostered to untreated dams that had given birth earlier that day (in order to avoid lactational exposure to endosulfan). After weaning at 21 days, two males from each of 3 litters per dose (6/dose) were housed together. At 100 days of age (adulthood) the males were sacrificed and examined for caudal epididymal sperm count, testis weights, intratesticular spermatid count, testicular marker enzymes (lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), gamma glutamyl transpeptidase and glucose-6-phosphate dehydrogenase). The seminal vesicles and prostate glands were also weighed. Treatment at both doses caused a reduction of sperm and spermatid counts, and decreases in testes, seminal vesicle and epididymis weights. There was also an increase in LDH and a decrease in SDH activities. The study results suggested that endosulfan might interfere in the process of spermatogenesis. The toxicity was dose-dependent, however there were several deficiencies (very small numbers of animals treated and assessed, the technical material contained almost 5% unknown impurities). **Supplemental.**

Wistar male prepubertal (45 day old) rats were treated by gavage with endosulfan (50%, unknown whether or not it was formulated product) at 0 (peanut oil) and 1.0 mg/kg/day (6/dose) for 30 days (Chitra, et al., 1999). Results at termination showed statistically significant decreases in body, testes, epididymal, ventral prostate, and seminal vesicle weights (all androgen-dependent organs) at 1.0 mg/kg/day. Biochemical parameters showed statistically significant increases in protein and decreases in DNA, RNA, ascorbic acid, lactate, pyruvate, 3- $\beta$ OH-steroid dehydrogenase, acid phosphatase (AP) and alkaline phosphatase (ALP) at 1.0 mg/kg/day. Changes in DNA, RNA and protein suggest a shift in synthetic activity in testis. The decrease in pyruvate, necessary for Sertoli cell function, along with lactate indicates a possible decrease in testicular metabolism. Decreased 3- $\beta$ OH-steroid dehydrogenase indicates a decreased steroidogenesis. Ascorbic acid, AP and ALP decreases in spermatogenic chamber, Leydig cells and semen acts to inhibit oxidative damage to sperm and this correlated with a decrease in testicular steroidogenesis. These findings suggest a possible connection between endosulfan treatment and steroidogenesis inhibition in male rats. Since no individual data were shown, the number of animals per group was small and there was a great deal of variation in

assay results, further studies would need to be performed to substantiate findings. **Supplemental.**

b) Subcutaneous injection to neonatal rats

Endosulfan technical was administered by subcutaneous injection to neonatal rats (7 days *post partum*, 8/sex/dose) at 0 (corn oil), 4.5 and 9.0 mg/kg/day for 15 consecutive days (Ahmad, et al., 1993). After 15 days of treatment, serum testosterone in males, 17 $\beta$ -estradiol (females), body weights and reproductive organ weights of both sexes were measured. Results showed statistically significantly decreased weights of testis, epididymus, vas deferens, prostate, seminal vesicles, ovaries, oviduct and uterus at both 4.5 and 9.0 mg/kg/day. Testosterone and 17 $\beta$ -estradiol were also statistically significantly decreased at 4.5 and 9.0 mg/kg/day. Body weights and mortality were not affected. Clinical signs, however, were not reported in this study. A NOEL was not achieved (i.e. < 4.5 mg/kg/day). **Supplemental.**

c) Gavage in Females During Pregnancy and Lactation, Sexual Development in Males

Endosulfan (97% pure) was administered by gavage to female Wistar rats (8/dose) at 0 (Tween 80 vehicle), 1.5 and 3.0 mg/kg/day from day 15 of gestation through day 22 of lactation (Dalsenter et al., 1999). Subsequently, the reproductive effects of endosulfan on male offspring (15/dose/time period) were investigated on postnatal days 65 and 140, which correspond to the pubertal and adult stages of male rat development (1-2 males/litter randomly selected for assessment). **At puberty (day 65), the absolute (data not included) and relative testes weights were significantly increased at 1.5 mg/kg/day or greater. By 140 days, however, this effect was only observed at 3.0 mg/kg/day. Testis descent and preputial separation occurred earlier in endosulfan-treated pups, compared to controls, but these effects were not statistically significant compared to controls at post-natal days 18 and 36, respectively. No effects were observed on epididymal, relative seminal vesicle or ventral prostate weights. Mean daily sperm production was decreased by 30% at 3.0 mg/kg/day and 21% at 1.5 mg/kg/day (puberty) but was only decreased by 13% at 140 days at 3.0 mg/kg/day (standard deviations were large for the control versus 3.0 mg/kg/day, perhaps overestimating the decrease). This effect at 65 days (3.0 mg/kg/day) corresponded with the decreased percentage of seminiferous tubules showing complete spermatogenesis, observed histopathologically at puberty at 1.5 mg/kg/day and greater. These effects, however, were no longer evident at 140 days at any dose. No treatment-related effects were observed on the developmental parameters or on the reproductive organ weights at any dose.** **Supplemental.**

In a follow up study, mated Wistar females (27/dose) were treated with endosulfan (97% pure) by gavage at 0 (sunflower oil), 0.5 and 1.5 mg/kg/day from 21 days prior to mating, then through mating, pregnancy and lactation (Dalsenter et al., 2003). For statistical parameters, 8 males per dose were used to assess body weight, and relative (to body weight) testis, epididymis, seminal vesicle and prostate weights. Eight litters per dose comprised the unit for assessment of age of testis descent and age of preputial separation. Pregnancy outcome was also 8 litters per dose to assess litter size, live births (%) and birth weight on postnatal day 1 and weaning rate (%) and body weight on postnatal day 21. Male offspring (8/dose) were also selected for assessment of daily sperm production, spermatid number count and sperm transit, sperm morphology, testosterone level on postnatal day (PND) 15, 21, 33, and at termination day 140. Results showed no toxicologically significant effects at any dose on the parameters measured, nor on the treated dams. The NOEL was greater than 1.5 mg/kg/day. **Supplemental.**

#### d) Gavage in Adult Male Rats, Assessment of Reproductive Tract

Endosulfan was administered by gavage to adult male rats at 0, 2.5 or 7.5 mg/kg/day, 6 days/week, for 60 days (Ansari and Gupta, 1981, abstract). No significant body weight changes were reported; however, testes weights were slightly decreased, and liver and kidney weights were significantly increased at 7.5 mg/kg/day. The NOEL was 2.5 mg/kg/day. In an abstract of a follow-up study, males treated with the same doses and dosing regimen showed decreased testes (7.5 mg/kg/day only), coagulating gland, ventral prostate, vas deferens and epididymal weights at 2.5 and 7.5 mg/kg/day (Gupta, et al., 1981). There was also a significant change in protein and RNA but not DNA content in testes. Sperm count in the vas deferens was significantly decreased and their motility was sluggish. Protein levels and alkaline phosphatase activity in the testes were increased. A hormonal imbalance was induced by endosulfan in the gonads. **Supplemental.**

### 3. Mouse - Gavage

Swiss albino male mice (6/dose/treatment) were gavaged with endosulfan at 0 (distilled water) or 3.0 mg/kg/day for 35 days (Khan and Sinha, 1996). Mean sperm counts were significantly decreased by 80% at termination (epididymal suspension). In addition, there was a significant increase in abnormal sperm and abnormal sperm heads in endosulfan-treated mice. When mice were treated concurrently with endosulfan (3.0 mg/kg/day) and vitamin C (in distilled water) at 10, 20, and 40 mg/kg/day, the decrease in sperm count was partially ameliorated. The decreases were 65% at 10 mg/kg/day, 26% at 20 mg/kg/day and 22% at 40 mg/kg/day. Vitamin C also decreased the number of abnormal sperm and sperm with head abnormalities. There was not a NOEL achieved. Supplemental.

### 4. Endocrine Effects on Human Reproductive Systems (Epidemiological Studies)

An epidemiological study was performed to assess potential effects of aerial spraying of endosulfan on sexual maturation in children (Saiyed, et al., 2003). Endosulfan was the only pesticide that had been used (sprayed 2 - 3 times/year for 20 years) on cashew nut plantations located on hilltops in villages in northern Kerala, India. The village school children were exposed to endosulfan via air, water (runoff from irrigation) and soil. Control children (comparable status) were from a village 20 km away without any history of aerial endosulfan spraying. Male children (study n = 117; controls n = 90) aged 10 -19 years were to receive an examination for sexual maturity rating (SMR, pubic hair, testes and penis), a blood test to assess testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and endosulfan residues ( $\alpha$ -,  $\beta$ - and sulfate). Non participation in the SMR was 57% for the study and 33% for controls; however, in the 43% (n = 50) and 76% (n = 68), respectively, that did participate there was a statistically significant decrease in SMR for pubic hair, testicular and penis development with regard to  $R^2$ , intercept ( $b_0$ ), age ( $b_1$ ) and aerial exposure to endosulfan (AEE  $b_3$ : Score =  $b_0 + b_{1age} + b_{2AEE}$ ). The study males with tested blood samples (n = 67) had lower than expected testosterone levels, considering age and LH in blood, compared to the control males (n = 46). In fact, the main study males had higher than expected LH levels, when compared to controls. Endosulfan residues  $\alpha$ -,  $\beta$ - and sulfate individually, as well as total endosulfan, were all statistically significantly increased in the study males (n = 70), compared to controls (n = 45). The authors concluded that a follow-up should be performed on the children to understand the implications of the findings, in addition to performing a study with a larger sample size to validate the study findings. However, it appears that endosulfan exposure may delay sexual maturity and interfere with hormone

synthesis in male children. **Supplemental.**

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). The adult women of reproductive age (149, mean age 44 years, range 33-57) had adipose tissue removed during surgery for different reasons. Placentas and cord blood were sampled from 200 women at term deliveries (mean age = 29 years, range 17-43 years). Breast milk from 23 healthy women (age 17-23 years; chosen at random from the 200 women donating placentas) was sampled. Results of adipose tissue assessments showed that endosulfan ether was the most frequently found residue (49.6% of samples) where endosulfan-sulfate was the most abundant (mean = 16.16 ng/g fat). In placenta, endosulfan sulfate was the most frequently detected residue (67.5%), and endosulfan lactone was in greatest abundance (15.62 ng/g fat). In cord blood, endosulfan diol was the most frequently detected (81% of samples), and endosulfan diol was most abundant (13.23 ng/g fat). In milk, endosulfan ether was found in 100% of samples, and  $\beta$ -endosulfan was most abundant (10.70 ng/g fat). 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). **Supplemental.**

### 5. *In vitro* Effects on Steroidogenesis and Spermatogenesis

Technical endosulfan, at 0.1 nM (0.041 ng/ml or 0.041 ppb) can inhibit the acrosome reaction (AR), which is essential to fertilization (Turner et al., 1997). The reaction, initiated *in vitro* in human sperm by progesterone or glycine, activates sperm GABA<sub>A</sub> receptor/chloride channels. The reaction can then be blocked by endosulfan. Endosulfan functions in insects to block neuronal GABA<sub>A</sub> receptor/ chloride channels. Results in this study were of interest in regard to endosulfan as an endocrine disruptor, due to the low doses of endosulfan at which the AR inhibition occurred. **Supplemental.**

Primary cultures of Sertoli and germ cells were obtained from 28 day old rats and were treated *in vitro* with 0, 2, 20, 40 and 80 uM endosulfan for 24 and 48 hours to assess aldose reductase and sorbitol dehydrogenase activity (Sinha, et al., 2001b). There was a dose-related increase in aldose reductase activity at  $\geq 20$  mM endosulfan after both 24 and 48 hours of treatment. Duration-dependent increases were observed only at the higher doses (40 and 80 uM). Sorbitol dehydrogenase showed a dose-dependent decrease at  $\geq 20$  uM endosulfan after 24 hours of exposure. In order for Sertoli and germ cells to interact for sperm production, the aldose reductase and sorbitol dehydrogenase enzymes must function normally. The trend was similar at 48 hours, but without a duration-dependent decrease in enzyme activity. Sertoli cell-germ cell interaction is critical for the maturation of male germ cells through seminiferous epithelium to the release of mature spermatozoon into the seminiferous tubules. Aldose reductase in Sertoli cells reduces aldols to polyols (e.g. glucose to sorbitol), which are taken up by germ cells and converted to fructose and other ketoses by sorbitol dehydrogenase. It appears that endosulfan interferes with this process. As testicular atrophy occurs, aldose reductase increases (overcompensating), resulting in a change in the metabolism of glucose to inositol, necessary for germ cells during spermatogenesis. Sorbitol dehydrogenase (a marker for germ cells) increases with the maturation of testis. This is because of enhanced fructose production for energy required by the testis (increases conversion of sorbitol to fructose). **Supplemental.**

## 6. *In Vivo* Effects on Steroidogenesis and Spermatogenesis

Endosulfan was administered to adult male Wistar rats (6/group) in 6 Groups (Zhu, et al., 2002). Groups 1 and 4 received 0, 2.5, 5.0 and 7.5 mg/kg/day, 6 times per week for 10 weeks. Groups 5 and 6 received i.p. injections of vitamin C at 20 and 40 mg/kg/day with 7.5 mg/kg/day of endosulfan. Daily sperm production (DSP), sperm count and morphology were studied after the treatments. Lipid peroxidation product (LPO) and 8-hydroxy-2'-deoxyguanosine (8-OhdG) in serum, liver and testis homogenates were determined with enzyme-linked immunosorbent assay (ELISA). DSP and epididymal sperm count were decreased and the number of abnormal sperm was increased significantly in all endosulfan treated groups. LPO and 8-OhdG in serum, liver and testis homogenates increased significantly in all endosulfan-treated groups. Vitamin C (antioxidant) administration protected against the endosulfan induced sperm toxicity and oxidative damage to liver and testes. Authors concluded that oxidative damage may be a large factor in the mechanism of endosulfan reproductive toxicities at the high doses used in this study. **Supplemental.**

Male rats were treated with endosulfan at 10 mg/kg/day, by gavage, for 60 days (Srivastava, et al., 1991). Results showed a significant decrease in hyaluronidase and acid phosphatase in the testes. Testicular seminiferous tubule degeneration, decreased epididymal tubular diameter in the caput region, marked reduction in tubular diameter and prominent intertubular connective tissue were also observed in the cauda region. This was only an abstract, and no data were presented. **Supplemental.**

Wilson and LeBlanc (1998) showed that mice treated with 7.5 mg/kg/day endosulfan for 7 days had an increase in  $16\beta$ ,  $6\alpha$ - and  $16\beta$ -hydroxytestosterone metabolites (females only). There was a 3.3-fold increase in hydroxylation of testosterone in the  $16\beta$ - position. There was also a 3.6-fold increase in the rate of urinary elimination of [ $^{14}\text{C}$ ]-androgen. The increase in androgen clearance was associated only with a small (not statistically significant) decrease in serum testosterone levels. Testosterone biotransformation from endosulfan exposure can result in increased elimination but homeostatic processes compensate for the effect and minimize consequences on serum hormone levels. **Supplemental.**

Endosulfan was administered by gavage to mated female Druckrey rats at 0 (peanut oil), 1.0 and 2.0 mg/kg/day from gestation day 12 (time when fetal gonad begins to differentiate) through parturition day 21, for a total of 9 treatments (Sinha, et al., 2001a). After birth, male pups were removed and foster nursed, to prevent further endosulfan exposure. At 100 days of age, male offspring were terminated and their epididymides and testes were removed. The following parameters were assessed on 6 males/dose: intratesticular spermatid count, lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH),  $\gamma$  -glutamyl transpeptidase (GGT), and glucose-6-phosphate dehydrogenase ( $\text{G}_6\text{PDH}$ ). Seminal vesicles and prostates were dissected out and weighed. Results showed a statistically significantly decreased testes, seminal vesicle and epididymal weights ( $p < 0.05$ ) at  $\geq 1.0$  mg/kg/day, with no increase in prostate weights. Sperm counts were statistically significantly decreased at 1.0 (53%) and 2.0 (62%) mg/kg/day, as were spermatid counts at 1.0 (37%) and 2.0 (51%) mg/kg/day ( $p < 0.05$ ). LDH was statistically significantly increased and SDH was decreased in a dose-related manner at 1.0 mg/kg/day and greater. These enzymes are markers of post-meiotic spermatogenic cell functions and the reversal in the pattern of activity suggests that endosulfan interferes with the process of spermatogenesis by changing the germinal epithelial cell function. Decrease in weights of epididymides and seminal vesicles in exposed groups may be due to interference in the process of differentiation and development of these organs. The decrease in

spermatids was related to the decreased number of sperm in the epididymis. Leydig and sertoli cell functions were not affected, as indicated by lack of effects to GGT and G<sub>6</sub>PDH, respectively. There was not a NOEL achieved in this study; however, the LOEL was 1.0 mg/kg/day. Muroño et al. (2001) confirmed that endosulfan had no effect on Leydig cells *in vitro*. **Supplemental.**

### 7. Estrogenicity in *In Vivo/In Vitro* Assays

Technical endosulfan (98% pure) showed no evidence of estrogenicity in three assays: (1) competitive binding with the mouse uterine receptor, (2) transcriptional activation in HeLa cells transfected with plasmids containing an estrogen receptor as a responsive element, and (3) the uterotrophic assay in mouse (Shelby et al., 1996). The first two assays were *in vitro* assays, and the third assay was *in vivo*. Immature mice were injected subcutaneously with endosulfan for three consecutive days. Uterine weights were determined on the fourth day. **Supplemental.**

Endosulfan was used on mouse mammary gland organ cultures (MMOC) *in vitro* to test for the ability to form nodule-like alveolar lesions (NLAL) that would demonstrate an estrogenic action (Je et al., 2005). Results showed no increases in NLAL in the endosulfan-treated mammary glands, however there were more alveolar buds induced than were observed in the control (vehicle) group. In addition, telomerase reverse transcriptase (TERT) mRNA expression levels showed a dose-related increase in activity when endosulfan was used on MCF-7 cells. MCF-7 cells are human mammary tumor cells that are estrogen receptor-positive and endosulfan was able to increase up-regulation of the TERT mRNA expression. Transient expression assays using reporter plasmids with fragments of the TERT promoter showed that this palindromic estrogen-responsive element might be responsible for the transcriptional activation by endosulfan. Despite these positive observations, there have been no endosulfan related effects in females in *in vivo* studies. **Supplemental.**

### 8. Physiological Compensation in Mammalian Females

Endosulfan technical (98% pure) was administered by gavage to female hemicastrated Swiss albino mice (10/dose) at 1.5, 3, 6 and 9.0 mg/kg/day for 15 days from the day of operation (Hiremath and Kaliwal, 2002). Sham operated and hemicastrated mice were treated with an equivalent volume of olive oil served as control groups. Results showed a statistically significant decrease in absolute and relative ovarian weights at 3 mg/kg/day and greater, a decrease in duration of cycle at 6 mg/kg/day and greater, a decrease in healthy ovarian follicles/ovary and an increase in atretic follicles/ovary at 3 mg/kg/day and greater in hemicastrated mice treated with endosulfan. These effects indicate an interruption of the hypothalamic-pituitary-gonadal axis. There were no effects on mortality, body weight, uterus, kidneys, liver, adrenals, thymus or thyroid after endosulfan treatment for 15 days. Clinical signs, however, were not described in this study. The NOEL was 1.5 mg/kg/day based on effects to reproductive organs at 3.0 mg/kg/day and greater. **Supplemental.**

In a subsequent study, by Hiremath and Kaliwal (2003), 5 groups of female Swiss albino mice (10/group) were treated by gavage as follows: Sham ovariectomized (sham OVX) + olive oil, OVX + olive oil; OVX + 4 mg/kg/day endosulfan (ES), OVX + 17 $\beta$ -estradiol (5 ug) and OVX + 17 $\beta$ -estradiol (5 ug) + ES (4 mg/kg/day) for 30 days. Results showed that there were no endosulfan-related effects on duration of vaginal cornification, estrus, diestrus, body weight change, relative uterine or liver weights and uterine protein, glycogen or total lipids. The authors concluded that there was no estrogenic or antiestrogenic activity by endosulfan observed. The NOEL was greater than 4

mg/kg/day, according to the parameters measured. **Supplemental.**

Wade et al. (1997) tested endosulfan in the rat uterotrophic assay which involves measuring uterine weights, peroxidase activity, progesterone and estrogen receptor levels; pituitary weights, thyroxine and pituitary hormone levels and stimulation of MCF-7 (estrogen-dependent) cells *in vitro*. There was no increase in uterine weights, peroxidase activity or progesterone and/or estrogen receptor levels after endosulfan treatment in rats. There was also no increase in circulating thyroxine levels, gross pituitary weight or the pituitary content of GH, FSH, LH or TSH. There was no *in vivo* evidence that endosulfan was an endocrine disruptor in female rats. **Supplemental.**

Ovariectomized rats treated at 0 or 1.5 mg/kg/day endosulfan for 30 days showed no effects on uterine, cervical, vaginal or pituitary weights (Raizada, et al., 1981). However, after treatment with 0 or 1.5 mg/kg/day endosulfan in addition to estradiol dipropionate (dose not stated) for 30 days, significant increases in uterine, cervical, vaginal and pituitary weights were observed. Glycogen in the uterus, cervix and vagina was significantly increased. This was an abstract (no data included). **Supplemental.**

## G. DEVELOPMENTAL TOXICITY

**Summary:** In acceptable FIFRA Guideline developmental studies, the rabbit had no fetal effects, only maternal neurotoxicity and death at greater than 0.7 mg/kg/day. Rats, however, had decreased fetal weights and percent of live fetuses at greater than 2.0 mg/kg/day. Maternal toxicity occurred at the same doses that fetal effects were observed. In a dermal developmental study, fetuses showed exencephaly at excessively toxic doses of 450 to 1000 mg/kg/day. In addition to the developmental studies summarized below, a dietary developmental neurotoxicity study in rat was reviewed (Gilmore et al., 2006, Sheets, and Hoss, 2006) and is summarized in H. NEUROTOXICITY. Of developmental importance is the finding of decreased body weight in neonates and pups and a delay in preputial separation in males at 10.8 mg/kg/day and greater. Dams had decreased body weight and food consumption at 3.74 mg/kg/day and greater. At the low dose, however the body weight decrease in dams was only 5 - 6% (GD 13 and 20 only) and food consumption was decreased 12% on GD 6 and 13 only.

### 1. Rat

#### a) Gavage

Initially a range-finding study was performed where pregnant CD Sprague-Dawley rats (6/dose) were gavaged with endosulfan at 0 (corn oil), 1.25, 2.5, 5.0, 10, 20 or 40 mg/kg/day during days 6 - 19 of gestation (Fung, 1980a). All animals died at 20 and 40 mg/kg/day and 4/6 died at 10 mg/kg/day. Clinical signs, observed at 2.5 to 40 mg/kg/day, were salivation, piloerection, hyperactivity, head-rubbing, hostility, spasticity, tremors and convulsions. Body weight gain was decreased at all dose levels. In the definitive study, Sprague Dawley rats were treated with endosulfan (97.3% pure) by gavage at 0 (corn oil), 0.66, 2.0 or 6.0 mg/kg/day during gestation days (GD) 6-19 (Fung, 1980b). Clinical signs were rough coat, lethargy, flaccidity, hyperactivity and face rubbing, observed primarily at 6.0 mg/kg/day. The maternal NOEL of 2 mg/kg/day was based on significantly decreased mean body weight change (GD 0 to 20; -33%; corrected = -40%), decreased absolute body weight (GD 20 = -13%; corrected = -13%) and increased clinical signs such as face rubbing (20/28)

and lethargy (2/28) at 6 mg/kg/day (Table 9). While there was a 14% decrease in body weight gain (corrected) on GD 20 at 2.0 mg/kg/day, this effect has no toxicological significance because the corrected body weight gain is derived from at least three calculations where there is ample room for error. Additionally, there were no other statistically significant effects that were noteworthy at this dose, so this effect was not considered to be sufficient to establish a NOEL lower than 2.0 mg/kg/day. The developmental NOEL was 2 mg/kg/day, based on decreased mean fetal weights (8%), and increased growth retardation and developmental skeletal anomalies (sternebrae: small #4 and unossified) at 6.0 mg/kg/day. While misaligned sternebrae number 4 was statistically significantly increased at 0.66 and 2.0 mg/kg/day (Table 9), it was not at 6.0 mg/kg/day. At 2.0 mg/kg/day, there was a statistically significant increase in number and percent of resorptions, as well as a decreased percent in live fetuses. This increase was represented by 2/25 litters at 2.0 mg/kg/day with 6 early resorptions/litter (28.6 and 33.3%) and was not representative of overall resorptions at this dose. Resorptions were not increased at 6.0 mg/kg/day, compared to controls. When tested statistically by Kendall's Tau B correlation coefficients (Hollander and Wolfe, 1973) there was not a dose-related response from 2.0 to 6.0 mg/kg/day. In addition, the observations at 2.0 mg/kg/day were within the historical controls for percent live fetuses ( $95.5 \pm 7.5\%$ ), number of resorbed fetuses/litter ( $0.6 \pm 1.04$ ) and percent-resorbed fetuses ( $4.6 \pm 7.5$ ). Therefore since these observations were concentrated within 2 litters and were also within historical control values, they were not considered to be treatment-related effects. At 6.0 mg/kg/day, however, fetuses showed a significant decrease in both body weight and length. These two measurements are fundamental measurements of fetal development stage and are therefore considered to be significant effects (Hughes and Tanner, 1970a,b). No malformations were reported. The definitive study was acceptable.

**Table 9. Developmental Effects Observed in Fetal Rats<sup>a</sup>**

Observations	Treatment Level (mg/kg/day)			
	0	0.66	2.0	6.0
<b>DAM EFFECTS</b>				
Number Dams on Study at Initiation of Dosing	30	25	25	35
Number of Dams on Study Day 20 of Gestation	29	25	25	28
Number of Dams with Implants	28	23	25	27
Number of Litters with Live Fetuses	28	23	25	27
Number of Deaths	1	0	0	7
Mean Weight (g) Gravid Uterine (# Weighed)	85 (28)	85 (23)	86 (25)	78 (27)
Mean GD 20 Body Weight (g) <sup>b</sup> (% decrease)	428	419	416	376** (-12%) <sup>e, f</sup>
Mean Weight Gain (g) – GD 0 to 20 <sup>b</sup> (% decrease)	160	155	151	108** (-33%) <sup>f</sup>
Corrected Body Weight (g) -- GD 20 <sup>c, b</sup> (% decrease)	343	335	330* (-1%) <sup>e, f</sup>	298** <sup>c</sup> (-13%) <sup>e, f</sup>
Corrected Body Weight Gain (g) --GD 20 <sup>d</sup>	75	70	64* (-14%) <sup>f</sup>	30* (-40%) <sup>f</sup>
<b>FETAL EFFECTS:</b>				
Percent Live Fetuses	97.2	96.4	91.0*	97.2
Number of Resorbed Fetuses per Litter	0.4	0.5	1.4*	0.3
Percent Resorbed Fetuses	2.8	5.2	8.5*	2.2
Mean Fetal Weight	3.8	4.0	3.9	3.5**
Mean Fetal Length (cm)	3.8	3.9	3.9	3.7*
<b>Number of Litters with DEVELOPMENTAL ABNORMALITIES:</b>				
Small 4th Sternebrae (% litters affected)	10 (45.5)	11 (50)	5 (20)	22 (84.6)*
Unossified 5th Sternebrae (% litters affected)	9 (41)	12 (54.5)	10 (42)	22 (84.6)**
Misaligned Sternebrae # 4 (% litters affected)	0	8 (36.4)*	8 (33)*	7 (27)*

\*, \*\* - Significantly different from control at  $p < 0.05$ ,  $0.01$ , respectively.

a - Fung, 1980b

b – Mean weights (grams) were calculated only for dams that were pregnant at C-section on GD 20.

c – Weight on GD 20 minus gravid uterine weight.

d – (GD 20 body weight) - (gravid uterine weight)

e – Parentheses = % decrease in body weights or % decrease in body weight gain.

f – Percent decrease of body weights were calculated using the mean body weights only for dams pregnant at C-section.

Endosulfan was administered by gavage to mated female albino rats at 0, 5 or 10 mg/kg/day during days 6-14 of gestation (Gupta et al., 1978). No body weight effects were reported for dams or fetuses. Dam mortality was increased at both doses and was significant at 10 mg/kg/day (5/32 dams died = 15.6%). Percent of litters with resorption sites was significantly increased with 5.5% of control, 20% at 5 and 22.8% at 10 mg/kg/day. The maternal LOEL was 5 mg/kg/day based on increased mortality and increased resorption sites. The developmental LOEL was 5 mg/kg/day, based on a significant increase in percent of fetal skeletal abnormalities at both treatment levels. This study was supplemental.

Endosulfan was administered via gavage to pregnant female Wistar rats (10/dose) at 0 (corn oil), 0.5, 1.0 or 2.5 mg/kg/day throughout the entire gestation period through postnatal day (PND) 28 in order to investigate the effects of endosulfan treatment on pups (Zhu, et al., 2000). Maternal decreased body weight gain and death (4/10) were observed at 2.5 mg/kg/day. Litter size and sex ratio were not affected, although birth weights and crown-to-rump ratios were somewhat decreased at birth, but later disappeared. Anogenital distances of males (measured days 1, 28 and 90, respectively) were not significantly decreased. There was no cryptorchidism or hypospadias in any male offspring. Apoptosis in testis germ cell was examined on PND 28 and was not significantly different and there

were no obvious histological changes in these organs. Daily sperm production, epididymal sperm count and morphology as well as male fertility were not significantly changed. Results further indicate that endosulfan does not induce endocrine disruption on male offspring of rats after this extended duration of treatment. Supplemental.

b) Dermal

Thionex was applied to clipped skin of pregnant Charles River-CD rats at 0, 450, 670, 1000, 1500 and 2250 mg/kg/day (5/group; non-occluded) from day 12 - 20 of gestation (Dashiell, 1973). The purpose of the study was to determine the approximate lethal dose (ALD) of endosulfan for pregnant rats as well its embryotoxic and teratogenic potential. Results showed increased mortality to dams, but not fetuses at 1500 mg/kg/day. Exencephaly was found in 5 and 3 fetuses from litters of dams treated at 670 and 1000 mg/kg/day, respectively. It was not stated whether or not effects were observed at 450 mg/kg/day. Results were in abstract form and the study was supplemental.

## 2. Rabbit

a) Gavage

In an oral gavage rangefinding study, pregnant New Zealand White rabbits were treated at 0, 0.5, 1.0 and 2.0 mg/kg/day with 0.5 ml/kg corn oil or 0 (corn oil), 0.625, 1.25, 2.5, 5.0, 10, 20, 40 and 80 mg/kg/day with 2.0 mg/kg/day corn oil during days 6-18 of gestation (Fung, 1981a). All rabbits at 5 mg/kg/day and greater, died in 20 minutes to 10 days after initial treatment on gestation day 6 and 2/6 died at 2 mg/kg/day. Clinical signs of neurotoxicity (hyperactivity, opisthotonos, convulsions and paralysis) were also observed from day 8 of gestation. The NOEL was 1 mg/kg/day, based on deaths and neurotoxic signs. Supplemental.

In a second rangefinding study, endosulfan was administered by gavage to mated New Zealand White rabbits (3 - 10/dose) at 0 (corn oil), 1, 2, 4, 8 or 12 mg/kg/day during days 6 - 28 of gestation (Fung, 1981b). All rabbits died at 8 mg/kg/day or greater, and 4 of 8 died at 4 mg/kg/day. However, two deaths at 4 mg/kg/day may have been due to errors in gavage treatment. Clinical neurotoxicity (hyperactivity, opisthotonos, convulsions and paralysis) was reported at 2 mg/kg/day or greater throughout the treatment period. The NOEL was 1.0 mg/kg/day, based on the deaths and neurotoxicity. Supplemental.

In a definitive study, mated New Zealand White rabbits (20/dose) were gavaged with endosulfan at 0 (corn oil), 0.3, 0.7 or 1.8 mg/kg/day during days 6 - 28 of gestation (Nye, 1981). At 1.8 mg/kg/day an additional 6 dams were added (total = 26 dams) due to an unexpectedly high mortality (Table 10). The maternal NOEL was 0.7 mg/kg/day based on increased mortality (4/20 dams died; one/day on days 7, 10, 21 & 29) and on clinical signs which occurred during treatment: convulsions/thrashing (3/26), noisy/rapid breathing (2/26), hyperactivity (1/26), salivation (1/26), and nasal discharge (3/26) at 1.8 mg/kg/day (Table 11). Deaths occurred at 1.8 mg/kg/day, beginning day 7 (4/26, not statistically significant). Clinical signs began on day 6 at 1.8 mg/kg/day (thrashing, phonation, coughing, cyanotic), where they began on day 18 in control (congestion/nasal congestion, 2/20) and day 14 at 0.7 mg/kg/day (nasal congestion; 2/20). There was no developmental toxicity at any dose. The study was acceptable.

**Table 10. Maternal Toxicity in Rabbits After Endosulfan Treatment <sup>a</sup>**

Observations <sup>b</sup>	Treatment Level (mg/kg/day)			
	0	0.3	0.7	1.8
<b>DAM EFFECTS:</b>				
Deaths <sup>b</sup>	0/20	0/20	0/20	4/26 <sup>c</sup> (1/26)
Clinical Observations <sup>d</sup>	0/20	0/20	0/20	10/26*
Total Litter Resorption	0/20	0/20	0/20	1/26
Hyperactivity	0/20	0/20	0/20	11/26**

a - Nye, 1981

b - Some deaths were due to misdosing (3 at 1.8 mg/kg/day); actual number of deaths = 1/26 at 1.8 mg/kg/day.

c - Six rabbits were added to this group due to deaths (total #dams = 26), two weeks after test initiation.

d - See Table 11 for clinical observations.

\*, \*\* - Statistically significantly increased compared to control at  $p < 0.05$  &  $0.01$ , respectively (Fisher's Exact Test).

**Table 11. Rabbit Dam Toxicity in a Developmental Study, After Treatment Gestation Days 6-28<sup>a</sup>**

Animal Number	Day of Gestation	Observations
<b>CONTROL</b>		
5	25 - 28	Nasal Congestion
8	18	Congestion
<b>0.3 mg/kg/day</b>		
--	--	No clinical signs occurred at this dose
<b>0.7 mg/kg/day</b>		
44	14 - 24	Nasal Congestion
52	19 - 22	Nasal Congestion
<b>1.8 mg/kg/day</b>		
64	24	Noisy breathing
66	10	Hyperactivity, tonic convulsions
67 (misdose)	7	Lying prone after dosing, died
70	24 - 29	Bloody nasal discharge
73	10	Clear nasal discharge
74 (misdose)	10	Clonic & tonic convulsions, rapid breathing, nictitating eyes, excessive salivation, died
76	6	Thrashing, phonation, coughing, cyanotic
82 (misdose)	21	Died on test
85	12 - 28	Nasal congestion
86	29	Died on test

a - Nye, 1981

NOTE: There were 20/group, except at 1.8 mg/kg/day where there were 26.

## H. NEUROTOXICITY

**Summary:** The primary target of endosulfan is the central nervous system, as was observed in numerous studies, primarily in the rat. Endosulfan is a strong neurotoxin in lower animals (rats, dog, mice, cow, cat, pig and lamb) as well as humans (see Illness Reports as well as studies throughout the Toxicology Profile) but it does not induce delayed neurotoxicity in hens.

The acute gavage neurotoxicity study in rat showed a systemic NOEL of 12.5 mg/kg for males and 1.5 mg/kg for females, based on an increase in clinical signs (mortality, tonic-clonic convulsions, coarse tremor, uncoordinated gait, increased salivation, stupor, prone position, increased fright reaction, squatting posture, stilted gait, irregular respiration, straddled hind limbs, decreased spontaneous activity, panting, bristled coat, flanks drawn in and narrowed palpebral fissure) in males at 25 mg/kg and greater and in females at 3 mg/kg and greater, lasting for 1 day. This difference between the sexes was also observed in the subchronic dietary neurotoxicity study in rats where the systemic NOELs were 37.2 mg/kg/day (HDT) for males and 16.6 mg/kg/day for females. The neurotoxicity parameters showed no treatment-related effects on FOB or motor activity in either sex at any dose. Other studies showed endosulfan interacts directly in the central nervous system to affect monoaminergic systems in different parts of the brain. This, in turn, affects memory and the learning operant paradigm. Immature (prepubescent) rats appear to be more susceptible to the effects of endosulfan than adults. Endosulfan decreased sleeping time induced by chlorpromazine and was also shown in 3 studies to induce kindling, a model of secondary generalized epilepsy from repeated, low intensity electrical stimulation of limbic foci in the brain. Endosulfan also was shown to inhibit a noncompetitive blocker site for the GABA<sub>A</sub> receptor in rat. Most of the open literature studies reported below were performed at toxic doses to examine specific effects in the nervous system, primarily brain, therefore NOELs and LOELs were not achieved. Endosulfan is a chlorine channel blocker in the CNS, and shows no direct effect on brain cholinesterase in rats. There was a decrease in serum ChE in female rats at toxic doses (50% and 49% at 13.7 and 37.3 mg/kg/day, respectively) but RBC and brain ChE remain unaffected (males also unaffected). These apparent effects on ChE are inconsistent, occur only at high doses and are likely secondary to systemic toxicity.

## 1. Hen

### a) Gavage

Domestic hens were administered endosulfan by gavage at 0 (10 hens) or 96 mg/kg/day endosulfan plus atropine and 2-PAM (40 hens) (Roberts and Phillips, 1983). After 21 days the birds were dosed again at the same dose. TOCP was used as the positive control (10 hens). Histopathological effects were performed on all the controls and on 9 of 17 surviving hens. No compound-related delayed neuropathological effects were observed. This study was acceptable.

## 2. Rat

### a) Gavage - Neonatal or Prepubescent Treatment

Effect of endosulfan on the concentrations of neurotransmitters in various regions of the Wistar rat brain was examined (Lakshmana and Raju, 1994). Weanling rats (6/dose/sacrifice time) were treated by gavage with endosulfan technical at 0 (peanut oil) and 6 mg/kg/day during post-natal days 2 - 25. Rat pups were sacrificed on day 10 and day 25. The effects on noradrenaline (NA), dopamine

(DA) and serotonin (5-HT) were assessed in olfactory bulb (OB), hippocampus (HI), visual cortex (VC), brainstem (BS) and cerebellum (CB) on days 10 and 25. Performance in operant conditioning for solid food reward was assessed in 25 day-old rats. The activity of acetylcholinesterase (ChE) was also estimated in the same parts of the brain. Results at 10 days showed increased NA in OB (12%  $p < 0.01$ ) and BS (10%  $< 0.05$ ) and in HI (20%  $p < 0.001$ ) and CB (12%  $p < 0.05$ ) at 25 days of age. DA was decreased in HI at both 10 (42%  $p < 0.001$ ) and 25 (45%  $p < 0.001$ ) days. Serotonin was increased in OB (12%  $p < 0.05$ ), HI (41%  $p < 0.001$ ), VC (30%  $p < 0.01$ ) and BS (15%  $p < 0.01$ ) at 10 days of age but at 25 days, levels were decreased in BS (20%  $P < 0.05$ ) and CB (31%  $p < 0.01$ ). There were no treatment-related effects on ChE. Data show that monoaminergic systems in developing rat brain are affected by endosulfan treatment (in the absence in body or brain weight effects). Chronic endosulfan ingestion also affected performance in the operant learning paradigm. Treated rats took significantly more time learning a task than controls. Even after learning a task, endosulfan treated rats were less able to retain the acquired task than controls. **Supplemental.**

“Immature” male rats ( $n = 10 - 12$ ; 60 - 70 g) were treated with 0 (water + tragacanth powder) and 2 mg/kg/day endosulfan by gavage for 90 days (Paul et al., 1994b). This treatment resulted in an inhibition of pole-climbing escape response to electric shock (unconditioned) and learning and avoidance response to buzzer (conditioned) memory processes. Endosulfan increased 5-HT concentrations in the cerebrum (165%) and midbrain (347%) regions. Protein and ChE activity in the brain were unaffected. Spontaneous motor activity was stimulated (counts increased days 75 - 90) but coordination on the rota-rod apparatus was not affected. 5-HT is involved in learning and memory and its endosulfan-induced increase resulted in decreased escape (learning) and avoidance acquisition (memory impairment). When p-chlorophenylalanine (PCPA), a 5-HT depletor, was used on endosulfan-treated animals, only learning (not memory) was restored. Food consumption and body weight gains (both measured over 15 day periods) were statistically significantly decreased from day 15 - 30 (food) and day 30 (body weight). Pole climbing was impaired in the absence of rota-rod (muscle) coordination impairment and none of the animals experienced tremors or convulsions. The decreased pole climbing as a result of shock stimulation may have resulted from a suppressed motivation. Endosulfan treatment resulted in a learning and memory inhibition, with 5-HT involved in the learning aspect. **Supplemental.**

Endosulfan was administered by gavage to immature (age not stated) male Wistar rats (60-70 g) for 90 days to test the effects of treatment on the therapeutic actions of drugs, such as chlorpromazine (CPZ) (Paul et al., 1994a). Treatment groups were as follows: A: controls (tragacanth + distilled water); B: 0 (tragacanth) or CPZ; C: endosulfan (2 mg/kg/day in distilled water); D: endosulfan (2 mg/kg/day) + CPZ. CPZ (4 mg/kg/day) was injected 24 hours post endosulfan treatment. Each treatment group was divided into 4 behavioral test groups ( $n = 10$ /dose/behavior group) as follows: 1) Spontaneous motor activity (SMA); 2) Pole climbing to avoid shock (conditioned avoidance response, CAR); 3) Muscle coordination by rota-rod apparatus; 4) Pentobarbital (40 mg/kg/day) sleeping time 15 mins after CPZ or distilled water. Endosulfan (E) statistically significantly increased SMA and CAR, but had no effect on rota-rod endurance. E increased latency to sleep and decreased duration of sleep at 2 mg/kg/day. E + CPZ showed statistically significantly decreased SMA and rota-rod endurance and increased CAR. E + CPZ showed decreased latency of sleep, with no effects on duration, compared with control. The suppression of pole climbing avoidance response in endosulfan-treated animals was not accompanied by motor coordination impairment. Therefore, endosulfan was considered to disrupt memory and not the somatic nerves for CAR-inhibition. The results of this study support those of Gupta and Gupta (1977) where endosulfan decreased sleeping time in adult rats

treated with pentobarbital (PB). Induction of P450s, as demonstrated by Tyagi, et al., 1984, was considered to be the reason for decreased sleeping time and duration in animals treated with CPZ + E. As an aside, in humans, workers who were exposed to endosulfan reported a memory loss (Aleksandrowicz, 1979). **Supplemental.**

#### b) Gavage - Adult

Endosulfan technical (98.6% pure) was administered by oral gavage in a single dose to fasted Wistar rats (10/sex/dose) at 0, 6.25, 12.5, 25, 50 or 100 mg/kg (males) and 0, 0.75, 1.5, 3, 6 or 12 mg/kg (females) (Bury, 1997). The vehicle was 2% starch mucilage (potato starch in deionized water). Endosulfan, in the vehicle, was stable for 4 hours and the duration of observation was 15 days. The neurotoxicological screening (Functional Observational Battery & motor activity) was performed 7 days prior to treatment initiation, 8 hours post-dosing (time of peak effect) and at 7 days and 14 days post-dosing. Three weeks post-dosing, controls (10/sex) and 5/sex (all other doses except 4/sex at 100 mg/kg) were terminated for neuropathological examination. The systemic NOEL was 12.5 mg/kg (males) and 1.5 mg/kg (females), based on an increase in clinical signs (mortality, tonic convulsions, coarse tremor, uncoordinated gait, increased salivation, stupor, prone position, increased fright reaction, squatting posture, stilted gait, irregular respiration, straddled hind limbs, decreased spontaneous activity, panting, bristled coat, flanks drawn in and narrowed palpebral fissure) in males at 25 mg/kg and greater and in females at 3 mg/kg and greater, lasting for 1 day. **Supplemental.**

Kindling, a model of secondary generalized epilepsy from repeated, low intensity electrical stimulation of limbic foci, is useful in the evaluation of human epilepsy because of its progressive nature, permanence and responsiveness to anticonvulsant therapies (Gilbert, 1992a, b, c; Gilbert and Mack, 1995; Goddard et al., 1969). Adult male Long-Evans rats (10 - 17/dose) treated by gavage with endosulfan 3 times per week for 21 days at 0, 2.5, or 5 mg/kg/day showed an increase in proconvulsant properties and kindled seizures (Gilbert, 1992a). The convulsions were considered by the authors to be related to an action on the GABA<sub>A</sub> receptor ionophore complex within the central nervous system. Therefore a second study was performed, following up on the proconvulsant properties of endosulfan and also testing for possible cumulative effects (Gilbert, 1992b). In Group 1, adult male Long-Evans rats (15/dose) received a single p.o. dose at 0 (corn oil), 10, 20, 30 and 40 mg/kg to establish an acute endosulfan dose that induces convulsions but not mortality. Detailed behavior observations and number of animals displaying myoclonic jerks (MCJ) were recorded for 1.5 hours post dosing. In Group 2, males (15-16/dose) received endosulfan p.o. at 0 (corn oil), 5 and 10 mg/kg/day 3 times/week for 21 dosing days. Detailed behavioral observations were performed on days 1, 10 and 21. To rule out cumulative toxicity of the sensitization to endosulfan and to determine the persistence of the enhanced state of seizure responsiveness, animals were challenged 2 weeks after the final dose of endosulfan, after cumulative effects had dissipated. In Group 3, the effects of repeated exposure over 10 versus 20 days was examined to test whether accumulation of endosulfan in the body, aging of animals, changes in weight or alterations in pharmacokinetics may lead to increased plasma and brain levels and enhanced behavioral responsiveness. Males (15/dose) were treated by gavage with 5 mg/kg/day of endosulfan daily for 20 days or 10 mg/kg/day 3 days/week for 10 dosing days (all eventually receiving 100 mg). Fourteen to 16 days after the final dose, animals were challenged with the same dose received throughout dosing and seizure responses were recorded. There were no alterations in threshold to induce a seizure or the duration of clonus upon the seizure generation. The faster kindling rates could not be attributed to transient toxicant-related increases in excitability of the nervous system. This study added further evidence that endosulfan has proconvulsant properties that

may be related to an action on GABA within the central nervous system. Dorough et al. (1978) indicated that endosulfan sulfate, the main metabolite, contributes to the acute endosulfan neurotoxicity, manifested by clonic convulsions in rats. Supplemental.

In an abstract by Kushwah and Dikshith (1981), a single dose of endosulfan (7.33 mg/kg) was administered by gavage to adult male albino rats. Results showed no change in brain (brain ChE) and RBC acetylcholinesterase (RBC ChE). When rats were gavaged with 7.33 mg/kg/day for 60 or 90 days, a significant decrease (no data given) was found in brain ChE activity; however, RBC ChE activity was unchanged. Concentration of a structural glycoprotein was significantly decreased (no data) after subchronic exposure to endosulfan. According to investigators, this indicated neuronal degeneration, which corresponded with the reported decrease in brain ChE activity. There appeared to be no direct interaction between brain ChE and endosulfan. Supplemental.

### c) Intraperitoneal -- Neonatal

Neonatal albino rats (1 day old, 4/sex/dose/time point, strain not stated) were treated intraperitoneally with endosulfan (purity not stated) at 0 (40% polypropylene glycol), 0.5 and 1.0 mg/kg/day for 3 or 5 weeks, followed by an 8-day recovery period without endosulfan treatment (Zaidi et al., 1985). Results showed statistically significantly increased <sup>3</sup>H-5HT binding to frontal cortical membrane at 5 weeks (1.0 mg/kg/day). This may have been due to increased maximum binding sites or alterations in the receptor affinity. At 1.0 mg/kg/day (after 5 weeks of treatment), there was a statistically significant increase in fighting behavior induced by endosulfan treatment that was reversed when the 5-HT-blocker, methysergide, was administered. The NOEL was 0.5 mg/kg/day. These effects were not reversed after 8 days of recovery. Data from this study were of limited value, however because the strain of rat was unknown and there was no information about purity of endosulfan used for dosing the animals (amount of endosulfan received by the animals versus impurities). Supplemental.

In a study by Seth et al., (1986) pregnant female rats (ITRC breeding colony, location not specified) were treated i.p. with endosulfan (purity unknown) in the following groups to examine the effects on dams and pups (in utero and post-natally): Group I: Vehicle control (40% propylene glycol) dams (5) and their pups (culled to 4/sex/litter) and observed for 2-3 weeks post-partum; Group II: Dams (5 at 3.0 mg/kg/day) treated through gestation until 2-3 weeks post-partum (6 pups/dose; either sex examined); Group III: Pups (6/dose) from untreated dams were cross-fostered with dams (5/dose) treated at 0 or 3 mg/kg/day for 2-3 weeks (lactation period to weaning); Group IV: Pups (6/dose; either sex) from dams receiving endosulfan at 3.0 mg/kg/day throughout pregnancy were cross-fostered with control dams (5/dose); Group V: Pups (4/dose; either sex) received endosulfan at 0, 0.5 and 1.0 (i.p.) for 5 days per week up to 2, 3 or 5 weeks old; Group VI: Adult males (8/dose; 8 weeks old) were given endosulfan i.p. at 1 mg/kg/day (1 day) or 3 mg/kg for 15 or 30 days. At termination, brains were excised and examined in high affinity binding assays with synaptic membrane preparations from several brain regions (corpus striatum, frontal cortex and cerebellum). Effects of endosulfan treatment on receptor binding in brain were compared in adults and pups (gestation, lactation and growth) using labeled ligands. Dopamine, brain cholinesterase (ChE), benzodiazepine, serotonin and GABA receptor binding was examined by use of <sup>3</sup>H-spiroperidol, <sup>3</sup>H-quinuclidinyl, <sup>3</sup>H-diazepam, <sup>3</sup>H-5HT and <sup>3</sup>H-muscimol, respectively. Results showed that <sup>3</sup>H-spiroperidol binding (dopamine) was increased in pups that had received endosulfan (Group II: 3 mg/kg/day) throughout gestation (p<0.05 at 2, 3 & 5 weeks post-partum) and in pups treated in utero (Group IV: 3 mg/kg/day)

but fosternursed to control dams ( $p < 0.05$  weeks 2 & 3). Pups exposed throughout gestation and lactation (Group II: 3 mg/kg/day) had an increase in  $^3\text{H}$ -spiroperidol binding at weeks 2, 3 and 5. Neonatal exposure at 0.5 mg/kg/day showed no effects from day 1 to 5 weeks (Group V: either sex) but at 1.0 mg/kg/day at 5 weeks there was a slight increase in 5-HT and benzodiazepine and a decrease in dopamine binding. Footshock fighting behavior was examined in 10 from control and 10 from treated groups randomly selected and was decreased in pups treated to 5 weeks of age (1.0 mg/kg/day). These changes were observed 8 days after cessation of treatment. Adults treated at 3 mg/kg/day for 15-30 days had increased  $^3\text{H}$ -5-HT binding along with increased footshock fighting (continuing 8 days posttreatment). Developing rats had increased sensitivity to endosulfan perhaps due to the interaction with developmental pattern of the neurotransmitter receptors that develop pre- and postnatally over a long period of time. The increased sensitivity of neonates to endosulfan may be due to their immature blood-brain barrier and excretory mechanisms and slow metabolism of the pesticide. Pup NOEL = 0.5 mg/kg/day. There were no effects on brain ChE in any group. Data from this study were of limited value, however because the strain of rat was unknown and there was no information about purity of endosulfan used for dosing the animals (amount of endosulfan received by the animals versus impurities). Supplemental.

#### d) Intraperitoneal - Adult

Anand, et al. (1980a) administered endosulfan intraperitoneally (i.p.) to adult rats at 5 mg/kg/day for 10 days. Results showed a brain seizure pattern, starting at 25 - 30 minutes post-treatment and persisting for 60 minutes. The clonic convulsions were more marked over the head region and were primarily localized in the upper extremities. Electrical activity in the brain showed a direct correlation with endosulfan concentration. In a subsequent study, endosulfan was administered i.p. to male rats (6/dose/timepoint) at 0 (propylene glycol), 1 and 3 mg/kg/day for 10, 30 and 60 days to examine effects on 5-HT uptake in blood platelets and thereby assess the cardiotoxic potential of endosulfan (Anand et al., 1985 and 1986). Results showed a statistically significant decrease in 5-HT uptake of platelets and an increase in number of platelets at 1 mg/kg/day and greater. A decreased uptake of 5-HT by platelets results in their increase in blood that could lead to blood wall abnormalities. Since endosulfan is neurotoxic and induces hypertension, these findings implicate 5-HT in possible obstruction of blood flow in small vessels. Supplemental.

Male rats (ITRC breeding colony, location not specified; 8/dose/timepoint) were treated in a single intraperitoneal dose of endosulfan (purity unknown) at 0 (40% propylene glycol), 1 and 3 mg/kg/day (single dose) or at 0 and 3 mg/kg/day for 15 or 30 days (Agrawal et al., 1983). There were no effects at either dose after a single treatment; however, there was increased binding of  $^3\text{H}$ -serotonin (5-HT) to frontal cortical membranes at 3 mg/kg/day after 30 days. There was an increased affinity of the receptor, with unchanged number of receptor sites. After 30 days at 3 mg/kg/day endosulfan induced aggressive behavior (foot-shock induced fighting behavior) that was blocked by methysergide (5-HT blocker). These results showed that serotonergic receptors were involved in endosulfan neurobehavioral toxicity. The NOEL was 1.0 mg/kg/day (single dose) and 3.0 mg/kg/day (treatment for 3 weeks). Supplemental.

ITRC albino male adult rats (origin of rats not specified; 5/dose/time point) received a single i.p. injection of endosulfan at 0 (alcohol:ground nut oil, ratio = 1:9) and 40 mg/kg to test the effect on neurotransmitters in the brain stem and cerebral cortex (norepinephrine - NE, serotonin - 5-HT, 5-hydroxyindolacetic acid (5-HIAA), GABA, dopamine, DA) and acetylcholinesterase (ChE) at 1, 3, 5

and 7 hours post-dosing (Ansari et al., 1987). At 7 hours DA and NE were statistically significantly increased and 5-HT, GABA and 5-HIAA were decreased in treated groups. There were no effects on brain ChE. Supplemental.

e) Subcutaneous Injection - Adult

Endosulfan was injected subcutaneously into male Wistar rats at 25 mg/kg/day for 10 consecutive days in order to test effects on GABAergic and cholinergic systems (main modulators of neuronal excitability in cortex and hippocampus), on spacial learning (water maze) and other parameters (Castillo et al., 2002). Animals were tested (4-8/test) before, during and at final treatment. Results showed no increases in the endosulfan treated groups, compared with controls, for glomerular flow rate, alanine aminotransferase activity, liver protein, triglyceride content, plasma cholinesterase levels, sensory evaluation, motor evaluation, mean escape latency, escape latency, times crossing the water maze, the percentage of failures to cross the water or the brain regional GABA content (hippocampus, cortex, striatum). Supplemental.

f) Intravenous Injection - Adult

Endosulfan competitively inhibited *t*-butylbicyclophosphorothionate (TBTS) that binds GABA<sub>A</sub> receptor in albino rat brain (Lawrence and Casida, 1984). Males were treated i.v. at the LD<sub>50</sub> doses of  $\alpha$ - and  $\beta$ -endosulfan and endosulfan sulfate (76, 240 and 76 mg/kg, respectively). Results showed these compounds were potent, competitive and stereospecific inhibitors of TBTS binding to brain-specific sites. TBTS binding site is hypothesized to be the same site on the GABA<sub>A</sub> receptor where endosulfan also binds. These data provide the first example of a GABA<sub>A</sub> antagonist with sufficient affinity to remain at the specific site during receptor preparation and assay in a mammalian system, after *in vivo* treatment with endosulfan. Supplemental.

g) Intraperitoneal and intracerebral vascularly

Subsequent work (Abalis, et al., 1986) showed that endosulfan inhibited *in vitro* GABA<sub>A</sub>-induced Cl<sup>-</sup> influx in rat brain membrane microsacs. The GABA<sub>A</sub> receptor for such insecticides as endosulfan is located on the Cl<sup>-</sup> channel. Cole and Casida (1986) used brain microsacs from male Swiss-Webster mice treated i.p. or intracerebral vascularly (i.c.v.) with endosulfan. Therefore, the magnitude of [<sup>35</sup>S]TBTS binding site inhibition correlated with the severity of the poisoning signs. This supported the hypothesis that the acute toxicity of endosulfan is due to the disruption of the GABA-regulated chloride ionophore. Supplemental.

h) *In vitro* brain cell cultures

The mechanism of neurotoxicity of  $\alpha$ -endosulfan was examined in primary cultures of cerebellar granule cells from 7-day old Wistar rats at doses of 0 (DMSO), 25, 50, 100, 150 and 200  $\mu$ M (Rosa et al., 1996). Disruption of cell membrane integrity, along with 3 endpoints of cellular status that were concentration of intracellular-free calcium, intracellular oxygen-free radical formation and mitochondrial transmembrane potential, were examined in parallel. Cytotoxicity, as measured by propidium iodide staining in cerebellar granule cells, was 50% at 100  $\mu$ M and approximately 35% at 50  $\mu$ M after an 18-hour exposure. At 2 hours, cytotoxicity, as measured by lactic acid dehydrogenase and propidium iodide staining, was approximately 25% at 200  $\mu$ M. Neither concentrations of

intracellular-free calcium nor intracellular oxygen-free radical formation was increased in treated cells at any dose. Changes in mitochondrial transmembrane potential after a 1-hour exposure was increased at all doses, compared to controls. This increase in mitochondrial transmembrane activity is associated with increase mitochondrial activity in rat liver mitochondria *in vitro*, after endosulfan treatment (Dubey et al., 1984). **Supplemental.**

#### i) Dietary - Developmental Neurotoxicity

Endosulfan technical (99.1% pure) was fed in diet to mated female Wistar rats (30/dose) at 0, 50, 150 or 400 ppm (0, 3.74, 10.8, and 29.8 mg/kg/day) from gestation day (GD) 6 through lactation day (LD) 21 (Gilmore et al., 2006). The concentration of endosulfan in the dietary preparations was adjusted to the expected food consumption during the lactation period in order to maintain a reasonably constant level of test material consumption. Offspring from 23 litters in the control, 50 and 150 ppm groups and pups from 21 litters in the 400 ppm groups were assessed neurologically up to 75 days post-natal in the functional observational battery (FOB), measurement of motor activity, 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. The neuropathologic examination and morphometric analysis 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. This effect persisted through the lactation period with the mean body weights of the dams at 150 and 400 ppm significantly less than that of the 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 treatment did not affect the gestation of the fetuses. 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 the live birth, viability or lactation indices. For the developmental landmarks, the preputial separation was marginally delayed (4-5%) for the male pups at 150 and 400 ppm (0 = 44.9 days; 50 = 44.8 d; 150 = 47.1 d; 400 = 46.8 d). The time to vaginal opening for the female pups was not affected in a dose-related manner. Sperm motility, count and morphology of the male pups were not affected by the treatment. No treatment-related effects were noted in the FOB for either the dams or the pups. The motor activity assessment of the pups did not reveal any treatment-related effects. The auditory startle response, passive avoidance learning and memory and water maze learning and memory assessments did not indicate any treatment-related effects on the pups. No neuropathological lesions were noted in either the 21-day old pups or the 70-day old adults. Morphometric analysis of the brain of these animals did not demonstrate any treatment-related effects. The maternal NOEL was less than 3.74 mg/kg/day, based upon lower mean body weights (5 - 6%) and lower food consumption (12%) at 3.74 mg/kg/day. While these decreases are marginal, the trend is dose-related and therefore considered to be a treatment-related effect. The developmental NOEL was less than 3.74 mg/kg/day based upon the lower mean body weights (8% on post-partum day 11 only) of the offspring at 50 ppm. Body weight gain for pups was also decreased on post-partum day 11, however this effect was reversed. The developmental neurotoxicity NOEL was 29.8 mg/kg/day, based upon the lack of neurological effects in the offspring at the highest dose tested. This study was acceptable.

#### j. Dietary – Subchronic

Endosulfan (purity = 98.1% (5/01) & 96.5% (12/03)) was fed in diet to Wistar Crl:WI[Glx/BRL/Han]IGS BR rats (12/sex/dose) at 0, 40, 225 and 600 ppm (equivalent to 0, 2.11, 13.7 & 37.2 mg/kg/day-M; 0, 2.88, 16.6 & 45.5 mg/kg/day-F) for 13 weeks to test for neurobehavioral, motor and systemic toxicity (Sheets et al., 2004). Of the 12/sex/dose treated and examined for neurotoxicity, 6/sex/dose were also examined for histopathology. One female had clonic convulsions week 1 of exposure and died week 8 and 3 females had red nasal stain at 45.5 mg/kg/day. Females had decreased body weights on day 7 (only) at 225 ppm and greater that was possibly due to palatability. Food consumption was decreased only week 1 in females at 16.6 mg/kg/day and greater and in males at 37.2 mg/kg/day. Plasma ChE activity was decreased in females at 16.65 mg/kg/day and greater. Absolute and relative kidney and liver weights were increased in both sexes at the mid-dose and greater. The kidneys of both sexes at all doses had an amorphous brown-to-yellow pigment in the cytoplasm of the proximal convoluted tubular epithelium, with pigment occasionally present in the lumen of proximal tubules. The systemic NOELs were 37.2 mg/kg/day (HDT; M) and 16.6 mg/kg/day (F). The neurotoxicity NOELs were: 37.2 mg/kg/day (M) and 45.5 mg/kg/day (F). There were no treatment-related effects on FOB or motor activity in either sex at any dose. This study is acceptable.

### 3. Mouse

#### a) Intraperitoneal (i.p.)

$\alpha$ -Endosulfan was injected i.p. with the LD<sub>50</sub> dose or twice the LD<sub>50</sub> dose (8 or 16 mg/kg; DMSO vehicle) and subsequently the regional modification of [<sup>3</sup>H]ethynylbicycloorthobenzoate ([<sup>3</sup>H]EBOB) binding in mouse brain GABA<sub>A</sub> receptor was assessed (Kamijima and Casida, 2000). [<sup>3</sup>H]EBOB blocks the noncompetitive binder site for the GABA<sub>A</sub> receptor and serves as a probe of functional changes in the chloride channel after endosulfan treatment. Areas of specific binding of [<sup>3</sup>H]EBOB in mouse brain were defined, then the binding modification was observed after treatment. At 4-8 minutes post-dosing, the mice were terminated. Results showed 10/10 mice had neurological signs at 8 mg/kg (myoclonic jerks, head twitching) and 8/9 at 16 mg/kg had generalized seizures (tonic extension of fore and hindlimbs).  $\alpha$ -Endosulfan (channel-blocker) inhibited [<sup>3</sup>H]EBOB binding in most brain regions in a dose-dependent manner; however, there were localized areas shown by autoradiography. Therefore, the noncompetitive blocker site was strongly inhibited by dose-dependence and regional selectivity of the channel-blocker  $\alpha$ -endosulfan, as demonstrated by the probe [<sup>3</sup>H]EBOB. **Supplemental.**

### 4. Cat

#### a) Intravenous

Intact cats and cats with spinal sections (spinal cord cuts made at the atlanto occipital level) were treated with 3 mg/kg endosulfan by i.v., in order to study brain bioelectrical activity (Anand et al., 1980b). Cerebral cortex acetylcholine was decreased 93% at 2 hours post-treatment. Seizure activity peaked at 30 minutes and subsided by 2 hours and at 4 hours the pattern was normal. Convulsive activity was associated with an accelerated release of acetylcholine from the brain. Convulsions ended when the acetylcholine content decreased to 50% of control activity. It was concluded by the investigators that depletion of acetylcholine without any change in acetylcholinesterase activity might be due to the direct effect of endosulfan on the synthesis of acetylcholine. The fall in blood pressure (hypotension) produced by endosulfan was followed by a

sustained increase in blood pressure (hypertension). Supplemental.

## 5. Humans

Endosulfan exposure in ambient air near agricultural applications to pregnant women was proposed to induce neurotoxicity in fetuses when exposure occurred during gestation weeks 1 through 8 (period of central nervous embryogenesis) (Roberts et al., 2007). Exposure was proposed to result in an increased incidence in autism spectrum disorder (ASD). Conclusions to this study that the association between endosulfan and pesticide applications during gestation and autism in children need further study. This study was very preliminary and numerous defects were discussed in a report by Erdreich and Morimoto (2007). This study cannot be used for regulatory purposes.

## 6. Cow, Pig, Lamb (additional studies)

Eight cows accidentally ingested Thiodan<sup>7</sup> that had been stored in 5 lb bags in a barn (Braun and Lobb, 1976). Upon discovery, 4/8 cows were dead and 4/8 were convulsing (1/4 convulsing died after 3 days). Total endosulfan residue (primarily endosulfan sulfate) levels in postmortem samples were 1.27 ppm in the rumen content and 4, 1.1 and 0.6 ppm in liver, kidney and muscle tissue respectively. Analysis of milk from surviving cows showed there was greater than 1 ppm endosulfan (primarily endosulfan sulfate) immediately following poisoning. After 35 days, this was reduced to 1 ppb and therefore the half-life for endosulfan in milk was calculated to be approximately 3.9 days.

Ataxia progressing to complete inability to stand (no convulsions) was observed in pigs and lambs that grazed in a field sprayed with endosulfan (Utklev and Westbye, 1971). Blindness was also reported in sheep. Blindness had previously not been reported in connection with endosulfan but had been observed with some other cyclodienes (Doman, 1971). Supplemental.

# IV. RISK ASSESSMENT

## A. HAZARD IDENTIFICATION

### 1. Acute Toxicity

#### a) Acute Oral NOEL

The adverse effects observed in laboratory animals with acute oral exposure to endosulfan are summarized in Table 12. In general, the effects considered to be adverse include clinical signs of neurotoxicity, deaths, neurobehavioral effects, reductions in body weight, and increased gross and histopathological effects. The possible acute oral effects from endosulfan included effects observed in the LD<sub>50</sub>/LC<sub>50</sub> studies and in a rabbit developmental study. The effects observed in the LD<sub>50</sub>/LC<sub>50</sub> studies included death, clinical signs, and liver, kidney, intestine, lung and adrenal toxicity. Liver changes were a granular-appearance, degeneration of hepatocytes with foamy cytoplasm and bile duct proliferation. Kidneys appeared congested and proximal convoluted tubules were necrotic and desquamated. Adrenal cortex showed swollen foamy cytoplasm, with eccentric nuclei. Congested lungs containing hemorrhagic areas were observed, along with irritation of the small and large intestine. Clinical signs were increased preening, salivation, excessive masticatory movements, lacrimation, exophthalmia, hyperresponsiveness to sudden sound and tactile stimuli, hyperexcitability,

dyspnea, decreased respiration, ataxia, depression of activity, discharge from eyes, nasal discharge, sprawling of the limbs, decreased reflexes (placement, pain, corneal, pupillary light, righting, startle, paw, cutaneous) and tremors, tonic and clonic convulsions and death.

The effects observed in an oral neurotoxicity study in rat occurred 4 to 8 hours post-dosing (Bury, 1997). Clinical signs were mortality, tonic convulsions, coarse tremor, uncoordinated gait, increased salivation, stupor, prone position, increased fright reaction, squatting posture, stilted gait, irregular respiration, straddled hind limbs, decreased spontaneous activity, panting, bristled coat, flanks drawn in and narrowed palpebral fissure. These signs were observed to a greater extent in females than in males. The NOEL for males was 12.5 mg/kg and 1.5 mg/kg for females based on increased clinical signs that lasted for 1 day. Like the oral and dermal LD<sub>50</sub> studies and the inhalation LC<sub>50</sub> study, this study showed females as the more sensitive sex (see Table 12).

The rat developmental study showed acute oral effects in dams (Fung, 1980b). Effects were also observed in fetuses. Clinical signs seen in dams were rough coat, lethargy, flaccidity, hyperactivity and face rubbing, observed post-dosing. Fetal effects were increased incidence in anomalies, including small 4<sup>th</sup>, and misaligned and unossified 5<sup>th</sup> sternbrae. Fetuses also showed an increased incidence in developmental malformations, including clubbed limb, skin of upper forelimb webbed to chest, edema, skeletal abnormalities including lordosis and cardiovascular abnormalities. The maternal NOEL of 2 mg/kg/day was based on significantly decreased mean body weight gain and increased clinical signs. The developmental NOEL was 2 mg/kg/day, based on decreased mean fetal weights, or increased growth retardation, and developmental anomalies and malformations. However, it is not known whether the fetal effects are due to an acute oral toxicity directly on the developing fetuses, or whether they developed only after multiple treatments or whether they were secondary to maternal toxicity.

The acute oral effects observed in a developmental toxicity study performed in the rabbit, included maternal signs within the first day of treatment (in the absence of fetal effects). Various clinical signs were observed in dams/does, including abortions, phonation, coughing, cyanosis, convulsions/ thrashing, noisy/rapid breathing, hyperactivity, salivation, and nasal discharge and death (Nye, 1981). Clinical signs began on gestation day 6 (day 1 of treatment) at 1.8 mg/kg/day. In particular, hyperactivity was observed at 1.8 mg/kg/day (no convulsions; thrashing, phonation, coughing, and cyanotic only). The NOEL for this study was 0.7 mg/kg/day. Similar effects were observed in 2 range-finding studies also performed in pregnant New Zealand rabbits (Fung, 1981a, b). In these studies the LOELs were 1.0 mg/kg/day, based on neurotoxicity and deaths beginning day 8 of gestation (treatment day 2).

There were no major deficiencies in the rabbit developmental study and it provided the lowest acute oral NOEL. The other studies described above (summarized in Table 12), showed that female rats are more sensitive to acute oral endosulfan treatment than are males and that pregnant female rabbits are more sensitive to endosulfan than are both non-pregnant and pregnant rats. Although the rabbit developmental study involved multiple dosing, rather than a single acute oral dose of endosulfan, the neurotoxic effects were seen on the first day of treatment and were therefore acute oral effects. Therefore, this study, with a critical NOEL of 0.7 mg/kg, was selected as the definitive study for evaluating acute dietary exposure and to calculate the MOE for potential acute single-day (non-inhalation) human exposures to endosulfan.

b) Acute Dermal NOEL

There were no FIFRA Guideline, nor open literature studies that were acceptable for acute dermal exposure to endosulfan technical. Technical material resulted in dermal erythema, and slight desquamation, however, the dose levels were too high to establish NOELs for these studies. Therefore, the oral acute NOEL (0.7 mg/kg) was used for determinations of MOEs for acute dermal occupational exposure and for swimmer exposure in surface water.

c) Acute Inhalation NOEL

An acute inhalation (LC<sub>50</sub>) study was performed (Hollander and Weigand, 1983), however, a NOEL was not achieved (LOEL = 0.567 mg/kg). Therefore, an acceptable subchronic rat inhalation study (based on a subchronic rangefinding study with a LOEL of 0.44 mg/kg reported within Hollander et al., 1984) with a NOEL of 0.0010 mg/L (0.194 mg/kg/day; LOEL = 0.387 mg/kg/day) was used to calculate the potential for acute single-day inhalation exposure to workers, and for exposure to endosulfan in ambient air or to bystanders (Hollander et al., 1984). The rationale for the use of the subchronic inhalation study for the Acute NOEL is that LOELs from all three studies were similar (0.567, 0.44 and 0.387 mg/kg/day), more animals treated in the subchronic (15/sex/dose subchronic versus 5/sex/dose in the acute), and the subchronic study used a 29 day recovery with 5 per sex per dose (acute 14d observation). The NOEL of 0.194 mg/kg/day is a reasonable selection based on the LOELs from the 3 studies. It is also a conservative estimate for an acute NOEL, since acute NOELs are usually higher than subchronic or chronic NOELs. It is also noted that all three studies were performed at the same laboratory and in the same timeframe (12/7/83—Acute; 8/15/83--Subchronics).

In this study, endosulfan was administered by aerosol (nose-only) for 21 days at 6 hours per day, followed by a 29-day recovery. The NOEL for inhalation was based on emaciation, pale skin, squatting position and high-legged position, decreased bodyweight gain and food consumption, increased water consumption, and clinical chemistry parameters (reversed during recovery). The advantages to using the subchronic study for the critical inhalation NOEL instead of the LC<sub>50</sub> are: A) a NOEL was achieved (0.194 mg/kg/day); B) there were 15 animals per sex per dose treated instead of 5 per sex per dose; C) the LOEL achieved in the subchronic study (0.387 mg/kg/day) was 2/3rds the dose of the LOEL in the LC<sub>50</sub> (0.567 mg/kg) and D) the NOEL in the subchronic study was, in general, more conservative, because the acute NOEL is usually higher than the subchronic NOEL. Both studies were acceptable according to FIFRA Guidelines. The subchronic inhalation NOEL was also selected instead of the oral NOEL of 0.7 mg/kg/day from the rabbit developmental study because not only is it lower, but more importantly, it is route-specific. Therefore the rat subchronic NOEL was used to estimate the MOE for acute inhalation (occupational and (non-occupational) ambient air and bystander exposure).

**Table 12. The Acute Effects of Endosulfan and the NOELs and LOELs**

Species	Exposure	Effect	NOEL mg/kg	LOEL mg/kg	Ref <sup>a</sup>
<b>ORAL</b>					
Rat <sup>b</sup> Male	Single Gavage	Death, clinical signs, irritation of stomach and small intestine; congestion of kidneys, lungs and adrenals, LD <sub>50</sub> = 48 mg/kg	--	31.6	1
Rat <sup>b</sup> Female	Single Gavage	Death, clinical signs, reddening of small intestine, LD <sub>50</sub> = 10 mg/kg	--	6.3	2
Rat M/F	Single Gavage	Death, clinical signs, neurotoxicity	M 12.5 F 1.5	M 25 F 3.0	3*
Rat Female	8 Days Gavage	Dams: Death, decreased body weight, clinical signs Fetuses: Increased anomalies and malformations	2.0	6.0 HDT	4
<b>Rabbit Female</b>	<b>12 Days Gavage</b>	<b>Death, clinical signs beginning the first day of treatment</b>	<b>0.7</b>	<b>1.8 HDT</b>	<b>5*</b>
<b>DERMAL</b>					
Rabbit <sup>b, c</sup>	Single Dermal	Death, erythema, atonia, desquamation, hemorrhagic lung, granular livers, irritation of large intestine, congested kidneys (clinical signs not described) LD <sub>50</sub> = 359 mg/kg	--	46.4	6
<b>INHALATION</b>					
Rat <sup>b, d</sup> M/F	Single 4 Hour Nose Only	LC50 M 4.6 (M); 1.2 (F); <u>28 Min</u> Clinical sign neurotoxicity 0.567 F; 1.19 M); <u>4 hr</u> (Clinical signs neurotoxicity all doses); <u>3.6 Hr F; Day 2 M</u> (Death ≥1.19 F; ≥6.42 M), <u>Post-dose Day 3 M; Day 14 F</u> (↓ body weight gain was determined.	--	0.567	7*

a - 1. Scholz and Weigand, 1971a; 2. Scholz and Weigand, 1971b; 3. Bury, 1997; 4. Fung, 1980b; 5. Nye, 1981; 6. Elsea, 1957; 7. Hollander and Weigand, 1983

b - LD<sub>50</sub>/LC<sub>50</sub> study

c - Gender unspecified

d - See Table 12, Subchronic Inhalation

\* - Designates studies that are acceptable, according to FIFRA Guidelines.

HDT = Highest Dose Tested

**Bold = Definitive test for the critical NOEL**

## 2. Subchronic Toxicity

The effects observed in laboratory animals after subchronic exposure to endosulfan are summarized in Table 13. Most commonly observed after subchronic treatment with endosulfan were death, clinical signs, increased liver and kidney weights and histopathological effects in liver and kidney, decreases in body weights and food consumption and effects in hematology and in clinical chemistry parameters, including decreases in acetylcholinesterase activities. Notably the effects to RBCs, Hb, and kidney (granular/clumped pigments and discolored pigment) were not observed in the chronic rat study. All of the studies selected to support critical dermal, oral and inhalation NOELs were performed in the rat and were acceptable according to FIFRA Guidelines.

### a) Subchronic Oral NOEL

Representative indicators of subchronic oral effects occurred in a rat subchronic dietary study, which included a 4-week, post-treatment recovery period (Barnard, et al., 1985). The dietary NOEL was established at 1.92 mg/kg/day and a LOEL of 3.85 mg/kg/day, based on decreased water consumption, increased kidney and liver weights, numerous hematological effects, the occurrence of granular brown pigment in the liver, centrilobular enlargement of hepatocytes, kidney pigmentation and dark urine with increased ketones. Females showed hair loss in the dorsal/scapular/cervical region (reversed during recovery). Males had significantly increased epididymal weights. Hematology showed effects in packed cell volume, hemoglobin, red blood cells, mean corpuscular hemoglobin concentration and mean cell volume, that were treatment-related. Both plasma and RBC cholinesterase activities were decreased in a dose-related manner. Many of the effects continued throughout the recovery period.

The study by Chitra et al. (1999) treated Wistar male prepubertal (45 day old) rats by gavage with endosulfan technical at 1.0 mg/kg/day (6 animals) for 30 days (Chitra, et al., 1999). Results at termination showed statistically significant effects in reproduction parameters (decreased testes, epididymal, ventral prostate, and seminal vesicle weights) and effects to 3- $\beta$ OH-steroid dehydrogenase among other biochemical parameters relating to testicular metabolism at 1.0 mg/kg/day. These findings suggest a possible connection between endosulfan treatment and steroidogenesis inhibition in male rats. However, there were major deficiencies in this study (only 6 animals treated, only a single dose, no individual data were shown, and there was a great deal of variation in assay results) that prevent its use as a critical endpoint study.

In addition to the standard subchronic oral toxicity studies, however, Table 13 includes a rat dietary reproduction study, where parental effects were observed after an exposure of 24 weeks throughout pre-mating, mating, gestation, lactation and weaning for 2 generations (Edwards et al., 1984). The oral, systemic NOEL was 1.18 mg/kg/day based on increased relative liver and kidney weights, decreased food consumption, and decreased body weights. The common endpoint for the reproduction and subchronic oral dietary study was an increase in both kidney and liver weights. Considering both studies are acceptable according to FIFRA Guidelines, the reproduction study is preferable because it provides the lower NOEL. Therefore, the reproduction study in rat was selected as the definitive study, with a critical NOEL of 1.18 mg/kg/day and a LOEL of 5.40 mg/kg/day for subchronic oral exposure. This NOEL will be used to estimate the subchronic dietary exposure to endosulfan.

#### b) Subchronic Dermal NOEL

There were no FIFRA Guideline, nor open literature studies that were acceptable for subchronic dermal exposure to endosulfan technical. There were two acceptable dermal studies performed with endosulfan as a water-dispersible powder (49.5% a.i.; Ebert et al., 1987) and as an emulsifiable concentrate (33.3% a.i.; Thevenaz et al., 1988), however the NOELs were 40 mg/kg/day (both sexes) and 27 mg/kg/day (M) or 9 mg/kg/day (F), respectively. Since the NOELs were much higher than the oral NOEL and studies were not performed with technical material, the oral NOEL (1.18 mg/kg/day) was used for determinations of MOEs for seasonal dermal occupational exposures and for exposures to swimmers in surface water.

#### c) Subchronic Inhalation NOEL

The definitive study for subchronic inhalation exposure was a study performed in rat, where endosulfan was administered by aerosol (nose-only) for 21 days at 6 hours per day, followed by a 29 day recovery (Hollander et al., 1984). The NOEL for inhalation was 0.0010 mg/L based on emaciation, pale skin, squatting position and high-legged position, decreased bodyweight gain and food consumption, increased water consumption, and clinical chemistry parameters (reversed during recovery). This study was acceptable according to FIFRA Guidelines and was the only one available for evaluation of subchronic endosulfan exposure by inhalation. It was therefore selected as the definitive study for the critical inhalation NOEL of 0.0010 mg/L (0.194 mg/kg/day; based on infant breathing rate) and a LOEL of 0.0020 mg/L (0.3873 mg/kg/day). This NOEL will be used to estimate the MOE for seasonal (non-occupational) ambient air and bystander exposure.

**Table 13. The Subchronic Effects of Endosulfan and the NOELs and LOELs**

Species	Exposure	Effect	NOEL mg/kg/d	LOEL mg/kg/d	Ref <sup>a</sup>
<b>Oral</b>					
Rat M/F	13 wk diet + 4 wk recovery	Hair loss, kidney pathological effects, hematology effects, increased liver and kidney weights, decreased RBC & plasma ChE <sup>b</sup> , decreased water & food intake	1.92	3.85	1*
Rat M/F	<b>2 Generation Diet</b>	<b>Maternal: Increased liver and kidney weights</b>	<b>1.18</b>	<b>5.4</b>	<b>2*</b>
<b>INHALATION</b>					
Rat M/F	<b>6 hr/d, 5 d/wk, 21d, Nose Only</b>	<b>Clinical signs, decreased body weight gain, food and water consumption, clinical chemistry parameters</b>	<b>0.194</b>	<b>0.3873</b>	<b>3*</b>

a - 1. Barnard et al., 1985; 2. Edwards et al., 1984; 3. Hollander et al., 1984

b - ChE = acetylcholinesterase activity, includes RBC (red blood cell) and serum (plasma)

\* - Designates studies that are acceptable, according to FIFRA Guidelines.

**Bold = Definitive tests for the critical NOELs for oral, dermal and inhalation effect**

### 3. Chronic Toxicity

#### a) Chronic Oral NOEL

The effects observed in laboratory animals from chronic dietary exposure to endosulfan are summarized in Table 14. Effects observed in the rat were different from those observed in the dog. In the rat, the chronic dietary NOEL was 0.6 mg/kg/day for males and 0.7 mg/kg/day for females based on decreased body weight gain, kidney enlargement, progressive glomerulonephrosis and glomerulonephritis, proteinuria, aneurysms (Ruckman et al., 1989). This study was acceptable according to FIFRA Guidelines.

In dogs, neurotoxicity was the most sensitive endpoint for chronic oral endosulfan toxicity. The chronic study in dogs was performed with endosulfan administered in diet. In the dog, the chronic dietary NOEL was 0.57 mg/kg/day for males and 0.65 mg/kg/day for females, based on clinical signs of violent contractions of the upper abdomen and convulsive movements, extreme sensitivity to noise, frightened reactions to optical stimuli and jerky or tonic contractions in facial muscles, chaps and extremities and impairment of the reflex excitability and postural reactions (Brunk, 1989). It was necessary to sacrifice some of the dogs prematurely due to the clinical signs of neurotoxicity. In addition, body weights and food consumption were decreased. This study was acceptable according to FIFRA Guidelines.

The dog appears to be very slightly more sensitive than the rat with regard to chronic effects. However, the dog study, with a critical NOEL of 0.57 mg/kg/day was similar to the NOEL obtained in the chronic dietary rat study (0.6 mg/kg/day). The two studies were performed by different methods, despite the fact that they are both considered oral, dietary studies. The dog study, with endosulfan administered in diet, was selected as the definitive study. However, mortality and neurotoxicity occurred in dogs at 2.0 mg/kg/day, where this dose was tolerated in rats. At 2.9 mg/kg/day in male rats and at 3.8 mg/kg/day in female rats (highest doses tested), kidney enlargement and glomerulonephritis in females and aneurysms in males were increased. Rat mortality, however, at these high doses was comparable to the controls. Rats received the endosulfan treatment in their diet, and this may account for the apparent interspecies sensitivity differential. The chronic dog study was selected as the definitive study with a critical NOEL of 0.57 mg/kg/day since it appeared to be the more sensitive species when tested in an acceptable FIFRA Guideline study. The chronic rat study NOEL, virtually the same at 0.6 mg/kg/day, served to support the value obtained in the dog study. The chronic dietary NOEL of 0.57 will be used to determine MOE for both dietary and worker exposure (Table 14).

b) Chronic Dermal NOEL

There were no FIFRA Guideline, nor were their open literature studies that were acceptable for chronic dermal exposure to endosulfan technical. Therefore, the procedure is to use the chronic oral NOEL in dog (0.57 mg/kg/day) for determinations of MOEs for chronic dermal occupational exposures and for exposures to swimmers in surface water.

c) Chronic Inhalation NOEL

An acceptable chronic inhalation exposure study was not available **from the open literature or studies submitted by registrants** to obtain a chronic inhalation NOEL. Therefore, an acceptable subchronic rat inhalation study with a NOEL of 0.0010 mg/L (0.194 mg/kg/day) was used to calculate the potential for chronic inhalation exposure to workers, and for exposure to endosulfan in ambient air or to bystanders (Hollander et al., 1984). In this study, endosulfan was administered by aerosol (nose-only) for 21 days at 6 hours per day, followed by a 29-day recovery. The NOEL for inhalation was based on emaciation, pale skin, squatting position and high-legged position, decreased bodyweight gain and food consumption, increased water consumption, and clinical chemistry parameters (reversed during recovery). A 10x uncertainty factor for extrapolation from subchronic to chronic was added to the NOEL of 0.194 mg/kg/day to give a final critical Estimated No Effect Level (ENEL) of 0.0194 mg/kg/day. This dose is lower than the chronic oral NOEL of 0.57 mg/kg/day from the chronic dog dietary study and more importantly, it is route-specific. The study was therefore selected as the definitive study for the critical NOEL with 0.0194 mg/kg/day. This NOEL will be used to estimate the MOE for chronic occupational and (non-occupational) ambient air and bystander exposure (Table 14).

**Table 14. The Chronic Effects of Oral Endosulfan Treatment and the NOELs and LOELs**

Species	Exposure	Effect	NOEL mg/kg/d	LOEL mg/kg/d	Ref <sup>a</sup>
Rat M/F	104 week (diet)	Aneurysms and progressive glomerulonephrosis & nephritis; enlarged kidneys, proteinuria; decreased body weight gain	0.6 M 0.7 F	2.9 M 3.9 F	1*
<b>Dog M/F</b>	<b>1 year (diet)</b>	<b>Premature termination, clinical signs of neurotoxicity, decreased body weight gain and food consumption</b>	<b>0.57 M 0.65 F</b>	<b>2.09 M 1.98 F</b>	<b>2*</b>

a - 1. Ruckman et al., 1989; 2. Brunk, 1989

\* - Designates studies that are acceptable, according to FIFRA Guidelines.

**Bold = Definitive test for the critical NOEL for dietary effects**

#### 4. Oncogenicity

##### a) Summary of Findings

When considering the results of all available *in vivo* studies performed in rats and mice, there is no evidence indicating endosulfan is oncogenic. There were **inconclusive findings with contradictory results from** genotoxicity induced by endosulfan (technical), as measured by the gene mutation, chromosomal aberration and other genotoxic effects tests submitted to DPR. Although there were some studies reported to be positive in the published literature (Chaudhuri et al., 1999; McGregor et al., 1988; Yadav et al., 1982; L'vova, 1984; Velazquez et al., 1984; Sobti et al., 1983; Sharma and Gautam, 1991; Martins, 2003, Daniel et al., 1986; Dubois et al., 1996), none was acceptable by FIFRA Guidelines (Tables 15 and 16). Results from the *in vitro* genotoxicity studies were equivocal and USEPA does not consider oral exposure (*in vivo* tests) of rats to be genotoxic; stating that “the data are inconclusive” (USEPA, 2000b). USEPA also states that induction of chromosomal aberrations and gene mutations in *Drosophila melanogaster* (Velazquez et al., 1984) and in mice (Usha Rana and Reddy, 1986) complicate data analysis because some of the formulations of endosulfan may have contained epichlorohydrin, a known genotoxin, as a stabilizer (see Tables 15 and 16; USEPA, 2000b; Hoechst, 1990).

The NOEL for mice in an acceptable FIFRA Guideline oncogenicity study was 0.84 mg/kg/day for males and 0.98 (females) mg/kg/day, based on mortality that occurred beginning week 45 in males and week 15 in females, primarily at the high doses of 2.48 (male) and 2.8 (female) mg/kg/day (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 104 weeks of dietary treatment. No pathological examinations were performed to identify reasons for high mortality or to identify target organs. No other effects were reported in the mice. Neurotoxic effects were not observed in the rat or mouse chronic studies.

**Endosulfan is categorized as “A4” (not classifiable as a human carcinogen) by the American Conference of Governmental Industrial Hygienists (Substances and Physical Agents and Biological Exposure Indices, Cincinnati, OH, 2005).**

**Table 15. Genotoxicity of Endosulfan (Gene Mutation Assays)**

System/Strain	Concentration/Dose	S9 <sup>a</sup>	Results	Ref <sup>b</sup>
<b><i>Schizosaccharomyces pombe</i></b>				
Haploid (4 hour exposure)	62.5, 125, 250, 500 ug/ml	+ / -	Negative	1
<b><i>Salmonella typhimurium</i> Strains</b>				
TA98, TA100, TA1978, TA1535	Spot test	+ / -	Negative	2
TA1535, TA1537, TA1538, TA98, TA100	5, 10, 50, 100, 500, 1000, 5000 ug/plate	+ / -	Negative	3
TA1535, TA1537, TA1538, TA98, TA100	5, 10, 50, 100, 500, 1000, 5000 ug/plate	-	Negative	4
TA1538, TA98, TA1538	Not stated	-	Negative	5
TA1535, TA1536, TA1537, TA1538	Spot test	-	Negative	6
TA100, TA98, TA97a	41, 3256 mg/L	+ / -	Negative	7
TA100	Not stated	+ / -	Negative	8
TA1535/pSK 1002	30, 50, 100, 150, 500 ug/ml	-	Positive	9
TA98, TA97a, TA102, TA104, TA100	1, 5, 10, 20 ug/plate	+/-	Positive	10
<b><i>Escherichia coli</i> Strains</b>				
WP2 hcr	5, 10, 50, 100, 500 ug/plate	-	Negative	4
Strain Not stated	Not stated	-	Negative	11
K12 (prophage λ) in WP2s (λ)	200, 400, 500 ug/ml	-	Positive	9
K12: AB1157, AB1886, AB2494, AB2463	10, 20, 30, 40, 50 mg/ml	-	Negative	9
K12 AB1157	10, 20, 30 mg/ml	-	Negative	9
<b>Mouse Lymphoma</b>				
L5178Y TK+/-	6.25, 12.5, 18.8, 25, 37.5, 50, 75, 100 ug/ml	+ / -	Negative	12
L5178Y TK+/-	3, 6, 12.5, 25, 50, 9, 14, 18.6, 23, 28 ug/ml	-	Positive	13
<b><i>Bacillus subtilis</i></b>				
H17, M45	20, 100, 200, 500, 1000, 2000 ug/disk	-	Negative	3
<b><i>Drosophila melanogaster</i></b>				
Male Germ Cells at 48 hours	0, 150, 200 ppm	NA <sup>c</sup>	Positive	14
Male Larvae	0, 50, 100 ppm	NA <sup>c</sup>	Positive	14

a - Supernatant fraction at 9,000 x g from homogenized rat livers (contains enzymes for metabolic activation).

b - References: 1. Mellano, 1984; 2. Dorrough et al., 1978; 3. Shirasu et al., 1978; 4. Moriya et al., 1983; 5. Quito et al., 1981; 6. Adams, 1978; 7. Pednekar et al., 1987; 8. Shirasu, 1982; 9. Chaudhuri et al., 1999; 10. Bajpayee et al., 2006; 11. Fahrig, 1974; 12. Cifone, 1984a; 13. McGregor et al., 1988; 14. Velazquez et al., 1984.

c - NA = Not applicable for some *in vivo* and/or *in vitro* tests.

**Table 16. Genotoxicity of Endosulfan (Chromosome Aberration and DNA Damage Assays)**

System/Strain	Concentration/Dose	S9	Results <sup>a</sup>	Ref <sup>b</sup>
<b>Micronucleus</b>				
NMRI Mice	0.02, 1.0, 5.0 mg/kg	NA <sup>c</sup>	Negative	1
Swiss Albino	43.3 mg/kg	NA <sup>c</sup>	Negative	2
<b>Dominant Lethal</b>				
Albino Mice	5, 10 mg/kg (i.p.)	NA <sup>c</sup>	Negative	3
<i>In vivo</i> Swiss Albino Mice	9.8, 12.7, 16.6, 21.6 mg/kg	NA <sup>c</sup>	Positive	4
<b>Human Lymphocytes</b>				
<b><i>In vitro</i> cytogenetics</b>	<b>1, 10, 100, 200 ug/ml</b>	+/-	<b>Negative</b>	<b>5*</b>
<i>In vitro</i>	5, 100 ug/ml	-	Negative	6
<i>In vitro</i> LAZ-007	0.37, 3.71, 37.1 mg/kg	+	Positive	5
<i>In vivo</i>	Unknown	-	Positive	7
<b>Mouse DNA Damage</b>				
Bone Marrow (in vivo treatment)	0.2, 1.0, 5.0 mg/kg	NA <sup>c</sup>	Positive	8
Bone Marrow (in vivo treatment)	1.75, 3.5, 5.25 mg/kg	NA <sup>c</sup>	Positive	9
Bone Marrow (in vivo treatment)	1.0, 10 mg/kg	NA <sup>c</sup>	Positive	10
<b>Chinese Hamster Ovary Cells (CHO)</b>				
CHO <i>in vitro</i>	Not Stated	Unk	Negative	11
CHO <i>in vitro</i>	Not Stated	Unk	Negative	9
CHO <i>in vitro</i> : DNA damage, Comet Assay Human Lymphocytes: DNA damage, Comet	0.01, 0.05, 0.25, 1.0, 10 uM	NA	Positive	12
<b><i>In vivo</i> Rat or Mouse</b>				
Albino Mouse Spermatocytes	22, 32, 42 mg/kg	NA <sup>c</sup>	Positive	13
Rat Bone Marrow/Spermatogonia	11, 22, 36, 55 mg/kg	NA <sup>c</sup>	Negative	14
Rat Spermatocytes	11, 22, 36.6, 55 mg/kg	NA <sup>c</sup>	Negative	15
<b><i>In Vivo</i> Syrian Hamster</b>				
<i>In vivo</i> intraperitoneal treatment	8, 16, 40, 80 mg/kg	NA <sup>c</sup>	Positive	16
<b>Unscheduled DNA Synthesis in Primary Rat Hepatocytes</b>				
<b><i>In vitro</i> treatment</b>	<b>0.1, 0.25, 0.5, 1, 5, 10, 26, 51 ug/ml</b>	<b>NA<sup>c</sup></b>	<b>Negative</b>	<b>17*</b>
<b>Human RBC <i>In vitro</i></b>				
<i>In vitro</i> treatment	0.001, 0.01, 0.1, 1.0 ug/ml	NA <sup>c</sup>	Positive	18
<b><i>Drosophila Melanogaster In vivo</i></b>				
Sex-Chromosome Loss after 24 hr	0, 50, 100, 200 ppm	NA <sup>c</sup>	Positive	19
<b>Fetal Hepatocytes <i>In vitro</i></b>				
Human Blastoma, Quail & Rat Cells	50 uM	NA <sup>c</sup>	Pos & Neg <sup>d</sup>	20
<b><i>Saccharomyces cerevisiae</i></b>				
<b>Strain not stated</b>	<b>100, 500, 1000, 5000 ug/ml</b>	+ / -	<b>Negative</b>	<b>4*</b>
D7	10, 100 ug/ml	-	Positive	21
T1/PG-154, T2/PG-155	10, 100 ug/ml	-	Positive	10
Strain not stated	Not stated	-	Negative	22
<b>Sister Chromatid Exchange (SCE), Micronuclei Test (MN), DNA Strand Breaks (SB): <math>\alpha</math>- &amp; <math>\beta</math>-isomers tested separately</b>				
<b>HepG2 Cells: Human Hepatocyte Cell Line</b>	<b>10<sup>-12</sup> to 10<sup>-3</sup> M; DMSO vehicle</b>	<b>-</b>	<b><math>\alpha</math>- SCE, MN: -- ; SB: + <math>\beta</math>- SCE, MN, SB: +</b>	<b>23</b>

a - Supernatant fraction at 9,000 x g from homogenized rat livers (contains enzymes for metabolic activation).

b - References: 1. Cifone, 1983; 2. Usha Rani et al., 1980; 3. Arnold, 1972; 4. Milone & Hirsch, 1986; 5. Sobti et al., 1983; 6. Shirasu et al., 1978; 7. Rupa et al., 1989a, b; 8. Kurinnyi et al., 1982; 9. Sharma & Gautam, 1991; 10. L'vova, 1984; 11. NTP, 1988; 12. Bajpayee et al., 2006; 13. Usha Rani & Reddy, 1986; 14. Dikshith et al., 1978; 15. Dikshith & Datta, 1977; 16. Dzwonkowska & Hubner, 1986; 17. Cifone, 1984b; 18. Daniel et al., 1986; 19. Velazquez et al., 1984; 20. Dubois et al., 1996; 21. Yadav et al., 1982; 22. Fahrig, 1974; 23. Lu et al., 2000.

c - NA = Not applicable for some *in vivo* and/or *in vitro* tests.

“\* “ and **Bold** depicts studies reviewed as acceptable under current FIFRA Guidelines.

## 5. Food Quality Protection Act

There is a discussion of issues related to the FQPA in E. ISSUES RELATED TO THE FQPA. Although endosulfan has effects in the male reproductive system as has been described in this document, doses that would protect for neurotoxicity would also protect for endocrine disruption (observed only at higher doses). The USEPA is currently evaluating their position on endosulfan as an endocrine disruptor and on the use of the FQPA SF. **DPR considers that the data do not warrant the use of additional safety factors at this time.**

## B. EXPOSURE ASSESSMENT

### 1. Introduction

Exposure estimates are provided for representative occupational exposure scenarios described below in addition to ambient air and bystander scenarios. For each scenario, estimates are provided for short-term (acute and up to 1 week), seasonal (intermediate-term intervals, one week to one year) and annual (approximately 1 year). Each use pattern for occupational exposures is described in the Worker Health and Safety Exposure Document (Beauvais, 2007; Appendix E [Volume II]).

Human dietary exposure was described by the use of residue levels detected in foods to evaluate the risk from total exposure, and use of tolerance levels to evaluate the risk from exposure to individual commodities. A critical commodity analysis, based on tolerance residue values, was also done for apples, melons and tomatoes.

### 2. Occupational Exposure Assessment

#### a) Acute, short term exposures

For short-term exposures, DPR estimates the highest exposure an individual may realistically experience during or following legal endosulfan uses. For this “upper bound” of daily exposure, the estimated population 95<sup>th</sup> percentile of daily exposure is used. A higher percentile is not used because the higher the percentile the less reliably it can be estimated and the more it tends to overestimate the population value (Chaisson et al., 1999).

Assumptions for all exposure scenarios, unless otherwise indicated, were 47.3% dermal absorption, based on a rat study (see Pharmacokinetics section; Craine, 1988), a 70 kg body weight (Thongsinthusak et al., 1993), and inhalation absorption of 100% (USEPA, 2001b).

#### b) Seasonal (1 week to 1 year) and annual (1 year) exposures

To estimate seasonal and annual exposures, the average daily exposure is of interest because over these periods of time, a worker is expected to encounter a range of daily exposures (i.e., DPR assumes that with increased exposure duration, repeated daily exposure at the upper-bound level is unlikely). To estimate the average, DPR uses the arithmetic mean of daily exposure (Powell, 2003). In most instances, the mean daily exposure of individuals over time is not known. However, the mean daily exposure of a group of persons observed in a short-term study is believed to be the best available

estimate of the mean for an individual over a longer period.

### 3. Agricultural Handlers

#### a) Exposure monitoring studies

Exposure of handlers to endosulfan was monitored in three studies (Baugher, 1989; Lonsway *et al.*, 1997; Hatzilarou *et al.*, 2004) that were described in detail in Beauvais (2006). In the first study, exposure monitoring was conducted of M/L/As and applicators during airblast applications to pears and plums in California (Baugher, 1989). Too few workers were monitored for each set of conditions described in the report, resulting in insufficient replication to develop a reliable estimate of exposure. Results from this study were not used in estimating dermal exposure of handlers to endosulfan. USEPA also found this study (submitted in two different reports) to be deficient and did not use it in their exposure assessment (U.S. EPA, 2002a).

Exposure of M/Ls and applicators to endosulfan during groundboom applications to tobacco was studied in Kentucky (Lonsway *et al.*, 1997). Due to numerous deficiencies described in Beauvais (2006) this study could not be used to estimate worker exposure. USEPA (2002a) apparently did not consider this study in their exposure assessment, nor was it mentioned in the RED (USEPA, 2002b). Hatzilazarou *et al.* (2004) monitored exposure to several pesticides, including endosulfan, using filter paper discs placed on the forehead and the chest of workers spraying pesticide solutions in a greenhouse. The amount of pesticide handled was not reported in this study, a single replicate was monitored, and only partial dermal exposure monitoring was done (head and chest only). Therefore, this study could not be used to estimate worker exposure.

#### b) Exposure Estimates Using Surrogate Data (short-term, seasonal and annual)

Although no acceptable studies were available in which handler exposure to endosulfan was monitored, one acceptable study was submitted in which dermal and inhalation exposure of airblast applicators to the surrogate compound, carbaryl, was monitored (Smith, 2005). This study provided acceptable data for estimating exposure of airblast applicators driving open-cab tractors. Carbaryl was applied in three orchard crops (peaches, apples, and citrus) in three states (Georgia, Idaho, and Florida). Applicators wore either Sou'wester rain hats (15 replicates) or hooded rain jackets (10 replicates) as chemical-resistant headgear; because the jackets provided an extra layer of clothing over the torso and arms, only data from the replicates wearing rain hats were used to estimate exposure. Dermal exposure was monitored with whole-body dosimeters, face/neck wipes, hand washes and patches on the inside and outside of headgear. Inhalation exposure was monitored with breathing zone air samplers consisting of OSHA Versatile Sampler tubes, each containing glass fiber filter and XAD-2 sorbent and connected to a sampler pump calibrated to 2 liters per minute. Applicators were monitored for 5 - 8 hours each, which is about the length of a typical workday for them. Actual spray times ranged 3.3 - 5.7 hours; applicators handled 24 - 90 pounds AI (11 - 41 kg), and treated 12 - 30 acres (5 - 12 ha). Quality assurance samples consisted of laboratory control samples of each matrix, laboratory-fortified samples of each matrix, and field fortified samples of each matrix. Field fortifications (FFs) consisted of each sample matrix spiked with formulated product, and with the exception of socks all FF recoveries were in the acceptable range (70 - 120%). Results were corrected for FF recoveries below 90%.

Exposure monitoring results for airblast applicators wearing Sou'wester rain hats are summarized in Table 17. Airblast applicators are required to wear chemical-resistant headgear, as product labels require chemical-resistant headgear for overhead exposures such as occur during airblast application.

**Table 17. Exposure of Open-Cab Airblast Applicators <sup>a</sup>**

<b>Dermal Exposure</b>	<b>Exposure Rate (µg AI/lb handled)</b>
Arithmetic Mean	70.2
Standard Deviation	65.4
95 <sup>th</sup> Percentile <sup>b</sup>	276
<b>Inhalation Exposure</b>	<b>Exposure Rate (µg AI/lb handled)</b>
Arithmetic Mean	3.41
Standard Deviation	3.65
95 <sup>th</sup> Percentile <sup>b</sup>	9.54

<sup>a</sup> Summary of data from open-cab airblast exposure monitoring study (Smith, 2005). Only the 15 replicates wearing Sou'wester rain hats were included; product labels require chemical-resistant headgear for overhead exposures such as occur during airblast application. Arithmetic mean exposure rates were used to calculate long-term exposures and 95<sup>th</sup> percentile exposure rates were used to calculate short-term exposures. All estimates were rounded to 3 significant figures.

<sup>b</sup> 95<sup>th</sup> percentile estimates calculated in Excel, assuming a lognormal distribution. First the natural logarithm (ln) was calculated for each value using the LN function; arithmetic mean and standard deviation was then calculated for the natural logarithms (am(lns) and asd(lns), respectively). The NORMSINV function, with a probability of 0.95, was used to get the inverse of the standard normal cumulative distribution, which was multiplied by asd(lns). This result was added to am(lns), and the sum taken as the power of e with the EXP function.

With the exception of airblast applicators and handlers dipping nursery stock (discussed later in this section), exposure estimates were derived using the Pesticide Handler Exposure Database (PHED, 1995). PHED was developed by the USEPA, Health Canada and the American Crop Protection Association to provide non-chemical-specific pesticide handler exposure estimates for specific handler scenarios. It combines exposure data from multiple field monitoring studies of different AIs. The user selects a subset of the data having the same or a similar application method and formulation type as the target scenario. The use of non-chemical-specific exposure estimates is based on two assumptions, that exposure is primarily a function of the pesticide application method/ equipment and formulation rather than the physical-chemical properties of the specific AI, and that exposure is proportional to the amount of AI handled (Reinert et al., 1986; Versar, 1992). These assumptions are supported by comparisons of exposure across several studies (Rutz and Krieger, 1992).

When using surrogate data to estimate short-term exposure, DPR uses the 90% upper confidence limit (UCL) on the 95<sup>th</sup> percentile. The UCL is used to account for some of the uncertainty inherent in using surrogate data and to increase the confidence in the estimate. (Confidence limits on percentiles, also called tolerance limits, are described by Hahn and Meeker (1991).) Estimating the confidence limit requires knowing the mean and standard deviation. PHED reports the mean of total dermal exposure, but only the coefficients of variation for separate body regions. Because the sample sizes per body region differ and because the correlations among body regions are unknown, the standard deviation of total dermal exposure cannot be calculated. In order to approximate the confidence limit for the 95<sup>th</sup> percentile, DPR makes the assumption that total exposure is lognormally distributed across persons and has a coefficient of variation of 100 percent. The approximation (Powell, 2002) uses the fact that in any lognormal distribution with a given coefficient of variation, the

confidence limit for the 95<sup>th</sup> percentile is a constant multiple of the arithmetic mean. The value of the multiplier depends only on sample size. To use the approximation with PHED data, the multiplier corresponding to the sample size is used (for dermal exposure, the median number of observations over body regions is used). If the sample size is between 20 and 119, the multiplier is 4; if it is between 12 and 19, the multiplier is 5 (Powell, 2002). Assumptions used in exposure calculations, results of PHED subsets, and short-term handler exposure estimates for workers handling endosulfan in support of aerial and ground applications are given in Table 18.

When using surrogate data to estimate seasonal or annual exposure, DPR uses the 90% UCL on the arithmetic mean. The 90% UCL is used for the reasons listed in the previous paragraph. As with short-term exposure estimates based on PHED subsets, a multiplier corresponding to the median sample size over body regions is used. If the median sample size is greater than 15, the multiplier is 1 (Powell, 2002).

Handlers of endosulfan are required to wear protective clothing and personal protective equipment (PPE), as described in the Label Precautions and California Requirements section. Clothing and PPE have been shown to reduce exposure to pesticides (Thongsinthusak *et al.*, 1991), and default protection factors are used by DPR to adjust exposure estimates. For M/Ls, exposure estimates were provided for WP in both WSP and non-WSP packaging. USEPA (2002a) would require all WP to be packaged in WSP, and non-WSP packaging is being phased out. However, as of September 2006, non-WSP products were available in California.

Surrogate data from the PUR also were used to estimate intervals for seasonal and annual exposures. Endosulfan is registered for use on several different crops, and for many crops repeated use is allowed within a growing season, suggesting that handlers may potentially be exposed throughout the year. Repeated exposures are especially likely for professional applicators and their employees, as these handlers can make the same treatment for several growers. However, PUR data show that in many parts of the state and in many crops endosulfan use does not occur throughout the year, and that at other times relatively few applications are made. It is reasonable to assume that an individual handler is less likely to be exposed to endosulfan during these relatively low-use intervals. Thus, rather than assume that handlers are exposed throughout the year, annual use patterns are plotted based on monthly PUR data from one or more counties with the highest use. Annual exposure to endosulfan is assumed to be limited to the months when use is relatively high (defined as 5% or more of annual use each month). Seasonal, annual, and lifetime exposure estimates for workers handling endosulfan in support of aerial and ground applications are given in Table 18. The occupational exposure values reported below are for total (dermal + inhalation), when applicable, for STADD, SADD and AADD.

USEPA (2002b) assumed that handler exposure durations would only be one day to one month. The basis for this assumption was not explained.

#### i. Aerial applications

Table 18 summarizes PHED data used in exposure estimates for mixer/loaders (M/Ls) and for applicators, as well as short-term exposure estimates. Separate dermal and inhalation exposures are provided along with total exposure estimates. Exposure estimates for handlers involved in aerial applications assumed a closed system for the M/L and that certain handlers (M/Ls and flaggers) wear the clothing specified on the product label: long-sleeved shirt and pants, waterproof gloves, and shoes

and socks (see Beauvais, 2007; Appendices 2-5). Applicators (pilots) are not required to wear gloves during an application (3 CCR 6738), and were assumed to wear no gloves. Open cockpits were assumed for pilots, as there is no requirement for closed cockpits during applications. STADD are 0.790 mg/kg/day and 0.373 mg/kg/day for aerial applicators and flaggers, respectively.

Short-term absorbed daily dosage (STADD) estimates for M/Ls range 0.185-2.63 mg/kg/day, for M/Ls handling EC and WP formulations (Note that mitigation measures proposed in USEPA (2002) would require all WP to be packaged in WSP) (Table 18).

To estimate seasonal and annual exposure of workers involved in aerial applications of endosulfan, temporal patterns were investigated by plotting percent of annual use based on pounds applied per month for the most recent five years for which data are available. Seasonal absorbed daily dosage (SADD) estimates for M/Ls range from 0.034-0.385 mg/kg/day, for M/Ls handling EC and WP formulations (Table 19).

Annual absorbed daily dosage (AADD) estimates for M/Ls range from 0.011-0.128 mg/kg/day, for M/Ls handling EC and WP formulations (Table 19).

#### ii. Airblast applications

Table 18 summarizes PHED data used in exposure estimates and STADD for handlers in support of airblast applications of endosulfan. Exposure estimates for handlers involved in airblast applications assumed an open system for the M/L and that all handlers wear the clothing and PPE specified on the product label: long-sleeved shirt and pants, waterproof gloves, shoes and socks, and a respirator. Open cabs were assumed for applicators, as there is no requirement for closed cabs during applications. STADD for M/Ls range 0.026 - 0.300 mg/kg/day. The applicator STADD is 0.188 mg/kg/day (Table 18).

Seasonal absorbed daily dosage (SADD) estimates for airblast range from 0.006 - 0.073 mg/kg/day, for airblast M/Ls handling EC and applicators (Table 19).

Annual absorbed daily dosage (AADD) estimates for airblast range from 0.001 - 0.012 mg/kg/day, for M/Ls handling EC and WP formulations and for M/Ls handling WSP, respectively (Table 19).

#### iii. Groundboom Applications

Exposure estimates for handlers involved in groundboom applications assumed a closed system for the M/L and that all handlers wear the clothing and PPE specified on the product label: long-sleeved shirt and pants, waterproof gloves, shoes and socks, and respirator. Open cabs were assumed for applicators, as there is no requirement for closed cabs during applications. STADD for M/Ls range 0.041 - 0.480 mg/kg/day. The applicator STADD is 0.045 mg/kg/day (Table 18).

Seasonal absorbed daily dosage (SADD) estimates for groundboom range from 0.005 - 0.088 mg/kg/day, for groundboom applicators and for M/Ls handling WP (Table 19).

Annual absorbed daily dosage (AADD) estimates for groundboom range from 0.002 - 0.037 mg/kg/day, for applicators and for M/Ls handling WP formulations (Table 19).

**Table 18. Exposure Rates Calculated from Surrogate Data and Short-Term Exposure Estimates for Workers Handling Endosulfan in Support of Aerial and Ground Applications <sup>a</sup>**

Scenario	# <sup>b</sup>	Short-Term Exposure Rates <sup>c</sup> (ug/lb AI handled)		Long-Term Exposure Rates <sup>d</sup> (ug/lb AI handled)		STADD <sup>e</sup> (mg/kg/day)		
		Dermal	Inhalation	Dermal	Inhalation	Dermal	Inhalation	Total
<b>Aerial<sup>f</sup></b>								
M/L - EC	3	37.0	0.512	9.24	0.128	0.219	0.006	0.225
M/L - WP <sup>g</sup>	4	392	24.7	98.0	4.94	2.32	0.309	2.63
M/L - WP/WSP	5	28.4	1.38	11.3	0.554	0.168	0.017	0.185
Applicator	6	133	0.286	44.3	0.115	0.786	0.004	0.790
Flagger	7	62.8	0.080	16.0	0.020	0.371	0.002	0.373
<b>Airblast<sup>h</sup></b>								
M/L - EC	3	37.0	0.512	9.24	0.128	0.025	0.001	0.026
M/L - WP	4	276	24.7	70.2	4.94	0.265	0.035	0.300
M/L - WSP	5	28.4	1.38	11.3	0.554	0.019	0.002	0.021
Applicator	--	70.2	9.54	276	3.41	0.187	0.001	0.188
<b>GB<sup>i</sup></b>								
M/L - EC	3	37.0	0.512	9.24	0.128	0.040	0.001	0.041
M/L - WP <sup>g</sup>	4	392	24.7	98.0	4.94	0.424	0.056	0.480
M/L - WSP	5	28.4	1.38	11.3	0.554	0.031	0.003	0.034
Applicator	8	40.6	0.472	6.04	0.118	0.044	0.001	0.045

<sup>a</sup> All scenarios except airblast applicator were based on data from the Pesticide Handlers Exposure Database (PHED, 1995). Airblast applicator exposure based on data from Smith (2005), shown in Table 17. Exposure rates and exposure estimates were rounded to three significant figures. Abbreviations: EC = emulsifiable concentrate. GB = groundboom. M/L = mixer/loader. WP = wettable powder. WSP = water-soluble packaging.

<sup>b</sup> Appendix number with details from PHED. Handlers were assumed to wear gloves as specified on product labels, except aerial applicators (exempt from wearing gloves under California law); respirator (except M/L using a closed system); and coveralls. M/L assumed to wear chemical-resistant apron. Protection factors given in appendices.

<sup>c</sup> These exposure rates were used to calculate STADD, as explained in Footnote <sup>e</sup>.

<sup>d</sup> These exposure rates used to calculate Seasonal Average Daily Dosage & Annual Average Daily Dosage in Table 18.

<sup>e</sup> Short-Term Absorbed Daily Dosage (STADD) is an upper-bound estimate calculated from the short-term exposure. Application rate is maximum rate on product labels, which varied for each scenario; acres treated per day varies by scenario. Estimates were rounded to three significant figures. Calculation: STADD = [(short-term exposure) x (absorption) x (acres treated/day) x (application rate)]/(70 kg body weight). Calculation assumptions include: Dermal absorption = 47.3% (Craine, 1988); Body weight = 70 kg (Thongsinthusak, *et al.*, 1993); Inhalation rate 16.7 L/min (Andrews and Patterson, 2000); Inhalation absorption = 100%.

<sup>f</sup> STADD estimates assumed 350 acres (142 ha) treated/day (USEPA, 2001D), and a maximum application rate of 2.5 lbs AI/acre (2.8 kg AI/ha), maximum rate on tree nuts.

<sup>g</sup> Data from open pouring mixing/loading used in exposure estimate. USEPA (2002a) would require all WP to be packaged in WSP, and non-WSP packaging is being phased out.

<sup>h</sup> STADD estimates assumed 40 acres (16 ha) treated/day (USEPA, 2001), and a maximum application rate of 2.5 lbs AI/acre (2.8 kg AI/ha), maximum rate on tree nuts.

<sup>i</sup> STADD estimates assumed 80 acres (32 ha) treated/day (USEPA, 2001D), and a maximum application rate of 2.0 lb AI/acre (2.2 kg AI/ha), maximum rate on strawberry, pineapple, or crucifers for seed only.

**Table 19. Seasonal and Annual Estimates for Workers Handling Endosulfan in Support of Aerial and Ground Applications**

Scenario <sup>a</sup>	SADD <sup>b</sup> (mg/kg/day)			AADD <sup>c</sup> (mg/kg/day)		
	Dermal	Inhalation	Total	Dermal	Inhalation	Total
<b>Aerial <sup>d</sup></b>						
M/L EC	0.033	0.001	0.034	0.011	0.0003	0.011
M/L WP <sup>e</sup>	0.348	0.037	0.385	0.116	0.012	0.128
M/L WSP	0.040	0.004	0.044	0.014	0.001	0.015
Applicator	0.157	0.001	0.158	0.053	0.0003	0.053
Flagger	0.057	0.0002	0.057	0.019	0.00005	0.019
<b>Airblast <sup>f</sup></b>						
M/L EC	0.006	0.0002	0.006	0.001	0.00003	0.001
M/L WP <sup>e</sup>	0.066	0.007	0.073	0.011	0.001	0.012
M/L WSP	0.007	0.001	0.008	0.001	0.0001	0.001
Applicator	0.047	0.0005	0.048	0.008	0.00008	0.008
<b>GB <sup>g</sup></b>						
M/L EC	0.008	0.0002	0.008	0.003	0.0001	0.003
M/L WP <sup>e</sup>	0.080	0.008	0.088	0.033	0.004	0.037
M/L WSP	0.009	0.001	0.010	0.004	0.0004	0.004
Applicator	0.005	0.0002	0.005	0.002	0.0001	0.002

<sup>a</sup> Abbreviations: EC = emulsifiable concentrate. M/L = mixer/loader. M/L/A = mixer/loader/applicator. WP = wettable powder. WSP = water soluble packaging containing wettable powder.

<sup>b</sup> Seasonal Average Daily Dosage is a 90% upper confidence estimate calculated from the long-term exposure estimate given in Table 16. Dermal absorption: 47.3% (Craine, 1988). Inhalation absorption assumed to be 100%. Body weight assumed to be 70 kg (Thongsinthusak *et al.*, 1993). Calculation: SADD = [(long-term exposure) x (absorption) x (acres treated/day) x (application rate)]/(70 kg body weight).

<sup>c</sup> Annual Average Daily Dosage = SADD x (annual use months per year)/(12 months in a year).

<sup>d</sup> Exposure estimates assumed 350 acres (142 ha) treated/day (USEPA, 2001D), and an application rate of 1.5 lbs a.i./acre (1.7 kg a.i./ha), maximum rate on collards, cotton, grapes, lettuce, sweet corn and tomatoes. Annual exposure estimate based on high-use period of 4 months.

<sup>e</sup> Data from open pour mixing/loading used in exposure estimate. USEPA (2002a) would require all WP to be packaged in WSP, and non-WSP packaging is being phased out. As of March 2005, non-WSP products were available in Calif.

<sup>f</sup> Exposure estimates assumed 40 acres (16 ha) treated/day (USEPA, 2001D), and a maximum application rate of 2.5 lbs a.i./acre (2.8 kg a.i./ha), max rate on tree fruits. Annual exposure estimate based on high-use period of 2 mos.

<sup>g</sup> Exposure estimates assumed 80 acres (32 ha) treated/day (U.S. EPA, 2001), and a maximum application rate of 1.5 lb a.i./acre (1.7 kg a.i./ha), maximum rate on sweet corn, collards, cotton, and lettuce. Annual exposure estimate based on high-use period of 5 months

#### iv. Backpack Applications

Table 20 summarizes PHED data used in exposure estimates and STADD for handlers applying endosulfan with a backpack sprayer. In its exposure scenarios for M/L/As using backpack sprayers, USEPA (2002) assessed use on three crops, greenhouse tomatoes, tobacco, and cherries. In California, the highest exposure estimates are associated with applications to macadamia nuts. Assumptions used in estimating the exposure, and estimates obtained from PHED, are summarized in Table 20. The STADD is 0.043 mg/kg/day.

Although the highest use rate for backpack sprayers is on macadamia nuts, examination of PUR data shows that endosulfan has infrequently been applied to these crops (DPR, 2005a; data not shown).

Because of this, ground applications of endosulfan to apricots, nectarines, peaches, and pecans were used instead for seasonal and annual exposure estimates.

Seasonal absorbed daily dosage (SADD) estimates for backpack M/L/A-EC were 0.011 mg/kg/day and AADD was 0.002 mg/kg/day (Table 21).

**Table 20. Data Used and Short-Term Exposure Estimates for Handlers Using Handheld Equipment**

Scenario <sup>a</sup>	Short-term Exposure <sup>b</sup> (ug/lb a.i. handled)		Long-term Exposure <sup>b</sup> (ug/lb a.i. handled)		STADD <sup>c</sup> (mg/kg/day)		
	Dermal	Inhalation	Dermal	Inhalation	Dermal	Inhalation	Total
<b>Backpack <sup>d</sup></b>							
M/L/A EC	16,000	10.5	5,320	3.50	0.043	0.0001	0.043
<b>High Pressure Hand Wand <sup>e</sup></b>							
M/L/A EC	7,400	75.5	2,960	30.2	0.501	0.010	0.511
<b>Low Pressure Hand Wand <sup>d</sup></b>							
M/L/A EC	4,720	13.7	1,570	4.56	0.013	0.0001	0.013
M/L/A WP	35,800	520	7,160	104	0.097	0.003	0.100
<b>Dip <sup>f</sup></b>							
M/L EC	37.0	0.512	--	--	0.00003	0.000001	0.00003
M/L WP	392	24.7	--	--	0.0003	0.00004	0.003
Applicator	--	--	--	--	41.4	0.005	41.4

- <sup>a</sup> -Abbreviations: EC = emulsifiable concentrate. M/L = mixer/loader. M/L/A = mixer/loader/applicator. WP = wettable powder. Handlers were assumed to wear gloves, respirator, & coveralls, as specified on product labels (Beauvais, 2007)
- <sup>b</sup> Dermal and inhalation exposure calculated from surrogate data using the Pesticide Handlers Exposure Database (PHED) database and software (PHED, 1995). Values from PHED were rounded to three significant figures.
- <sup>c</sup> Short-Term Absorbed Daily Dosage (STADD) is an upper-bound estimate calculated from the short-term exposure. Application rate is maximum rate on product labels, which varied for each scenario; acres treated per day varies by scenario. Estimates were rounded to three significant figures. Calculation: STADD = [(short-term exposure) x (absorption) x (acres treated/day) x (application rate)]/(70 kg body weight). Calculation assumptions include: Dermal absorption = 47.3% (Craine, 1988); Body wt = 70 kg (Thongsinthusak, *et al.*, 1993); Inhalation absorption = 100%
- <sup>d</sup> STADD estimates assumed handling of 40 gal/day (150 l/day; USEPA, 2001D), containing 1.0 lb a.i./100 gal (0.12 kg a.i./100 liters; maximum application for macadamia nuts), for a total of 0.4 lb a.i./day (0.2 kg a.i./day).
- <sup>e</sup> STADD estimates assumed handling of 1,000 gal/day (3,800 l/day; USEPA, 2001D), containing 1.0 lb a.i./100 gal (0.12 kg a.i./100 l; maximum application for macadamia nuts), for a total of 10 lb a.i./day (4.5 kg a.i./day).
- <sup>f</sup> STADD estimates assumed handling of 40 gal/day, containing 1.25 lb a.i./40 gal (0.15 kg a.i./40 l), for a total of 1.25 lb a.i./day (0.56 kg a.i./day). M/L estimates from PHED. Applicator dermal exposure estimates based on RAGS-E equations (USEPA, 2004a). Applicator inhalation exposure estimates based on SWIMODEL (USEPA, 2003), assuming a saturated endosulfan vapor concentration. See Beauvais (2007) for calculations of applicator exposure estimate<sup>a</sup> Abbreviations: EC = emulsifiable concentrate. M/L = mixer/loader; M/L/A = mixer/loader/applicator; WP= wettable powder; WSP = water soluble packaging containing wettable powder.

v. High Pressure Handwand Applications

Table 20 summarizes PHED data used in exposure estimates and STADD for handlers applying endosulfan with a high-pressure handwand. High-pressure handwands can be used to apply endosulfan to the same crops as backpack sprayers. Exposure was estimated for this scenario using the same assumptions as for the backpack sprayer, except that greater amounts are typically handled with high-pressure handwands. Assumptions used in estimating the exposure, and estimates obtained from PHED, are summarized in Table 21. The STADD is 0.511 mg/kg/day, the SADD is 0.153 mg/kg/day and the AADD is 0.026 mg/kg/day.

**Table 21. Seasonal & Annual Exposure Estimates for Endosulfan Handlers Using Handheld Equipment**

Scenario <sup>a</sup>	SADD <sup>b</sup> (mg/kg/day)			AADD <sup>c</sup> (mg/kg/day)		
	Dermal	Inhalation	Total	Dermal	Inhalation	Total
<b>Backpack<sup>d</sup></b>						
M/L/A	0.011	0.00002	0.011	0.002	0.000003	0.002
<b>High Pressure Handwand<sup>e</sup></b>						
M/L/A	0.150	0.003	0.153	0.025	0.001	0.026
<b>Low Pressure Handwand<sup>d</sup></b>						
M/L/A EC	0.003	0.00002	0.003	0.0005	0.000003	0.0005
M/L/A WP	0.015	0.0004	0.015	0.003	0.0001	0.003

<sup>a</sup> No seasonal or annual exposure is anticipated for workers dipping nursery stock; that scenario is omitted from this table. Abbreviations: EC = emulsifiable concentrate. M/L = mixer/loader. M/L/A = mixer/loader/applicator. WP = wettable powder. Handlers were assumed to wear gloves, respirator, and coveralls, as specified on product labels (Beauvais, 2007)

<sup>b</sup> Seasonal Average Daily Dosage is a 90% upper confidence estimate calculated from the long-term exposure estimate (Beauvais, 2007). Application rate is maximum rate on product labels, which varied for each scenario; acres treated per day varies by scenario. Dermal absorption assumed to be 47.3% (Craine, 1988). Inhalation absorption assumed to be 100%. Body weight assumed to be 70 kg (Thongsinthusak *et al.*, 1993). Calculation: SADD = [(long-term exposure) x (absorption) x (acres treated/day) x (application rate)]/(70 kg body weight)

<sup>c</sup> Annual Average Daily Dosage = SADD x (annual use months per year)/(12 months in a year).

<sup>d</sup> Estimates assumed handling of 40 gal/day (150 l/day; US EPA, 2001), containing 0.75 lb a.i./100 gal (0.09 kg a.i./100 l; maximum application for apricots, nectarines, peaches, and pecans), for a total of 0.3 lb a.i./day (0.14 kg a.i./day). Annual exposure estimate based on high-use period of 2 months.

<sup>e</sup> Estimates assumed handling of 1,000 gal/day (3,800 l/day; USEPA, 2001D), containing 0.75 lb a.i./100 gal (0.09 kg a.i./100 l; maximum application for apricots, nectarines, peaches, and pecans), for a total of 7.5 lb a.i./day (3.4 kg a.i./day). Annual exposure estimate based on high-use period of 2 months.

vi. Low Pressure Handwand Applications

Table 21 summarizes PHED data used in exposure estimates and STADD for handlers applying endosulfan with a low-pressure handwand. Low-pressure handwands can be used to apply EC endosulfan products to the same crops as backpack sprayers. Exposures were estimated using the same assumptions as for the backpack sprayer. Other assumptions used in estimating the exposure, and estimates obtained from PHED, are summarized in Table 21. The STADD is 0.013 mg/kg/day for M/L/As handling EC products and 0.100 mg/kg/day for M/L/As handling WP endosulfan products. The SADDs are 0.003 and 0.015 mg/kg/day for M/L/A with EC and WP, respectively. AADDs are 0.005 and 0.003 for M/L/A with EC and WP, respectively.

vii. Nursery Stock Dip

Nursery stock dipping may be done for treatment of cherry, peach and plum seedlings for peachtree borer. Examination of PUR data shows that endosulfan is infrequently applied to nursery stock (DPR, 2005b). Therefore, seasonal and annual exposures to endosulfan are not anticipated to occur during activities in these crops, and only short-term exposures were estimated. For M/L exposure estimates a closed-system was assumed, as required under California law (3 CCR 6746). Most of the applicator exposure is anticipated to be to hands and inhalation exposure is also anticipated to occur, assuming that dipping tanks have a free liquid surface from which chemicals can volatilize

into the air. There are; however, no dermal or inhalation monitoring data available for workers dipping nursery stock.

STADD for M/Ls are 0.0001 mg/kg/day and 0.002 mg/kg/day for M/Ls handling EC and WP products, respectively. STADD are 41.4 mg/kg/day for applicators (Table 21).

#### viii. Reentry Exposure

##### 1) Overview

Representative exposure scenarios for reentry workers were selected as described above in the Exposure Scenarios section. As exposure data were not available for workers reentering crops treated with endosulfan, exposures were estimated from DFR values summarized in Beauvais (2006) from studies with surrogate chemicals (i.e., it was assumed that residue transfer is not chemical-specific). For summary data, see Tables 22 and 23, below.

The major route of pesticide exposure for reentry workers is the dermal route; contact with treated surfaces, especially foliage, causes pesticide residues to be transferred to the skin. The TC is a parameter- estimating rate of contact between the worker and treated surface, based on empirical data from studies in which both DFR and dermal exposure have been measured. The TC for an activity is calculated by dividing DFR from a treated crop into the dermal exposure measured for workers performing reentry activities in the crop:  $TC (cm^2/hr) = [dermal\ exposure\ (\mu g/hr)]/[DFR\ (\mu g/cm^2)]$ . As the TC depends on the intensity of contact with the contaminated surface, it is activity- and surface-specific; however, TCs are only available for a limited number of activities and crops. When specific TCs were not available, TCs from similar crops and activities were used instead.

The absorbed daily dosage (ADD) was calculated as shown in the equation below (Zweig *et al.*, 1984; Zweig *et al.*, 1985), using the dermal absorption rate (DA) of 47.3%, based on Craine (1988); default exposure duration (ED) of 8 hours; and default body weight (BW) of 70 kg (Thongsinthusak *et al.*, 1993). Short-term exposure estimates for fieldworkers are given in Table 22, reported as mg/kg/day (a conversion of 1 mg = 1,000  $\mu$ g was done).

$$ADD (ug/kg/day) = \frac{DA \times DFR (ug/cm^2) \times TC (cm^2/hr) \times ED (hrs/day)}{BW (kg)}$$

Reentry workers are not required to wear protective clothing unless entering fields before expiration of the restricted entry interval (REI). Because a lot of reentry work occurs in hot weather and for several hours per day, protective clothing is often not worn by fieldworkers unless required for early reentry. Therefore, fieldworker exposure estimates were based on an assumption that no protective clothing or equipment was used.

Scouting may occur at any time, and was assumed to occur after all applications. Information about when other reentry activities might occur was obtained from crop profiles prepared by the University of California Cooperative Extension and the Vegetable Research and Information Center (UCCE, 2004; VRIC, 2004), and from the California Farm Worker Activity Profile (CFWAP; Edmiston *et al.*, 1999). CFWAP is a DPR database compiled from a number of sources, including the California Employment Development Department, U.S. Department of Agriculture, California

Department of Food and Agriculture and the University of California Cooperative Extension. CFWAP includes information on harvested acreage, cultural practices necessary to grow a crop, and the dates of peak and overall activity periods for work activities such as harvesting and thinning, based on data from 1994. More recent data are not available at the present time.

Short-term exposures were estimated at the expiration of the 2-day restricted entry interval (REI) for all activities except hand harvesting, which was estimated at the expiration of the pre-harvest intervals (PHI); if PHI was less than 2 days, then the REI was used. For seasonal and annual exposure estimates, it was assumed that workers would enter fields at some average time after the expiration of the REI or PHI, based on how frequently specific activities generally occur in general crop types (UCCE, 2004). For longer-term exposure estimates it was assumed that workers would not always enter fields at the expiration of the REI. Seasonal and annual exposures were estimated at an assumed average reentry of REI (or PHI, if longer than REI) plus 7-10 days. These assumed averages were not based on data; rather, they were based on the reasonable, conservative assumption that workers may enter fields an average of 7-10 days after expiration of the REI or PHI. Table 23 contains seasonal and annual exposures estimates for reentry activities.

Most reentry activities are not expected to result in pesticide exposure throughout the year. This is true because pesticides like endosulfan are not necessarily applied all year in all crops, and because many activities are performed only seasonally. To estimate when endosulfan applications might occur throughout the year, five-year averages were plotted of monthly PUR data (numbers of acres treated) for endosulfan applications to the crops of interest in one or more high-use counties. These average use patterns were compared to information about when reentry activities might occur. Annual exposure to endosulfan is assumed to be limited to the months when activities overlap relatively high use (defined as 5% or more of annual use each month). See Table 23.

## 2) Thinning Almonds

The REI following endosulfan applications to almonds is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 1,500 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.009 mg/kg/day.

Examination of PUR data shows that endosulfan is infrequently applied to almonds and other tree nuts (DPR, 2006; data not shown). Therefore, seasonal and annual exposures to endosulfan are not anticipated to occur during activities in these crops.

## 3) Hand Harvesting Broccoli

The PHI following endosulfan applications to broccoli is 7 days. For exposure estimates, the estimated DFR 7 days post-application was used, as well as a TC of 5,000 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.030 mg/kg/day.

The SADD for hand-harvesting broccoli was 0.008 mg/kg/day and the AADD was 0.001 mg/kg/day.

4) Scouting Broccoli

The REI following endosulfan applications to broccoli is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 4,000 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.084 mg/kg/day.

SADD for broccoli scouting was 0.012 mg/kg/day and the AADD was 0.004 mg/kg/day.

5) Thinning Citrus

The REI following endosulfan applications to citrus is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 3,000 cm<sup>2</sup>/hr (Dawson, 2003). The STADD is 0.055 mg/kg/day.

Examination of PUR data shows that endosulfan is infrequently applied to citrus (DPR, 2006; data not shown). Therefore, seasonal and annual exposures to endosulfan are not anticipated to occur during activities in these crops.

6) Hand Harvesting Sweet Corn

The PHI following endosulfan applications to sweet corn is one day. However, the REI is 2 days, and workers would not reenter a treated field to harvest before expiration of the REI without wearing additional PPE. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 17,000 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.533 mg/kg/day.

The SADD for hand-harvesting sweet corn was 0.075 mg/kg/day and the AADD was 0.006 mg/kg/day.

7) Scouting Cotton

The REI following endosulfan applications to cotton is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used. Transfer factors were derived from a series of studies in which several organophosphates were applied to cotton (Ware et al., 1973, 1974, 1975). Geometric mean transfer factors were computed for bare hands (950 cm<sup>2</sup>/hr), the clothed upper body (102 cm<sup>2</sup>/hr), and the clothed lower body (964 cm<sup>2</sup>/hr). The potential dermal transfer factor for the whole body of cotton scouts (2,000 cm<sup>2</sup>/hr) was calculated by summing these individual geometric mean transfer factors (Dong, 1990). STADD for scouting in cotton is 0.063 mg/kg/day.

The SADD for scouting cotton was 0.009 mg/kg/day and the AADD was 0.001 mg/kg/day.

8) Hand Harvesting Cucumbers

The PHI following endosulfan applications to cucumbers is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 2,500 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.053 mg/kg/day.

The SADD for hand-harvesting cucumbers was 0.007 mg/kg/day and the AADD was 0.001 mg/kg/day.

9) Cane Turning/Leaf Pulling in Grapes

The REI following endosulfan applications to grapes is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 10,000 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.335 mg/kg/day.

The SADD for cane turning/leaf pulling in grapes was 0.141 mg/kg/day and the AADD was 0.047 mg/kg/day.

10) Scouting Lettuce

The REI following endosulfan applications to lettuce is 2 days. To calculate exposure estimates, a DFR of 2.0 µg/cm<sup>2</sup> was used, as well as a TC of 1,500 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.162 mg/kg/day.

The SADD for scouting lettuce was 0.004 mg/kg/day and the AADD was 0.002 mg/kg/day.

11) Hand Harvesting Ornamentals - Flowers

There is no PHI specified following endosulfan applications to ornamental plants, as these are not used for food (PHI are based on residue levels in food crops). The REI following endosulfan applications is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 7,000 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.159 mg/kg/day.

Examination of PUR data suggests that endosulfan is infrequently applied to nursery and greenhouse-grown flowers (DPR, 2006; data not shown). Therefore, seasonal and annual exposures to endosulfan are not anticipated to occur during activities in these crops.

12) Hand Harvesting Ornamental Plants - Trees and Shrubs

There is no PHI specified following endosulfan applications to ornamental plants, as these are not used for food (PHI are based on residue levels in food crops). The REI following endosulfan applications is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 400 cm<sup>2</sup>/hr (Klönne et al., 2000). The STADD is 0.009 mg/kg/day.

Examination of PUR data suggests that endosulfan is infrequently applied to container-grown ornamentals (DPR, 2006; data not shown). Therefore, seasonal and annual exposures to endosulfan are not anticipated to occur during activities in these crops.

13) Thinning Peaches

The REI following endosulfan applications to peaches is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 3,000 cm<sup>2</sup>/hr (Dawson, 2003). STADD is 0.055 mg/kg/day.

The SADD for thinning peaches was 0.028 mg/kg/day and the AADD was 0.005 mg/kg/day.

14) Scouting Potatoes

The REI following endosulfan applications to potatoes is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 1,500 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.032 mg/kg/day.

The SADD for scouting potatoes was 0.004 mg/kg/day and for AADD was 0.002 mg/kg/day.

15) Hand Harvesting Strawberries

The PHI following endosulfan applications to strawberries is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 1,500 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.067 mg/kg/day.

Examination of PUR data suggests that endosulfan is infrequently applied to strawberries (DPR, 2006; data not shown). Therefore, seasonal and annual exposures to endosulfan are not anticipated to occur during activities in these crops.

16) Hand Harvesting Tomatoes

The PHI following endosulfan applications to tomatoes is 2 days. For exposure estimates, the estimated DFR 2 days post-application was used, as well as a TC of 1,000 cm<sup>2</sup>/hr (USEPA, 2000a). The STADD is 0.021 mg/kg/day.

SADD for hand-harvesting tomatoes was 0.009 mg/kg/day and the AADD was 0.003 mg/kg/day.

**Table 22. Short-term Exposures to Endosulfan Estimated for Reentry Workers**

Exposure scenario	DFR ( $\mu\text{g}/\text{cm}^2$ ) <sup>a</sup>	TC ( $\text{cm}^2/\text{hr}$ ) <sup>b</sup>	STADD ( $\text{mg}/\text{kg}/\text{day}$ ) <sup>c</sup>
Almond, Thinning	0.34	500	0.009
Broccoli, Hand Harvesting	0.22	5,000	0.030
Broccoli, Scouting	0.39	4,000	0.084
Citrus, Thinning	0.34	3,000	0.055
Sweet Corn, Hand Harvesting	0.58	17,000	0.533
Cotton, Scouting	0.58	2,000	0.063
Cucumber, Hand Harvesting	0.39	2,500	0.053
Grape, Cane Turning	0.62	10,000	0.335
Lettuce, Scouting	2.00	1,500	0.162
Ornamental Plants, Hand Harvesting	0.42	400	0.009
Peach, Thinning	0.34	3,000	0.055
Potato, Scouting	0.39	1,500	0.032
Strawberry, Hand Harvesting	0.83	1,500	0.067
Tomato, Hand Harvesting	0.39	1,000	0.021
Ornamental Cut Flowers, Hand Harvest	0.42	7,000	0.159

<sup>a</sup> Dislodgeable foliar residue (DFR) values from Table 12 in Beauvais, 2007.

<sup>b</sup> Transfer coefficient (TC) is rate of skin contact with treated surfaces. TC references: Cotton scouting (Dong, 1990); citrus and peach (Dawson, 2003); ornamental plants (Klonne *et al.*, 2000); all other crops (U.S. EPA, 2000).

<sup>c</sup> Short-term Absorbed Daily Dosage (STADD) calculated as described in text. Exposure estimates are for dermal route, as inhalation route assumed to be insignificant. Assumptions include: Exposure duration = 8 hr; Dermal Absorption = 47.3% (Craine, 1988); Body weight = 70 kg (Thongsinthusak *et al.*, 1993)

**Table 23. Seasonal and Annual Exposures to Endosulfan Estimated for Reentry Workers<sup>a</sup>**

Exposure scenario	DFR ( $\mu\text{g}/\text{cm}^2$ ) <sup>b</sup>	SADD ( $\text{mg}/\text{kg}/\text{day}$ ) <sup>c</sup>	AADD ( $\text{mg}/\text{kg}/\text{day}$ ) <sup>d</sup>
Broccoli, Hand Harvesting <sup>e</sup>	0.029	0.008	0.001
Broccoli, Scouting <sup>f</sup>	0.055	0.012	0.004
Sweet Corn, Hand Harvesting <sup>g</sup>	0.082	0.075	0.001
Cotton, Scouting <sup>e</sup>	0.082	0.009	0.002
Cucumber, Hand Harvesting <sup>e</sup>	0.055	0.007	0.001
Grape, Cane Turning <sup>f</sup>	0.26	0.141	0.047
Lettuce, Scouting <sup>h</sup>	0.055	0.004	0.002
Peach, Thinning <sup>e</sup>	0.17	0.028	0.005
Potato, Scouting <sup>i</sup>	0.055	0.004	0.002
Tomato, Hand Harvesting <sup>f</sup>	0.17	0.009	0.003

a - No seasonal or annual exposure estimates were prepared for workers reentering treated almond or citrus orchards or strawberry fields. Infrequent endosulfan use is reported on these crops.

b - Dislodgeable foliar residue (DFR) values in Beauvais (2007).

c - Seasonal Average Daily Dosage is a mean estimate of absorbed dose, calculated as described in text. Exposure estimates are for dermal route, as inhalation route assumed to be insignificant. Transfer coefficients in Beauvais (2007).

d - Annual Average Daily Dosage = ADD x (annual use months per year)/(12 months in a year).

e - Annual exposure estimate based on high-use period of 2 months.

f - Annual exposure estimate based on high-use period of 4 months.

g - Annual exposure estimate based on high-use period of 1 month.

h - Annual exposure estimate based on high-use period of 5 months

i - Annual exposure estimate based on high-use period of 6 months.

#### 4. Dietary Exposure

##### a) Overview

DPR evaluates the risk of human exposure to an active ingredient in the diet using two processes: (1) use of residue levels detected in foods to evaluate the risk from total exposure, and (2) use of tolerance levels to evaluate the risk from exposure to individual commodities (Carr, 2006; Appendix C see Section VI). For evaluation of risk to detected residue levels, the total exposure in the diet is determined for all label-approved raw agricultural commodities, processed forms, and animal products (meat and milk) that have established USEPA tolerances. The potential exposure from residues in the water and certain commodities without tolerances are also assessed in some cases. Tolerances may be established for the parent compound and associated metabolites. DPR considers these metabolites and other degradation products that may be of toxicological concern in the dietary assessment.

The dietary exposure to endosulfan and its metabolites was assessed initially in 1998 by Medical Toxicology Branch staff (Carr, 1998, Appendix B). The 1998 assessment used the TAS, Inc EX<sup>TM</sup> acute and chronic dietary exposure software (TAS, 1996a, b). All of the acute and chronic dietary margins-of- exposure (MOEs) were greater than 100. The need for a complete revision of the 1998 dietary exposure assessment for endosulfan was subsequently evaluated and presented as an addendum (Carr, 2006, Appendix C). A revised DPR dietary exposure assessment would preferentially use Pesticide Data Program (PDP) data for distributional analyses when appropriate. PDP data are acquired from the U.S. Department of Agriculture through a nationwide cooperative monitoring program (USDA, 1996b). A distributional analysis holds the potential to significantly refine the dietary exposure assessment to more accurately reflect the actual commodity residue. Only high value deterministic estimates were used to represent commodity residues in the previous 1998 dietary exposure assessment.

A complete reassessment is considered unnecessary for three reasons: 1) The existing 1998 dietary exposure assessment resulted in acceptable margins of exposure (MOEs) for both the acute and chronic scenarios; 2) A revised dietary exposure assessment would also likely result in acceptable MOEs because of post- 1998 USEPA endosulfan product label changes, commodity tolerance revocations, decreased use, and residue data changes; 3) The USEPA draft 2002 RED presented a dietary exposure assessment using methodology similar to current DPR methods. The USEPA draft RED assessment resulted in acceptable acute and chronic dietary exposure. The changes in residue determination methodology in the current DPR methods and the differences between the residue databases since the 1998 DPR dietary exposure assessment are presented in Carr (2006; Appendix C, Section V); 4) The USEPA recently put forth “Endosulfan: Notice of Receipt of Requests to Voluntarily Cancel Uses of Certain Pesticide Registrations,” whereby registrants have requested to voluntarily terminate use of certain endosulfan products on succulent peas, succulent beans, spinach, grapes and pecans (Federal Register, 2005). Cancellation of endosulfan products on grapes comprises a large percentage of use that ultimately eliminates potential occupational as well as dietary exposure.

The estimated national average annual usage of endosulfan in 1998 was 1.4 million pounds (USEPA, 2001b). This was also the national average annual usage for the period between 1987 - 1998 (USEPA, 2001b). The multi-year 1.4 million pounds national annual average through 1998 represents the most recent information (USEPA, 2001b, 2002). According to the draft 2002 USEPA endosulfan

RED, the top 4 national commodities, based on pounds (lbs.) of endosulfan active ingredient used annually, are cotton (286,000 lbs.), tomatoes (194,000 lbs.), potatoes (120,000 lbs.), and apples (110,000 lbs.) (USEPA, 2001b). Pears, a frequently consumed commodity by infants and children, averaged approximately 35,000 lbs. of endosulfan applied per year nationally. The national rank for endosulfan use on pears was 12, just below the average annual use on cantaloupe (39,000 lbs./year) (USEPA, 2001b, 2002). Overall, national endosulfan use remained fairly stable during the 1992-2001 period for the above commodities examined individually for individual years.

The United States Department of Agriculture (USDA) National Agricultural Statistics Services (NASS) conducts annual national crop and pesticide use surveys. The USDA NASS crop surveys are used to estimate national endosulfan use since the release of the draft 2002 USEPA RED. The most recent USDA-NASS information for the 5 crops (cotton, tomato, potato, apple and pear) previously noted in the draft 2002 USEPA endosulfan RED are summarized. The NASS survey results for cotton indicates approximately 7,000 lbs. was used nationally in 2005 (USDA, 2006a). The NASS results for tomato (fresh and processed) show approximately 99,000 lbs. were used nationally in 2004 (USDA, 2005). The results for potatoes indicate that approximately 14,000 lbs. was used nationally in 2005 (USDA, 2006a). Survey results for apple indicates approximately 59,000 lbs. was used nationally in 2005 (USDA, 2006b). The NASS results for pear shows that approximately 54,000 lbs. was used nationally in 2005 (USDA, 2006b). Compared to the estimates in the draft 2002 USEPA RED, USDA-NASS statistics indicate that recent national endosulfan use is lower for 4 crops (cotton, tomato, potato, and apple) and higher for one (pear). Based on the NASS statistics, the combined use of endosulfan nationally on cotton, tomato, potato, apple and pear declined by 69% (233,000 lbs. vs. 745,000 lbs) from the total reported in the draft 2002 USEPA RED.

The average annual use of endosulfan in California is summarized in Table 24. The 1996-2000 California annual average use was 187,900 lbs. of active ingredient (DPR, 2002). The most current 4 years (2001-2004) of endosulfan annualized use in California averaged 147,968 lbs. per year (DPR, 2006). The top 4 crops receiving endosulfan applications in California are alfalfa, cotton, head lettuce, and tomato (Table 23). The top 4 crops for endosulfan applications comprise about 77% of the total California use (2001-2004 average). California applications represent about 13% of the total national average. Cotton and tomato are on both the national and California top 4 commodity use lists. Applications on California cotton represent about 13% of the national use on cotton. California accounts for approximately 8% of the national endosulfan tomato applications and the 2001-2004 average annualized use is ca. 78% of the 1996-2000 period and 41% of the 1993-1995 period. Total endosulfan use in California has decreased 42% over the last 12 years (1993: 366,008 lbs. vs. 2004: 153,339 lbs.). Pear had very little California acreage treated with endosulfan during either the 1996-2000 or 2001-2004 periods (Table 23, footnote #2). A measurable downward trend in California endosulfan use is apparent even when considering annual variability due to weather and pest pressure.

**Table 24. Average Annual Endosulfan Use in California**

<b>Time Period</b>	<b>Average Annual Use<sup>a</sup></b>	<b>Highest Use Crops<sup>b</sup> (lbs.)</b>
1993-1995	356,970 lbs.	Cotton (192,000), grapes (47,000), cantaloupe (20,000), head lettuce (20,000)
1996-2000	187,900 lbs.	Alfalfa (46,420), cotton (36,000), head lettuce (23,800), tomato (15,900)
2001-2004 <sup>c</sup>	147,968	Cotton (61,460), tomato (19,850), head lettuce (17,675), alfalfa (14,470)

a - Average of California DPR Pesticide Use Report (PUR) data.

b - The top 4 crops for each time period based on average annualized pounds used from DPR PUR. Endosulfan use on

California pears averaged 1,197 lbs/yr 1993-95; 259 lbs/yr 1996-00; 59 lbs/yr 2001-2004. c- 2004 is the most recent year for complete data on individual products on a year by year basis.

A review of the tolerance, usage, residue, consumption, prior MOEs, and Continuing Survey of Food Intakes by Individuals (CSFII) information indicates that an update of the endosulfan dietary exposure assessment is not necessary. The CSFII is an annual survey that reflects the current consumption pattern and has a greater focus on consumption data for vulnerable population subgroups (e.g. infants and children). While the average consumption rates increased for some commodity/population sub-group combinations in the 1994-98 CSFII survey versus the 1989-92 CSFII, this would likely be mitigated by existing tolerance cancellations and proposed revocations, and reduced annual maximum applications. In addition, if the DPR acute dietary exposure analysis were updated there would be a change to Monte Carlo distributional iterations for many commodities where acute %CT estimates could be incorporated. Using updated residue, consumption, and the USEPA endosulfan RED tolerance and use information, the DPR acute dietary exposure assessment would likely be lower. This conclusion would hold even though MOEs at the 99.9<sup>th</sup> percent level of acute exposure would be reported instead of the 95<sup>th</sup> percent level of exposure MOEs used in the existing point estimate DPR dietary analysis. This conclusion is supported by the acceptable acute MOEs reported in the 2002 USEPA draft endosulfan RED (USEPA, 2002).

A new DPR acute dietary exposure analysis would: 1) update the residue data of the remaining commodities with tolerances, 2) delete the 9 commodity tolerances from the dietary residue file that have been canceled by the registrants since 1998, 3) delete the USEPA draft endosulfan RED proposed tolerance revocations (succulent bean and pea, grape, pecan, and spinach), 4) use more realistic residue data (e.g. exclude melon rinds), 5) use processed food forms when available instead of the raw forms as surrogates (e.g. apple juice instead of raw apples representing apple juice) for processed forms, 6) use Monte Carlo distributional iterations to replace point estimates whenever appropriate, and 7) incorporate acute %CT data into any distributional analysis (Monte Carlo). The updated DPR acute dietary exposure analysis would be very similar to the acute dietary analysis referenced in the 2002 USEPA endosulfan RED (USEPA, 2002)

The existing DPR 1998 endosulfan dietary exposure assessment reports MOEs greater than 100 for both the acute and chronic scenarios (Carr, 1998). Acute MOEs at the 95<sup>th</sup> percent level of exposure ranged from 212 for children 1-6 years to 513 for males 13-19 years population subgroups. The chronic MOEs ranged from 1,407 for children 1-6 years to 7,421 for nursing infants at less than or equal to 1 year old.

The USEPA 2002 endosulfan draft RED concluded that infants and children acute dietary exposure risk is mitigated by their tolerance revocations and proposed label changes and therefore, no longer a concern. The USEPA endosulfan RED concluded that adult acute and chronic dietary exposures are also not a concern. The conclusions resulting from an updated DPR dietary exposure assessment would likely be similar to those reported in the USEPA 2002 endosulfan RED. Therefore, a complete revision of the DPR 1998 dietary exposure assessment would appear unnecessary and the 2006 dietary exposure addendum suffices when combined with the prior 1998 DPR dietary exposure assessment.

b) Residue Data (General)

The sources of residue data for dietary exposure assessment include DPR and federal monitoring programs, field trials, and survey studies. In absence of data, surrogate data from the same crop group as defined by USEPA or theoretical residues equal to USEPA tolerances are used. Residue levels that exceed established tolerances are not utilized in the dietary exposure assessment because over-tolerance incidents are investigated by DPR Pesticide Enforcement Branch and are relatively infrequent. DPR evaluates the potential risk from consuming commodities with residues over tolerance levels using an expedited acute risk assessment process.

DPR has two major sampling programs: priority pesticide and marketplace surveillance. The priority pesticide program focuses on pesticides of health concern as determined by DPR Enforcement and Medical Toxicology branches. Samples are collected from fields that have been treated with the specific pesticides. For the marketplace surveillance program, samples are collected at the wholesale and retail outlets, and at the point of entry for imported foods. The sampling strategies for both priority pesticide and marketplace surveillance are similar and are weighted toward such factors as pattern of pesticide use; relative number and volume of pesticides typically used to produce a commodity; relative dietary importance of the commodity; past monitoring results; and extent of local pesticide use. DPR had two additional monitoring programs prior to 1991. The pre-harvest monitoring program routinely examined the levels of pesticides on raw agricultural commodities in the field at any time during the growth cycle. Commodities destined for processing were collected in the field no more than 3 days prior to harvest, at harvest, or post-harvest before processing.

The U.S. Food and Drug Administration (FDA, 1998) has three programs for examining residues in food: 1) regulatory monitoring; 2) total diet study; 3) incidental/level monitoring. For regulatory monitoring, surveillance samples are collected from individual lots of domestic and imported foods at the source of production or at the wholesale level. In contrast to the regulatory monitoring program, the total diet study monitors residue levels in the form that a commodity is commonly eaten or found in a prepared meal. The incidence/level monitoring program is designed to address specific concerns about pesticide residues in particular foods.

The USDA, responsible for the PDP (USDA, 1996b), is designed to collect objective, comprehensive pesticide residue data for risk assessments. Several states, including California, collect samples at produce markets and chain store distribution centers close to the consumer level. The pesticide and produce combinations are selected based on the toxicity of the pesticide as well as the need for residue data to determine exposure. In addition, USDA is responsible for the National Residue Program that provides data for potential pesticide residues in meat and poultry. These residues in farm animals can occur from direct application, or consumption of commodities or by-products in their feed.

The PDP program analyzes residues with the intent to provide residue information for dietary risk assessment. The primary mission for the DPR program is enforcement of USEPA tolerances.

These dissimilar mandates result in two main programmatic differences between the PDP and DPR residues. Commodities in the PDP program are prepared into a ready-to-eat form (inedible rinds removed, fruit destemmed, etc.) before the pesticide analysis is conducted. The DPR program includes rinds and peels. Also, the PDP's limit-of-detection (LOD) levels are generally much lower by design

than the DPR market basket program.

The raw agricultural commodity cantaloupe illustrates the preparation differences between the DPR market basket and USDA PDP analytical programs. The DPR analysis cuts a number of melons into slices with the outer rind attached before analysis of the composite sample. The PDP program cuts cantaloupes in half with the seeds and outer rind removed from each melon before the composite sample is analyzed. For non-systemic pesticides (those that stay only on the surface of the plant), discarding inedible rinds prior to commodity pesticide analysis reduces and more accurately reflects the actual residues likely to be consumed. The current DPR dietary guidelines recommend using ready-to-eat residue data when available. This was not a criterion in the earlier 1998 dietary exposure assessment.

The raw agricultural commodity, pear, provides another example of preparation differences between the DPR and PDP programs. The DPR analysis uses the entire pear including the outer peel and attached stem for each analyzed composite sample. The PDP program washes, cores and removes the stems from each pear before the sample is analyzed. Commodity-processing studies have demonstrated that many non-systemic pesticide residues can be significantly lowered by removal through washing prior to consumption (Elkins, 1989; Hamilton, et al., 2004). Current DPR guidelines prioritize using ready-to-eat residue data that includes washing and de-stemming as minimal processing efforts.

The PDP preparation methods would have the potential to remove residues of non-systemic pesticides but also better reflect how people eat cantaloupes and pears. The typically lower PDP analytical method LODs would have a greater impact on chronic rather than acute dietary residues. This occurs because the chronic non-detect values contribute directly into the overall commodity mean residues. The lower LODs for the non-detected residues would not have a meaningful impact on upper bound deterministic acute values. The lower LODs would mainly affect acute residues in a distributional assessment. A revised DPR dietary exposure assessment would likely use a distributional analysis and combine the lower LOD values with the detected residues. This would probably result in a lower distributional residue than the deterministic value. This was not done in the 1998 endosulfan assessment.

#### c) Residue Data - Endosulfan Specific

##### i. Residue Data, 1998

Acute and chronic dietary exposure assessments and an acute tolerance assessment were conducted for endosulfan (40 CFR #180.182). All available endosulfan raw agricultural commodity (RAC) residue data were evaluated (Table 25). The 40 CFR 180.182 tolerance is characterized as total endosulfan which includes the  $\alpha$ -,  $\beta$ - and sulfate forms (CFR, 1997).

All of the federal and state regulatory pesticide residue-monitoring programs analyze for endosulfan and its main isomers and sulfate form. The detections are reported as endosulfan. The Food and Drug Administration (FDA) monitoring program analyzes for all endosulfan  $\alpha$ -,  $\beta$ - and sulfate). The United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) and the Pesticide Data Program (PDP) monitor for the same forms. The California Department of Pesticide Regulation (DPR) residue screens can also detect the same endosulfan forms.

Residues analyzed by the FDA regulatory monitoring surveillance program (domestic commodities) from July 1, 1995 through June 30, 1997 were considered for use in the DPR dietary exposure analysis. The FDA multiple residue screen minimum detection level (MDL) for endosulfan is 0.1 ppm for many RACs (FDA, 1998). The lowest reported FDA MDL was 0.03 ppm for tomato. The FDA data were not utilized, but instead, DPR, PDP and registrant data were used.

The DPR total endosulfan MDL was between 0.03 and 0.09 for program 4 (the market basket surveillance), the years 1993 to 1995, residue data. There were extensive findings of total endosulfan residues detected on label approved RACs in the DPR programs during 1993, 1994 or 1995 (DPR, 1994, 1995, 1997).

The USDA also monitors for endosulfan including  $\alpha$ -,  $\beta$ - and sulfate forms with their multi-residue screen analytical program and the results are reported in two different annual surveys, the Pesticide Data Program (PDP) and the Food Safety Inspection Service. The PDP program targets raw agricultural commodities that are likely to be heavily consumed by infants and children. The FSIS looks for residues on various commercial meat animals such as cattle, sheep and poultry. The USDA Food Safety Inspection Service (FSIS) meat monitoring program LOD for total endosulfan is 0.06 ppm (0.02 ppm each for endosulfan  $\alpha$ -,  $\beta$ - and sulfate forms) and over 2,400 samples of meat were tested during 1995 (USDA, 1994c, 1995). No endosulfan residues were detected.

The USDA PDP, established May 1991, has monitored for endosulfan using the multi residue methods (MRMs) since 1992. Only the 1994 and 1996 PDP data were used since the 1994 had no detected residues on RACs that are no longer examined and the 1996 program characterizes the residues as  $\alpha$ -,  $\beta$ - and sulfate forms. The endosulfan residue limit of detection (LOD) range is generally between 0.002 - 0.006 ppm for each of the analytes (USDA, 1996b, 1998b). The upper end of the LOD range (0.011 ppm total endosulfan) was used as the “total non-detect value” for California analyzed samples. The 0.011 ppm value was also reported for all samples without any detected endosulfan residues in the DPR dietary analysis. The following RACs were included from the PDP apple, carrot, celery (1994), grapes, green beans, lettuce (1994), peaches, potatoes (sweet), spinach, tomatoes and sweet corn. The following processed commodities from the PDP surveys were also used: sweet pea, apple juice, milk and wheat.

The FMC Corporation endosulfan compound name used in the submitted field residue studies is: Thiodan® (6,7,8,9,10,10 hexachloro-1, 5, 5 $\alpha$ , 6, 9, 9 $\alpha$ -hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide) and endosulfan sulfate (FMC, 1990). The potential dietary exposure from residues with the endosulfan and its metabolite was evaluated together with residues expressed as total endosulfan residues by FMC and reported in the few, submitted field studies. The registrant LOD for endosulfan ranged from 0.05 - 0.1 ppm depending on the commodity or the age of the submitted field study.

In 1994, 475,740 lbs of endosulfan was used on California commodities. During 1995 the total usage was 229,160 lbs. (DPR, 1996 a and b). The evident decrease in pounds applied in 1995 versus 1994 is due almost completely to a decreased use on melons. About 4,800 lbs of endosulfan were applied to melons in 1995 while the use on melons was 152,350 lbs during 1994. The primary endosulfan residue data are presented in Table 25.

**Table 25. Summary of Endosulfan ( $\alpha$ -,  $\beta$ - and Sulfate) Expressed as Total Endosulfan Residues (July, 1998).**

Raw Agricultural Commodity (RAC)	Source <sup>a</sup> (Reference Year)	Tolerance <sup>b</sup> (ppm)	Residue Used (ppm)		N <sup>c</sup>	Additional Information	%CT <sup>d</sup>
			Acute	Chronic			
Almond	REG-f (Beckman, 1962)	0.2(N) <sup>e</sup>	0.20	0.10	2	LOD = EPA tolerance	
Apple, whole	PDP (1996)	2.0	0.021	0.0064	530	1 yr. Acute 95th %.	33%
Apple, juice	PDP (1996)	2.0	0.002	0.002	179	0.002 (2 LOD) mix, all ND.	33%
Apricot	DPR (1993-1995)	2.0	0.15	0.031	95	Acute = hi #, LOD = 0.06 ppm	
Artichoke	REG-f (Gowen, 1967)	2.0	0.10	0.05	4	all nondetect (ND)	
Barley (grain)	PDPsur (1996)	0.1(N)	0.021	0.0105	340	wheat as surrogate, all ND	
Beans, dry & succulent.	PDP (1996)	2.0	0.010	0.004	531	green bean sur., 0.008 LOD	
Beet (sugar)	PDPsur (1996)	0.2	0.0082	0.0042	500	carrot as surrogate RAC	
Blueberry	PDPsur (1996)	2.0	0.065	0.0077	525	grape as surrogate RAC	
Broccoli	DPR (1993 - 1995)	2.0	0.1	0.0303	312	0.06 LOD, Acu = high #	12%
Brussels Sprouts	DPRsur (1993 - 1995)	2.0	0.1	0.0303	312	broccoli as surrogate RAC	
Cabbage	DPR (1993 - 1995)	2.0	0.12	0.045	266	0.09 LOD, Acute = high #	
Carrot	PDP (1996)	0.2	0.0082	0.0042	500	1 yr. Acute=95th %	
Cauliflower	DPR (1993 - 1995)	2.0	0.1	0.0303	312	broccoli as surrogate RAC	
Celery	PDP (1994)	2.0	0.005	0.0015	176	0.003 LOD, Acute = high #	
Cherry	DPR (1993 - 1995)	2.0	0.18	0.033	59	0.06 LOD, Acute = high #	
Collards	DPRsur (1993 - 1995)	2.0	0.1	0.0303	312	broccoli as surrogate RAC	
Corn (sweet)	PDP (1996)	0.2	0.007	0.0035	173	0.007 LOD, all ND	
Cottonseed meal/oil	REG-fp (FMC, 1967)	1.0	0.03	0.015	6	0.03 LOD, all ND	
Cucumbers	DPRsur (1993 - 1995)	2.0	0.57	0.049	211	0.06 LOD, cantaloupe as sur	
Eggplant	DPRsur (1993 - 1995)	2.0	0.092	0.027	580	0.03 ppm LOD, squash as sur	
Grape fresh/dry/juice	PDP (1996)	2.0	0.065	0.0077	525	CA LOD=0.011, Acute=95%	7%
Kale	DPRsur (1993 - 1995)	2.0	0.1	0.0303	312	broccoli as surrogate RAC	
Lettuce, head & leaf	PDP (1994)	2.0	0.108	0.014	546	0.009 LOD, ac = 95th %	
Melons, all types	DPR (1993 - 1995)	2.0	0.57	0.049	211	0.06 LOD, cantaloupe hi # = ac	
Milk, fat	PDP (1996)	0.5	0.0015	0.0015	575	0.0015 ppm (2 LOD) mixture	
Mustard Greens/Seed	DPRsur (1993 - 1995)	2.0	0.1	0.0303	312	broccoli as surrogate RAC	
Nectarine	PDPsur (1996)	2.0	0.053	0.009	329	peach as surrogate RAC.	6%
Nuts (all but almond)	REG-f (1962)	0.2(N)	0.2	0.1	--	almond as surrogate, all ND	
Oat (grain)	PDPsur (1996)	0.1(N)	0.021	0.0105	340	wheat as surrogate, all ND	
Peach	PDP (1996)	2.0	0.053	0.009	329	0.011 ppm LOD, Acu=95%	6%
Pear	DPR (1993 - 1995)	2.0	0.077	0.0202	402	0.03 LOD, Ac=95th%.	54%
Peas, succulent	PDP (1996)	2.0	0.0071	0.0037	355	0.007 ppm LOD, Ac=95th%	
Peppers, bell & chili	DPR (1993 - 1995)	2.0	0.18	0.037	745	0.03 ppm LOD, Acute=95th%.	
Pineapple	DPR (1993 - 1995)	2.0	0.09	0.0454	43	0.03 ppm LOD, Acute=high #	
Plum	DPR (1993 - 1995)	2.0	0.07	0.0302	217	0.06 ppm LOD, Acute=high #	
Potato	PDP (1996)	0.2(N)	0.00703	0.0036	507	0.007 ppm LOD, Acu=high #	
Prune	DPR (1993 - 1995)	2.0	0.12	0.045	11	0.06 ppm LOD, Acute=high #	
Pumpkin	DPRsur (1993 - 1995)	2.0	0.092	0.027	580	0.03 ppm LOD, squash surrog	
Rape seed (canola)	DPRsur (1993 - 1995)	2.0(N)	0.1	0.0303	312	broccoli as surrogate RAC	
Raspberry	PDPsur (1996)	2.0	0.065	0.0077	525	grape as surrogate RAC.	
Red meat, fat <sup>f</sup>	FSIS (1996)	0.2	0.06	0.03	2,484	0.06 LOD, all ND.	2,484
Red meat, mby	FSIS (1996)	0.2	0.06	0.03	2,484	combined N for cattle, hogs	
Red meat, meat	FSIS (1996)	0.2	0.06	0.03	2,480	sheep & goats together.	
Rye (grain)	PDPsur (1996)	0.1(N)	0.021	0.0105	340	wheat as surrogate, all ND	
Safflower seed (oil)	REG-fp (Gowan, 1967)	0.2(N)	0.05	0.008	6	0.05 ppm LOD, Acute=high #	
Spinach	PDP (1996)	2.0	0.357	0.026	525	0.011 CA LOD, Acute=95th%	
Squash-sum/winter	DPR (1993 - 1995)	2.0	0.092	0.027	580	0.03 LOD, Acute = 95 <sup>th</sup> %	
Strawberry (& juice)	DPR (1993 - 1995)	2.0	0.18	0.0603	228	0.12 ppm LOD, Acu=hi #	14%
Sugarcane (sugar)	EPA (1997)	0.5	0.5	0.25	--	U.S. EPA tolerance as acute #	
Sunflower seed (oil)	REG-fp (Gowan, 1967)	0.2(N)	0.05	0.008	6	safflower as surrogate RAC	
Sweet Potato	PDP (1996)	0.2(N)	0.00703	0.0036	507	0.007 ppm LOD, Acu=high #	
Tomato	PDP (1996)	2.0	0.048	0.0105	179	0.011 CA LOD, Ac=hi#	46/10%
Turnip (greens)	PDP (1994)	2.0	0.108	0.014	546	lettuce as surrogate RAC	
Watercress	PDP (1994)	2.0	0.108	0.014	546	lettuce as surrogate RAC	
Wheat (grain)	PDP (1996)	0.1(N)	0.021	0.0105	340	0.021 ppm LOD, all ND	

a - DPR = California Department of Pesticide Regulation, EPA = U.S. Environmental Protection Agency, FSIS = U.S. Department of

Food and Agriculture Food Safety Inspection Service, **PDP** = U.S. Department of Agriculture Pesticide Data Program residue monitoring program. **PDPsur** = USDA Pesticide Data Program residue monitoring program - surrogate data used (similar crop types), **REG-f & REG-fp** = Registrant supplied field or field and processing residue studies.

b - **USEPA** = Tolerances for U.S. EPA 40 CFR 180.182.

c - **N** = The number of RAC composite samples analyzed from the selected submitted studies or monitoring programs.

d - **%CT** = Percent of the crop treated adjustment made to chronic dietary residues when sufficient use data are available.

e - **(N)** = USEPA determined that residue is expected to be a negligible residue as defined.

f - The red meat tissues are all at limit of detection (LOD) based on USDA FSIS monitoring data.

The majority of the endosulfan residue data for the 1998 dietary exposure assessment was taken from either the DPR market basket or the USDA Pesticide Data Program (PDP) monitoring programs. There were 56 separate residue values for endosulfan listed in the 1998 DPR dietary commodity file. The commodity sources were as follows: 20 commodity residues obtained from DPR, 6 residues from the endosulfan registrants, the USDA (PDP and FSIS) provided 29 residue values, and one USEPA tolerance was used.

If the endosulfan dietary exposure assessment were revised, there would be an almost complete replacement of DPR residue data with USDA PDP data. This is because the statistically valid PDP data are more valid, and therefore more suitable, for use in distributional analysis. The USDA PDP annual summaries, published since the 1998 endosulfan dietary exposure assessment, contain residue data representing all 20 commodities that had previously used DPR data (USDA, 1998a, 2000-2006). These 20 commodity residues originating from the DPR monitoring program would be replaced by PDP values. This could have a meaningful impact on detected residue values primarily due to the differences in PDP sample preparation versus the DPR methods. The following commodities currently use DPR origin data and could be replaced by USDA PDP residue data in an updated dietary exposure analysis. The commodities are broccoli, cantaloupe, cherry, cucumbers, pear, peppers, pineapple, squash, and strawberry. In addition, broccoli would be used as a surrogate to represent 5 other Brassica varieties with endosulfan tolerances, cantaloupe would be a surrogate for all melons, squash would represent pumpkins, and cherry could serve as a surrogate for apricot, plum, and prune. Several of these commodities recorded frequent consumption by infants and children in the CSFII databases. The current DPR dietary exposure guidelines allow for use of representative surrogate residue data. The 1998 dietary exposure assessment did not use this currently accepted DPR and USEPA method. Instead, marginal, and possibly non-representative residues or tolerance values were used, leading to greater exposure.

The complete distribution of the new, replacement PDP commodity residues is unknown. Their impact on the commodity residue contribution is also unknown unless a revised dietary exposure assessment is conducted. However, the commodities that previously used DPR residues in the 1998 dietary exposure assessment are compared with replacement PDP residues (Table 25). Table 25 presents the maximum measured value (non-distributional) for each monitoring program.

Cantaloupe and pear are used to illustrate the DPR and PDP residue monitoring program comparisons. Both cantaloupe and pear are frequently consumed commodities each with significant national percent crop treated rates (USDA, 1994-98a, U.S. EPA, 2001b). Both of these commodities were represented by deterministic values in the 1998 DPR acute dietary exposure assessment but in a revised assessment would be replaced by distributional estimates using PDP data, thus reducing exposure.

The USEPA endosulfan RED reported that nationally on average, 31% of the cantaloupe and

20% of the pear acreage were treated (USEPA, 2002). The 1998 DPR value of 0.57 ppm represents the maximum measured from the detected cantaloupe residues. The residue of 0.091 ppm represents the maximum measured PDP cantaloupe value. The maximum PDP cantaloupe residue is 6 fold lower than the DPR maximum value. The 1998 DPR value of 0.25 ppm represents the maximum measured pear residue. The 0.16 ppm pear residue represents the maximum measured PDP value. The maximum PDP pear residue is 36% lower than the DPR maximum value.

Both of the PDP cantaloupe and pear endosulfan maximum residues are lower than the 1998 DPR values they would replace. All but three of the commodity residues in Table 26 are lower when PDP data replace the 1998 DPR values. Broccoli, pepper and strawberry are the three out of nine PDP residues that are higher (Table 25). If the screening level deterministic estimate margins of exposure (MOEs) were inadequate at the 95<sup>th</sup> percent level of exposure in a revised dietary exposure assessment, then a distributional analysis (Monte Carlo) would be performed to better characterize the acute anticipated residues. These new data would most likely consist of PDP data. This is because the commodity sampling protocol for the PDP program is statistically more representative of residues found in grocery stores in both California and nationally. Refined Monte Carlo iterations using PDP data at the same 95<sup>th</sup> percent of exposure as the 1998 assessment would result in lower dietary exposures. Based on the Table 24 examples and previous residue comparisons between PDP and DPR data for other pesticides, the PDP residues would be lower than the DPR values the majority of the time.

The PDP data reported in the 1998 DPR dietary exposure assessment could also be revised using food form data. Both the deterministic and distributional processes allow for the incorporation of specific food form data (i.e. canned tomato versus raw, whole tomato) when available. Such PDP data now exist and a revised DPR acute dietary exposure assessment would use these food forms in a similar manner to the USEPA methodology (see Table 26) (Carr, 2006). Specific food form data were not used in the 1998 DPR dietary exposure assessment

Recent PDP data include both whole and canned tomato food form residue data. The whole tomato food form residue was used to represent the other tomato food forms in the 1998 DPR dietary exposure assessment. The whole tomato deterministic residue used in the assessment was 0.048 ppm (95<sup>th</sup> percent of the detected residues). The maximum detected whole tomato residue value was 0.132 ppm. The canned tomato Monte Carlo estimation residue would be based on a distribution of several hundred samples with the maximum detected value of 0.008 ppm. The canned tomato maximum value is 6-fold lower than the tomato value used in the 1998 dietary assessment and 16.5 fold lower than the maximum whole tomato residue. In a revised DPR assessment, canned tomato residue values would be used to represent commercially prepared canned juice, ketchup, paste, puree and canned whole tomatoes. This method would be used for additional PDP origin commodities with both raw and processed residue data in a revised DPR dietary exposure assessment.

**Table 26. Comparative DPR and PDP Maximum Measured Endosulfan Residues**

Commodity	Monitoring Program Residue (PPM) <sup>a</sup>		
	DPR <sup>b</sup>	USDA PDP <sup>b</sup>	Comparison
Broccoli <sup>c</sup>	0.1	0.19 (2002)	PDP Residue is higher
Cantaloupe <sup>c</sup>	0.57	0.091	DPR Residue is higher
Cherry	0.18	0.041	DPR Residue is higher
Cucumber <sup>c</sup>	0.57	0.44 (2003)	DPR Residue is higher
Pear <sup>c</sup>	0.25	0.16	DPR Residue is higher
Pepper <sup>c</sup>	0.71	1.1	PDP Residue is higher*
Pineapple <sup>c</sup>	0.09	0.005	Non detect for each program
Squash <sup>c</sup>	0.031 NA <sup>d</sup>	Fresh: 0.048 Processed: 0.02	DPR residue is higher
Strawberry <sup>c</sup>	0.18 NA	Fresh: 0.68 Processed: 0.008	PDP fresh value higher *

a - Maximum detected deterministic values represent both monitoring program’s residues.

b - DPR = California Department of Pesticide Regulation. PDP = USDA Pesticide Data Program. The DPR residues used in the 1998 analysis came from the 1993 -1995 market basket program. The PDP data are from the 1994 (broccoli only) and 1997-2004 annual summaries.

c - Frequently consumed commodity and a primary candidate for a distributional analysis.

d - N.A. - not applicable.

\* - The DPR program analyzed only raw agricultural commodities.

A processing study performed by the registrant, primarily for pineapple bran, was reviewed; however, it provides information relevant to pineapple juice residue reductions based on the general processing method used. Therefore, this processing reduction factor from the processed commodity measurement was used to modify the default adjustment factor. Residues derived from registrant field studies using the maximum label rates and minimum pre-harvest intervals are considered the appropriate situation on which to apply any commercial processing effect changes to default adjustment values.

A 1968 FIVIC Corporation pineapple bran study for animal feed processing included supplemental information relevant to pineapple juice (Hintridge, 1968). The processing method contained information regarding endosulfan residue reduction in pineapple juice. The data indicated that when using maximum application rates that the label allows, the endosulfan residues in juice were 77% lower than the whole fruit (Hintridge, 1968). Therefore, the TAS program food form adjustment factor is 1 for pineapple juice and juice concentrate were set to 0.23 from 1.7 times and then combined with the DPR monitoring program for whole pineapple residues in the dietary exposure analysis.

ii. Residue Data - Percent of the Crop Treated

The Biological and Economic Analysis Division of the USEPA (USEPA BEAD) can generate percent-of-the-crop-treated (%CT) estimates based on USDA National Agricultural Statistical Service (NASS) and registrant product use and sales information (USEPA, 2001b). When appropriate, commodity residue values in a dietary exposure analysis that come from registrant field trial data, state, or federal residue monitoring programs can be considered for %CT adjustments.

A commodity that has distributional residue data can be considered for modification by acute %CT information (DPR, 2002c). Unless specifically modified by an acute %CT adjustment, all other commodities in a DPR acute dietary exposure analysis will use the default assumption that 100% of the commodity has been treated with the pesticide active ingredient. When quality data are available that indicate that less than 100% of a commodity is treated with a specific pesticide, then on an individual commodity by pesticide combination basis, exceptions to the default assumptions can be made. The USEPA BEAD estimate can be factored into the acute residue distribution to represent untreated commodity (true zeros) instead of the limit of detection (LOD). Incorporation of %CT estimates into a distributional estimate would likely result in lower exposure than deterministic estimates using a 100 %CT assumption. The %CT treated method was not used for any acute residues in the 1998 DPR dietary exposure assessment. A revised DPR dietary exposure assessment would extensively use USEPA BEAD data for both the acute and chronic duration scenarios.

With regard to subchronic or chronic exposure, the assumption that people, under normal eating conditions, would be continuously exposed to the average residue level of a pesticide for every labeled commodity for 1 year (chronic) is unrealistic based on available substantial dietary information. This assumption does not take into account the fact that a significant amount of a commodity is often untreated with the pesticide under consideration. This is not reflective of actual practices and is borne out by the lower residue levels encountered in various market basket surveys versus the registrant field studies. The actual percentage of the crop treated with a specific pesticide varies from year to year depending upon biotic and abiotic factors. Using the existing percent crop treated data it is reasonable to revise the 100% treated assumption downward using more realistic pesticide treatment rates and use patterns. Commodities that used residues obtained from registrant field trial or state and federal monitoring data in the chronic dietary exposure assessment were considered for percent crop treated adjustments.

The percent of the crop treated adjustment method has been employed as a comparison to the standard chronic dietary exposure assessment using 7 commodities that have endosulfan tolerances. The following commodities have reported endosulfan use at the federal and state levels: apple, broccoli, grape, peach, pear, strawberry and tomato. DPR Pesticide Use Reports and CDFA crop statistics together with USDA Ag Field Crops Summary annuals were used. Conservative, but realistic, assumptions were made when setting the percentage of crop treated adjustment factors for the chronic dietary exposure section for each commodity. Multiple years of endosulfan use and acreage harvested data were evaluated at the federal and state levels.

#### 1) Apple

The total California apple acreage during 1993 was 33,000 acres and 40,000 acres for 1995 (USDA, 1994a, 1996a). The California apple acreage represents approximately 10% of both the 1993 and 1995 U.S. apple crops. Endosulfan was applied to less than 1,300 acres of California apples in both the 1994 and 1995 seasons (DPR, 1996a,b). The United States apple acreage, based on the eight major production states (California, Michigan, New York, North Carolina, Oregon, Pennsylvania, South Carolina and Washington), harvested during 1993 from 332,000 acres and 345,000 acres during 1995 (USDA, 1994a, 1996a). Based on USDA Agriculture Marketing Statistics and DPR data, endosulfan was applied to 31% (1993) and 16% (1996) of the national acreage in the major production states (USDA, 1994a, 1996b). Therefore the National 1993 treated acreage rate of 31% of the crop will be used to represent the domestic apple information. There was one year of data, 1994, showing

that 243 million pounds of apples were imported into the United States compared to the 11,000 million pounds from domestic 1994 production (USDA, 1994b). The imported apples represented about 2% of the total U.S. apple market. Based on the USDA domestic production value and the imported apple information, a 33% crop adjustment factor to conservatively represent the 31% national higher annual value plus the 2% imported apples overall total will be used in the calculations of the chronic dietary assessment.

## 2) Broccoli

The total California broccoli acreage during 1994 was 95,000 acres and 106,000 acres for 1996 (USDA, 1996c, 1997). The California broccoli acreage represents approximately 89% of both the 1994 and 1996 U.S. broccoli crops (USDA, 1996c, 1997). Endosulfan was applied to between 4,000-6,000 acres of California broccoli during both the 1994 and 1995 seasons (DPR, 1996a,b). The United States broccoli acreage, based on the four major production states (Arizona, California, Oregon and Texas), harvested 111,000 acres during 1994 (USDA, 1996c). Based on USDA Agriculture Marketing Statistics and DPR data, endosulfan was applied to about 12% of the 1994 national acreage in the major production states (USDA, 1996c). Therefore, the residues for broccoli will be reduced (adjusted) to 12% of the amount of the national crop treated values in the assessment of chronic dietary exposure.

## 3) Grape (all)

California bearing grape acreage totaled 651,000 acres during 1993 and 701,000 during the 1995 seasons (USDA, 1994a, 1996a). The United States acreage originates from six principal states; California, Michigan, New York, Oregon, Pennsylvania and Washington, which during 1993 had bearing grapes on 743,000 acres and during 1995 from 796,000 acres (USDA, 1994a, 1996a). The California acreage represented approximately 88% of the total production for both 1993 and 1995 U.S. grapes (all types). Endosulfan was applied to about 11,000 acres, 1% of the California grapes in 1993 and to 13,000 acres (about 1%) of grapes during the 1995 season (USDA, 1994a, 1996a). The 1993 USDA data indicated that endosulfan was applied to less than 1% of the total national grape acreage. The 1995 USDA data also show endosulfan application on less than 1% of the national acreage (USDA, 1994a, 1996a). Therefore the California 1995 treated acreage rate of 1% of the crop will be used to represent the domestic grape information. There is also imported grape data available from the USDA (USDA, 1994b). The most recent single year of data, 1992, showing that 370,568 U.S. tons of grapes were imported into the United States compared to the 6,051,650 tons from domestic 1992 production (USDA, 1994b). The imported grapes represented about 6% of the total U.S. grape market. A grape residue decrease (adjustment) to 7% (based on USDA domestic production value and imported grape data) was used to conservatively represent the 1% California annual use plus the 6% imported overall total to calculate chronic dietary exposure residues.

## 4) Peach

Peaches were planted to a total of 60,200 acres during 1993 and 72,600 acres for the 1995 season in California (USDA, 1994a, 1996a). The United States primary acreage originates from four states; California, Georgia, New Jersey and South Carolina, which during 1995 produced peaches on 127,400 acres (USDA, 1996b). California production represented approximately 57% of the total 1995 U.S. peach crop. Endosulfan was applied to less than 900 acres in 1994 and 500 acres during the 1995

season (DPR, 1995, 1996b). Endosulfan was applied to about 6% of the total 1993 and 1995 acreage from the major production states based on the USDA data (USDA, 1994a, 1996a). Based on the national data, a 6% crop residue adjustment factor will be used to represent the endosulfan treatment total. This 6% reduced (adjustment) value will be used for peaches to estimate the chronic dietary assessment.

#### 5) Pear

Pears were planted to a total of 67,000 acres during 1993 and 68,000 acres for the 1995 season in the major production states (USDA, 1994a, 1996a). The United States primary national acreage originates from four states; California, New York, Oregon and Washington (USDA, 1996a). California production represented approximately 36% of the total 1995 U.S. pear crop. Endosulfan was applied to less than 300 acres in 1994 and 400 acres during the 1995 season (DPR, 1995b, 1996b). Endosulfan was applied to about 46% of the 1993 national acreage and to about 21% of the total 1995 acreage from the major production states based on the USDA data (USDA, 1994a, 1996a). Therefore the national 1993 treated acreage rate of 46% of the crop will be used to represent the domestic pear information. There is also imported pear data available from the USDA (USDA, 1994a). The most recent year of data, 1992, showed that 71,300 U.S. tons of pears were imported into the United States compared to the 926,000 tons from domestic 1992 production (USDA, 1994b). The imported pears represent about 8% of the total U.S. pear market. Based on the combined USDA domestic production value (48%) and the imported pear values (8%), a 54% crop adjustment factor will be used to conservatively represent all annual (chronic) pear dietary residues in chronic dietary assessment calculations.

#### 6) Strawberry

The California strawberry acreage totaled 23,300 acres during 1994 and 25,200 acres during the 1996 season (USDA, 1996c, 1997). The California acreage represented 51% and 57% of the total 1994 and 1996 U.S. strawberry crops respectively. The United States acreage originates from six main states; California, Florida, Michigan, New York, North Carolina and Oregon, which during 1994, produced strawberries from 45,800 acres and 44,500 acres in 1996 (USDA, 1996c, 1997). Endosulfan was applied to 14% of the total 1994 acreage and 11% of the 1996 acreage from the major production states based on the USDA data (USDA, 1996c, 1997). An assumption was made that the total planted 1996 California acreage was the about the same as the 1995 surveyed acreage so that the 1995 DPR percent crop treated data could be combined together with the 1994 data to arrive at a hypothetical two season amount, which is more representative. Endosulfan was applied to about 300 acres of California strawberries during both the 1994 and 1995 seasons (DPR, 1996 a,b). There are no USDA California specific endosulfan data for 1994 or 1996 strawberries. The 1994 and 1996 strawberry values, as reported by USDA marketing data, are based on national records (multiple applications to the same acreage are not counted again as is the case for DPR data) and are 14% and 11% respectively (USDA, 1996c, 1997). An adjustment factor of 14% to represent the 1994 percent of the endosulfan treated U.S. strawberry crop will be used in the calculations for the chronic dietary assessment.

#### 7) Tomatoes (fresh market and processed)

The United States fresh market acreage originates from eight main states; California, Florida, Georgia, Michigan, New Jersey, New York, North Carolina, and Texas which during 1994 produced

tomatoes from 104,000 acres and 89,000 acres during 1996 (USDA, 1996c, 1997). The USDA endosulfan fresh market tomato records indicate that there was use on 41% of the acres during 1994 and 29% during 1996 (USDA, 1996c, 1997). The California fresh market tomato acreage totaled 37,000 acres during 1994 and 33,000 acres during 1996 (USDA, 1996c, 1997). The 1994 and 1996 California acreage represented approximately 36% of the total U.S. fresh market tomato crop. Endosulfan was applied to less than 15% of the California fresh market tomatoes in 1994 and 1995 seasons (DPR, 1996a,b). Therefore for the fresh market aspect of tomato use was 41% (1994 national use) will be the residue value for the calculations for chronic dietary assessment. This value (41%) will also incorporate the portion of tomatoes used for processing.

The California processed tomato acreage totaled 318,000 acres for both 1994 and 1996 seasons (USDA, 1996c, 1997). The 1994 and 1996 California tomato acreage represented virtually 100% of the total U.S. processed tomato harvest. The DPR and USDA processed tomato records for California indicate that 2% of the 1994 crop and 5% of the 1996 crop were treated with endosulfan (DPR, 1996a,b, USDA, 1996c, 1997).

The total U.S. domestic tomato production (fresh and processed combined) amounted to 11,451,490 tons during 1993 (USDA, 1994b). Foreign imports as fresh, canned and pureed tomatoes, during 1993, amounted to 556,440 tons that constitute about 5% of the total U.S. market (USDA, 1994b). Based on the U.S. domestic tomato endosulfan treatment rate 1994 value of 41 % for fresh market plus 5% for foreign imports for a combined total of 46% will represent whole tomatoes. The combined value of 10% of the processed crop (5% value for domestic processed tomatoes plus 5% for imported tomatoes, 1996) will be used as a value for "all processed tomato food codes" used to calculate chronic dietary exposure.

#### d) Consumption Databases

The United States Department of Agriculture (USDA) 1989-92 and 1994-98 Continuing Survey of Food Intake by Individuals (CSFII) consumption databases constitute 2 of the dietary consumption surveys available (USDA, 1989-92, 1994-98). The TAS Exposure<sup>TM</sup> program used residue data, acute/chronic NOELs, and the 1989-92 CSFII consumption database to estimate dietary exposure and resulting margins of exposure (MOEs) (TAS, 1996). The TAS Exposure<sup>TM</sup> program is inactive and not currently supported by any organization. However, the 1989-92 CSFII consumption data within the TAS program are still relevant and available in other dietary exposure software. The 1998 DPR dietary exposure assessment used the 1989-92 CSFII consumption data from the TAS Exposure<sup>TM</sup>

The DEEM<sup>TM</sup> program is the current<sup>1</sup> standard dietary exposure software used by DPR in 2002 when the USEPA draft endosulfan RED was released (Novigen, 2001; USEPA, 2002). The DEEM<sup>TM</sup> program can access either the 1989-92 or the 1994-98 CSFII databases. The USEPA also used the DEEM<sup>TM</sup> dietary exposure software. The USEPA 2002 draft endosulfan RED used the

---

<sup>1</sup> The DEEM<sup>TM</sup> software was in use by DPR at the time the endosulfan dietary assessment was performed. In the future DEEM-FCID is the software that will be used for dietary exposure assessments performed by DPR.

DEEM™ 1989-92 CSFII consumption database.

The DEEM-FCID™ program is the current standard dietary exposure software used by the California DPR (Exponent, 2004b). In August 2002, the DPR adopted the 1994-98 CSFII consumption database within the DEEM-FCID™ program for all new or revised dietary exposure assessments. The DEEM-FCID™ program only uses the 1994-98 CSFII database. The USEPA also currently uses the DEEM-FCID™ dietary exposure software. The USEPA 2002 draft endosulfan RED used the DEEM™ 1989-92 CSFII consumption database. The USEPA under went a transition to the 1994-98 CSFII from the 1989-92 consumption database within DEEM™ software between 2000 and 2002. The USEPA then converted to the DEEM-FCID™ dietary exposure software and the 1994-98 CSFII consumption database in 2003 (Exponent 2004a, b). Primarily, the USEPA did this to use the revised and more organized USEPA commodity codes and the individual food form codes.

The USDA 1989-92 CSFII database contains the consumption survey results from 10,383 participants over 3 complete days of dietary intake. There were a total of 3,132 individuals between the ages of 0 and 11 surveyed for the 1989-92 CSFII (Exponent, 2004a, USDA, 1989-92). The 1994-96 and 1998 (1994-98 CSFII) USDA survey reflects the results from 15,303 participants during the 1994-96 period plus an additional 5,304 children (0-9 years of age) surveyed in 1998 (Exponent, 2004b, USDA, 1994-98a). The 1994-98 CSFII is a 2-day complete consumption intake survey. The 1994-96 CSFII surveyed the dietary intake of 4,253 children between birth and 9 years of age. Therefore, the combined total of infants and children between the ages of 0 and 9 surveyed for the 1994-98 CSFII was 9,557. The 1994-98 CSFII represents a 2-fold increase in the number of infants and children surveyed over the 1989-92 CSFII (Exponent, 2004b, USDA, 1994-98a). Infants and children constitute approximately 46% of the 1994-98 participants.

There are two main differences between the CSFII databases besides the total number of participants and survey days. First, the 1994-98 CSFII reflects the most recent regional, gender, and ethnic commodity consumption patterns. Second, the survey contains additional targeted infants and children consumption information to address USDA concerns regarding statistically valid population sampling (NRC, 1993; USDA, 1994-98a). These concerns arose during evaluations of the preceding 1987-88 National-Food-Consumption-Survey (NFCS) and the 1989-92 CSFII survey by the National Research council (USDA, 1994-98, NRC, 1993). After review by governmental statisticians, the 1994-98 CSFII survey was deemed more statistically reliable and reflective of current consumption patterns by the USDA and the Government Accounting Office (GAO) than either the 1987-88 NFCS or the 1989-92 CSFII (USDA, 1994-98a, Exponent, 2004a). The 1998 DPR assessment used the 1989-92 CSFII with its GAO and NRC identified sampling deficiencies. DPR currently uses the 1994-98 CSFII consumption database associated with the DEEM-FCID™ dietary exposure software (USDA, 1994-98, Exponent, 2004b).

The consumption rates for the 4 highest use crops treated with endosulfan nationwide were analyzed. Pear was also included because it is a frequently consumed commodity by infants and children. When the 2002 USEPA RED was published, the 4 crops with the highest endosulfan use at the national level were cotton, tomato, potato, and apple (USEPA, 2001b, 2002). Pear has the 12<sup>th</sup> highest use nationally. The consumption rates for cottonseed are not discussed since there was

very low intake and also little difference in mean consumption between the two CSFII surveys<sup>2</sup>. Consumption rates based on age, gender, and ethnicity for each of the 3 highest use crops plus pears were compared for the 1989-92 and 1994-98 CSFII surveys. The consumption rates of apple, pear, potato, and tomato from the population sub-group western United States (western U.S.), children 1-6 years, nursing, and non-nursing infants of less than 1 year for both the 1989-92 and 1994-98 CSFII are summarized. There are significant differences in mean consumption rates between the 2 CSFII surveys (1989-92 and 1994-98) for the selected commodities and infant/children combinations. The differences between the 2 surveys' consumption rates ranged from a 63% decrease in tomato consumption by nursing infants from the 1989-92 group levels to a 71% increase in potato consumption by non-nursing infants relative to the 1989-92 rates.

The 1989-92 CSFII survey results were determined using the DEEM<sup>TM</sup> program while the 1994-98 CSFII consumption results were obtained using the DEEM-FCID<sup>TM</sup> software. Children 1-2 years of age population subgroup available in the 1994-98 CSFII of the DEEM-FCID<sup>TM</sup> software was not used. This exclusion from the 1994-98 CSFII was done because a standard Children 1-2 years population subgroup is unavailable in the 1989-92 CSFII consumption survey.

There are significant differences in mean consumption rates between the two CSFII surveys (1989-92 and 1994-98) for the selected commodities and infant/children combinations. The differences between the 2 surveys consumption rates ranged from a 50% decrease in pear consumption by both nursing and non-nursing infants from the 1989-92 group levels to a 238% increase in potato consumption by non-nursing infants relative to the 1989-92 rates. Additionally, the increased number of surveyed infants and children in the 1994-98 CSFII appears to have had an impact on percent user day rates (The definition of user day is found in Section VI Consumption Databases of the Endosulfan Dietary Exposure Addendum; Carr, 2006). Overall, the percent user day rates for the four surveyed groups in the 1994-98 CSFII database were about equal or higher than the rates seen in the 1989-92 populations with the exception of non-nursing infants (Table 27).

**Table 27. Comparison of Percent User Day Rates Between the 1989-92 and 1994-98 CSFII<sup>a</sup>**

RAC ----	Apple		Pear		Potato		Tomato	
	1989 <sup>b</sup>	1994 <sup>b</sup>	1989	1994	1989	1994	1989	1994
Population Sub-groups								
Western US	33%	34%	7%	11%	43%	71%	60%	67%
Children age 1-6	46	61	8	22	48	78	54	64
Nursing Infant	15	25	5	8	11	17	5	8
Non-Nursing Infant	47	44	15	15	30	35	24	17

a - A Percent User Day rate is the ratio of actual consumers divided by the combination of consumers and non-consumers (*per capita*) for each population subgroup Definition in Appendix C, Section VI Consumption Databases of the Endosulfan Dietary Exposure Addendum

b - The 1989-92 CSFII survey used DEEM<sup>TM</sup> and the 1994-98 CSFII used the DEEM-FCID<sup>TM</sup> program.

i. Apple: The mean consumption of apple from the 1994-98 CSFII survey ranged from a 39% decrease from 1989-92 levels to a 37% increase over 1989-92 levels depending on the population subgroup. The 1994-98 CSFII consumption by the western US population subgroup was 4% higher than rates from the 1989-92 survey. The 1994-98 consumption by the nursing infants sub-group was 39% lower than 1989-92 rate. The 1994-98 CSFII consumption by the

<sup>2</sup> Western U.S. population mean cottonseed consumption rates were 0.03 grams 0.05 g for the 1989-92 survey and 0.04 g 0.07 g for the 1994-98 CSFII. Cottonseed consumption rates were similar for the other groups.

non-nursing infants subgroup was 37% higher than the 1989-92 rate. The 1994-98 CSFII consumption by the children 1-6 years population subgroup was 9% lower than the 1989-92 rate.

ii. Pear: Mean pear consumption from the 1994-98 CSFII survey ranged between decreases of 45% to 60% when compared to the 1989-92 levels. The 1994-98 CSFII consumption by the western U.S. population sub-group was 54% lower than 1989-92 rate. The 1994-98 consumption by the nursing infants subgroup were 60% lower than 1989-92 rates. The 1994-98 consumption by non-nursing infants was 51% lower than 1989-92 rates. The 1994-98 CSFII consumption by the children 1-6 years population sub-group was 45% lower than 1989-92 rate.

iii. Potato: Overall mean potato consumption from the 1994-98 CSFII survey ranged from a 37% decrease to a 71% increase over 1989-92 levels. The 1994-98 CSFII consumption by the western U.S. population sub-group was 37% lower than 1989-92 rate. The 1994-98 consumption by the nursing infants subgroup was 24% higher than the 1989-92 CSFII level. The 1994-98 CSFII consumption by the non-nursing infants population sub-group was 71% higher than the 1989-92 level. The 1994-98 CSFII potato consumption level by the children 1-6 years population sub-group was 32% lower than the 1989-92 rate.

iv. Tomato: The mean consumption of tomato from the 1994-98 CSFII survey ranged from a 63% decrease from 1989-92 levels to a 71% increase over 1989-92 levels. The 1994-98 CSFII consumption by the western U.S. population subgroup was 7% lower than 1989-92 rate. The 1994-98 consumption by the nursing infants subgroup was 63% lower than 1989-92 rate. The 1994-98 CSFII consumption by the non-nursing infants subgroup was 71% higher than the 1989-92 rate. Finally, 1994-98 CSFII tomato consumption by the children 1-6 years population subgroup was 18% lower than 1989-92 rate.

The increased number of surveyed infants and children included in the 1994-98 CSFII database appears to have had an impact on percent user day rates. A Percent User Day rate is the ratio of actual consumers divided by the combination of consumers and non-consumers (*per capita*) for each population subgroup. Per capita consumption includes both consumers and non-consumers. Therefore, percent user day basis consumption reports only active consumers. The higher the percentage of active consumers relative to per capita consumption, the lower the variability and higher the reliability of the survey commodity consumption rates. Per capita consumption can often result in lower exposure in individual commodities when compared with active consumers (user days). The differences between per capita and active consumer consumption results become less relevant in frequently eaten, ubiquitous commodities (i.e., corn and other grain products, milk, soybean and refined sugar). Therefore, there is no difference between the intake rate of per capita and active consumers (user days) when consumption approaches 100% of the total person days. Over-all, the percent user day rates for the 4 surveyed groups in the 1994-98 CSFII database were generally equal to or higher than the consumption rates seen in the 1989-92 populations (Table 27). The exception was the non-nursing infants subgroup (apple and tomato only).

#### e) Exposure Analysis

Acute and chronic dietary exposure analyses were conducted with the Exposure-4<sup>TM</sup> and Exposure-1<sup>TM</sup> software programs, respectively (TAS, Technical Assessment Systems, Inc.). The

Exposure -4<sup>TM</sup> program estimates the distribution of user-day (consumer-day) exposure for the U.S. population and specific subgroups (TAS, 1996a). A user-day is any day in which at least one food from the labeled-approved commodities is consumed. The Exposure-1<sup>TM</sup> program estimates the annualized average exposure for all members of a designated population subgroup (TAS, 1996b). The rationale for this process is that an alternative to conducting seasonal exposure analysis is to closely examine both the acute and chronic dietary exposures for the possibility of using them as bounding range for the seasonal exposure. In a subchronic exposure scenario, individuals in a population subgroup could potentially have higher than chronic (average) exposure depending on the consumption pattern and residues on the seasonal commodities. The overall exposure for the group is, however, expected to be closer to the chronic than acute exposure because it is highly unlikely that individuals would consume commodities containing residue levels at the highest detected residues for the entire season. On the other hand, the exposure for a shorter-term (*e.g.*, 2-week) can be closer to the acute than the chronic exposure especially if the same or similar batch of food could be consumed over this period of time.

#### i. Acute (daily) Exposure

Potential acute dietary exposures were estimated using the highest measured residue values, the 95th percentile of all values, or the MDL for each commodity (Table 28; TAS, 1996a, USDA, 1989-91). For commodities with residues at “below detection limit,” a value equal to the MDL is assigned to each commodity. When the residue values were derived from monitoring programs, the assumption is that the data represent high-end residue levels in the diet. When data were available, residue levels for the raw agricultural commodities and processed forms were reduced to account for the loss of residues due to washing and other processing methods.

The potential acute dietary exposure of endosulfan from all labeled uses ranged from 1.37 ug/kg/day, males 13-19 years (females 13-19 years = 1.37) to 3.30 ug/kg/day, children 1-6 years for the 95<sup>th</sup> percentile of user-days exposures (Table 28). Male and female values (13-19 years), when rounded to two significant figures, were both 1.37 ug/kg/day. The complete acute dietary exposure analysis includes all current USEPA label approved endosulfan uses.

The exposure to endosulfan through the diet was also considered for pesticide workers in combination with occupational exposure (Tables 32-34). For acute dietary exposure, the value for Females (13+), nursing was used for adult acute occupational, adults in the general public for ambient air and bystanders and for adult swimmers in surface water. This population subgroup was selected, since it was a relatively high exposure in a population that would be found amongst all exposure scenarios for adults. The potential acute dietary exposure was estimated to be 2.06 ug/kg/day, based on the 95<sup>th</sup> percentile of user-day exposure for females age 13+ years, nursing (Table 28). The acute dietary exposure levels for infants (non-nursing, < 1 year) was selected to represent infants exposed to endosulfan in ambient air and to bystanders (95<sup>th</sup> percentile, 3.18 ug/kg/day). Children exposed to endosulfan while swimming in surface water had the acute dietary component of 3.30 ug/kg/day from the population subgroup of Children (1-6 years).

#### ii. Subchronic (seasonal) Exposure

The TAS program does not perform a subchronic dietary analysis; therefore, potential subchronic dietary exposures were estimated using the chronic exposure data (average measured

residue values of all values for each commodity). The subchronic NOEL, however, was different from the chronic. Therefore, subchronic dietary exposure is likely different even when using chronic RAC residues. For commodities with residues at "below detection limit," a value equal to one-half (50%) of the MDL was assigned to each commodity. When the residue values are derived from monitoring programs, the assumption is that the data represent annual average level in the diet (%CT). Therefore, for subchronic dietary exposure, the chronic value for females (13+), nursing was used for adult subchronic occupational, adults in the general public for ambient air and bystanders and for adult swimmers in surface water. The potential subchronic dietary exposure was estimated to be 0.17 ug/kg/day, based on the %CT annualized average for females age 13+ years, nursing (Table 28). The dietary subchronic exposure levels for infants (non-nursing, < 1 year) was selected to represent infants exposed to endosulfan in ambient air and to bystanders (0.28 ug/kg/day). Children exposed to endosulfan while swimming in surface water had the subchronic dietary component of 0.41 ug/kg/day from the subgroup of Children (1-6 years) (Table 28).

### iii. Chronic (annual) Exposure

Potential chronic dietary exposures were estimated using the average measured residue values of all values for each commodity. For commodities with residues at "below detection limit," a value equal to one-half (50%) the MDL is assigned to each commodity. When the residue values are derived from monitoring programs, the assumption is that data represent annual average level in the diet (%CT). Potential chronic dietary exposure, the chronic value for females (13+), nursing was used for adult chronic occupational, adults in the general public for ambient air and bystanders and for adult swimmers in surface water. The potential chronic dietary exposure was estimated to be 0.17 ug/kg/day for females age 13+ years, nursing (Table 27). The dietary chronic exposure levels for infants (non-nursing, < 1 year) was selected to represent infants exposed to endosulfan in ambient air and to bystanders (0.28 ug/kg/day). Children exposed to endosulfan while swimming in surface water had the chronic dietary component of 0.41 ug/kg/day from the subgroup of Children (1 - 6 years) (Table 28).

**Table 28. Acute and Chronic Dietary Exposure <sup>a</sup> to Anticipated Endosulfan Residues on Raw Agriculture Commodities.**

Population Subgroups	Acute Exposure <sup>a,b,c,e</sup> 95 <sup>th</sup> Percentile (ug/kg/day)	Chronic Exposure Annualized Ave <sup>a,b,c,d,e</sup>	
		No %CT	With %CT
US Population, all seasons	1.85	0.22	0.19
US Population, spring season	N/A	0.22	0.19
US Population, summer season	N/A	0.24	0.21
US Population, autumn season	N/A	0.22	0.19
Western Region	1.87	0.21	0.18
Pacific Region	1.86	0.21	0.18
Hispanics	1.94	0.22	0.19
Non-Hispanic Whites	1.79	0.22	0.19
Non-Hispanic Blacks	2.29	0.23	0.20
Non-Hispanic Other	2.34	0.24	0.20
All Infants	3.08	0.22	0.22
Infants (nursing, < 1 year)	1.90	0.11	0.08
<b>Infants (non-nursing, &lt; 1 year)</b>	<b>3.18</b>	0.34	<b>0.28</b>
<b>Children (1 - 6 years)</b>	<b>3.30</b>	0.47	<b>0.41</b>
Children (7 - 12 years)	2.09	0.33	0.29
Females (13 - 19 years), not pregnant, not nursing	1.37	0.20	0.18
Females (20+ years), not pregnant, not nursing	1.51	0.16	0.14
Females (13 - 50 years)	1.39	0.17	0.15
Females (13+ years), pregnant, not nursing	1.57	0.17	0.15
Females (13+ years), nursing	2.06	0.20	0.17
Males (13 - 19 years)	1.37	0.24	0.21
Males (20+ years)	1.38	0.18	0.15
Seniors (55+ years)	1.65	0.16	0.14

a - Exposure levels have been rounded off to 3 significant figures and are based on the 1989-1992 Continuing Survey of Food Intakes of Individuals (CSFII) and residue data from DPR, FDA and USDA.

b - The acute and chronic residue files used anticipated residue values for the commodities (Table 25).

c - No %CT = No adjustments were made for % crop treated. With %CT = Adjustments were made for % crop treated.

d - Chronic exposure data were used for subchronic exposures

e – “Acute” users were consumers. Chronic was “*per capita*” (consumers + non-consumers).

## 5. Air -- All Populations

### 1) Overview

Ambient air and application site air monitoring detected endosulfan, suggesting that the public may be exposed to airborne endosulfan. Individuals might be exposed to endosulfan if they are working adjacent to fields that are being treated or have recently been treated (bystander exposure). In addition, air monitoring conducted in Fresno County suggests that airborne endosulfan exposures are possible in areas that are far from application sites (ambient air exposure). Public exposure to airborne endosulfan was estimated based on monitoring studies of endosulfan at application sites and in ambient air. See Appendix E: Environmental Concentrations section for study details.

## 2) Ambient air

Table 29 summarizes ambient air exposure estimates to endosulfan based on ambient air monitoring in Fresno County, as well as on inhalation rate defaults documented by Andrews and Patterson (2000). These defaults are listed in Table 29, along with the original references. Seasonal and annual exposures were estimated according to DPR policy, based on the arithmetic mean of concentrations from Site SJ, where the highest concentrations were measured. Short-term exposures to ambient air are anticipated to be equal to or less than the acute bystander exposure, which addresses exposure of an individual who is adjacent to an application.

The endosulfan use pattern shown for Fresno County showed that the highest use occurred in June and July and most use occurred during seven months (February - March and June - October). This pattern is similar to the use pattern observed during ambient air monitoring in 1996. Annual exposure estimates shown in Table 29 assumed exposure occurred during the seven high-use months. SADD is 0.000037 mg/kg/day for infants and 0.000017 mg/kg/day for adults. Annual ADD is 0.000021 mg/kg/day for infants and 0.000010 mg/kg/day for adults.

Lee et al. (2002) estimated subchronic (> 14 days) and chronic (> 1 year) exposures for children and adults. For children, subchronic exposure estimates ranged 0.014 - 0.070  $\mu\text{g}/\text{kg}/\text{day}$  and chronic exposure estimates ranged from 0.0006-0.0035  $\mu\text{g}/\text{kg}/\text{day}$ . For adults, subchronic exposure estimates ranged from 0.006-0.049  $\mu\text{g}/\text{kg}/\text{day}$  and chronic exposure estimates ranged from 0.0003 - 0.0014  $\mu\text{g}/\text{kg}/\text{day}$  (Lee et al., 2002). Seasonal exposure estimates in Table 29 are in the range of the subchronic estimates reported by Lee et al. (2002). The annual ADD estimates reported in Table 29 are higher than the chronic estimates, as they are based on assumed constant inhalation rates and ambient air concentrations for 7 months, while the probabilistic estimates reported by Lee et al. (2002) assumed a gamma distribution for inhalation rates and a lognormal distribution for air concentrations.

## 8) Bystanders at application sites

To estimate bystander exposure to endosulfan in air, data were used from application site monitoring in a 1997 study in San Joaquin County (ARB, 1998). Stations (one each east, west and south, and two north) were located 6 - 16 m from the edge of the orchard. The application took place on April 8 between 5:45 and 7:45 AM. See Appendix E, Table 14 for a summary of endosulfan concentrations during several monitoring periods at each of these stations (Beauvais, 2007).

Bystander exposure estimates are given in Table 29. The 24-hour time-weighted average (TWA) for the east monitoring station ( $\text{TWA} = 1.26 \mu\text{g}/\text{m}^3$ ) was used to estimate exposure. The application rate used in the study (1.5 lbs AI/acre, or 1.7 kg AI/ha) was below the maximum rate allowed on apples (2.5 lbs AI/acre, or 2.8 kg AI/ha), suggesting that bystanders near fields where the maximum allowed rate is used would be exposed to higher concentrations than were found by ARB (1998). Exposure is assumed to be directly proportional to application rate. Each exposure estimate received a 1.67 multiplication factor (2.5 divided by 1.5). STADD for bystanders was 0.00160 mg/kg/day for infants and 0.00076 mg/kg/day for adults. Seasonal ADD estimates for bystander exposures to endosulfan were 0.00056 mg/kg/day for infants and 0.00027 mg/kg/day for adults. Annual ADD estimates for bystanders were 0.000047 mg/kg/day for infants and 0.000022 mg/kg/day for adults.

**Table 29. Ambient Air and Bystander Exposure Estimates for Persons Exposed to Endosulfan <sup>a</sup>**

Site	Air Concentration <sup>b</sup> µg/m <sup>3</sup>		STADD <sup>c</sup> mg/kg/d		SADD <sup>d</sup> mg/kg/d		AADD <sup>e</sup> mg/kg/d	
	Short-term <sup>c</sup>	Long-term <sup>d</sup>	Infants	Adults	Infants	Adults	Infants	Adults
Ambient Air								
Site SJ <sup>f</sup>	NA <sup>g</sup>	0.062	NA	NA	0.000037	0.000017	0.000021	0.000010
Bystander								
East Station <sup>g</sup>	2.10	0.952	0.00160	0.00076	0.00056	0.00027	0.000047	0.000022

<sup>a</sup> Estimates based on total endosulfan concentrations from monitoring conducted in Fresno County (ambient air) in 1996, and San Joaquin County (application site for bystander exposure) in 1997 (ARB, 1998).

<sup>b</sup> Arithmetic mean and standard deviation (SD). Calculated using 2 limit of quantitation (LOQ) for samples <LOQ. (See Table 14 for endosulfan concentrations in ambient air monitoring, and Table 15 for application site monitoring in Beauvais, 2007).

<sup>c</sup> Short-Term Absorbed Daily Dosage (mg/kg/day) = (short-term concentration) x (inhalation rate), where 24-h TWA was used for Short Term Concentration calculations. Calculation assumptions include:

- Infant inhalation rate = 0.59 m<sup>3</sup>/kg/day (Layton, 1993; U.S. EPA, 1997)
- Adult inhalation rate = 0.28 m<sup>3</sup>/kg/day (Wiley *et al.*, 1991; U.S. EPA, 1997; OEHHA, 2000)
- Inhalation absorption is assumed to be 100%

<sup>d</sup> Seasonal ADD = (long term concentration) x (inhalation rate), where 3-day TWA was used for Long Term Concentration calculations. Calculation assumptions are as described above.

<sup>e</sup> Annual ADD = (Seasonal ADD) x (annual use months per year)/12. Annual exposure estimates are based on high-use period of 7 months for ambient air, based on use reported in San Joaquin County. Annual bystander exposure estimates are based on high-use period of 1 month, as repeated applications adjacent to any one individual are considered unlikely for longer intervals.

<sup>f</sup> Site SJ = San Joaquin Elementary School, San Joaquin. This was the site with most samples above the LOQ.

<sup>g</sup> East station was the application air monitoring site with the highest endosulfan TWA concentrations. Short-term exposure estimates were multiplied by 1.67, because the application rate used in the study (1.5 lbs AI/acre, or 1.7 kg AI/ha) was below the maximum rate allowed on apples (2.5 lbs AI/acre, or 2.8 kg AI/ha). Seasonal and annual exposure estimates were not adjusted for differences in application rate.

## 6. Water

### a) Surface Water

Historically, endosulfan has been detected numerous times in California surface waters. Guo and Spurlock (2000) summarized historical monitoring data, reported by nine different agencies between 1990 and July 2000, for pesticides in surface water in California. Monitoring for α-endosulfan, β-endosulfan, and endosulfan sulfate was conducted between August 1990 and July 1996; no monitoring has been reported since 1996 (DPR, 2004).

As shown in Table 30, endosulfan sulfate has been detected more frequently in surface water samples than α- or β-endosulfan, and generally at higher concentrations. Endosulfan residues have been detected in California surface waters in the Central Valley (Ross *et al.*, 1996 and 2000) and in the Sierra Nevada Mountains (Fellers *et al.*, 2004). Movement of endosulfan into surface water via rainfall runoff and irrigation drainage has been documented (Gonzalez *et al.*, 1987; Fleck *et al.*, 1991). No endosulfan residues have been detected in drinking water in California in the past three years for which data are available (USDA, 2003; 2004; 2005). These results suggest that drinking water systems in California, and household water used for showering and bathing, are not likely to be a source of human exposure to endosulfan.

**Table 30. Summary of Historical Surface Water Sampling Data for Endosulfan in California Through July 2000**

Chemical	# Analyses <sup>a</sup>	# Detections <sup>a</sup>	Detection Frequency (%) <sup>a</sup>	Concentration (µg/L) <sup>b</sup> Percentiles		
				50 <sup>th</sup>	75 <sup>th</sup>	95 <sup>th</sup>
α-Endosulfan	764	40	5.2	0.0025	0.005	0.05
β-Endosulfan	764	41	5.4	0.0025	0.036	0.05
Endosulfan Sulfate	661	114	17.2	0.005	0.029	0.05

<sup>a</sup> Adapted from Guo and Spurlock (2000), which summarizes water sampling conducted between August 1990 and July 2000. However, no monitoring for endosulfan has been reported since July 1996 (DPR, 2004), nor does the database differentiate between surface water systems that are sources of drinking water and those that are not (F. Spurlock, personal communication, June 7, 2005).

<sup>b</sup> Values were calculated using the Percentile function in Excel, from data in DPR (2004). Calculated using 2 LOQ for samples < LOQ. Nine samples collected before introduction of permit conditions were omitted.

In surface water systems, endosulfan residues have also been detected in sediment (Gonzalez et al., 1987; Fleck et al., 1991; Ganapathy et al., 1997; Weston et al., 2004); mussels (Singhasemanon, 1996; Ganapathy et al., 1997); amphibians (Sparling et al., 2001); and fish (Singhasemanon, 1995; Brodberg and Pollock, 1999). Because endosulfan has been detected in surface water, sediment and aquatic organisms, and in response to concerns about endosulfan's toxicity, in 1991 DPR began requiring permit conditions to prevent use of endosulfan where it might be allowed to reach surface water (Okumura, 1992).

#### b) Swimmer Exposures

As summarized previously in the Environmental Concentrations section, endosulfan residues have been detected in surface waters in California. Exposures of adults and children swimming in surface waters were estimated based on equations listed in U.S. EPA (2003). These calculations are summarized below.

The endosulfan dose absorbed dermally was estimated with the following equation:

$$ADR = C_w * SA * ET * K_p * CF1$$

where ADR = absorbed dose rate (mg/day);  $C_w$  = concentration of AI in water (mg/L); SA = surface area exposed ( $\text{cm}^2$ ); ET = exposure time (hours/day);  $K_p$  = permeability coefficient; and CF1 = volume unit conversion factor ( $\text{L}/1,000 \text{ cm}^3$ ). The 95<sup>th</sup> percentile total endosulfan concentration of 0.15 µg/L ( $C_w = 0.00015 \text{ mg/L}$ ), calculated from the 95<sup>th</sup> percentile concentrations (reported in Table 15, Beauvais, 2007) was used in estimating short-term swimmer exposure (STADD). For long-term exposures, the median total endosulfan concentration of 0.010 µg/L ( $C_w = 0.000010 \text{ mg/L}$ ) was calculated from the 50<sup>th</sup> percentile concentrations (Beauvais, 2007; Table 15). Default values were used for SA and ET. For adults, SA = 18,150  $\text{cm}^2$  and for a 6 year-old child, SA = 8,545  $\text{cm}^2$  (U.S. EPA, 1997). For short-term exposures, the ET was assumed to be 5 hours (U.S. EPA, 2003). For long-term exposures, the ET was assumed to average 2.3 hours/day for children and 1.3 hours/day for adults (U.S. EPA, 2003). Weather was assumed to be suitable for outdoor swimming for 100 days each year. The permeability coefficient for endosulfan was 0.0112 cm/hr and was used for  $K_p$  (See calculations in Appendix 13, Beauvais, 2007).

The endosulfan dose absorbed from incidental non-dietary ingestion was estimated with the following equation:

$$PDR = C_w * IR * ET$$

where ADR = absorbed dose rate (mg/day);  $C_w$  = concentration of AI in water (mg/L); IR = ingestion rate of pool water (L/hour); and ET = exposure time (hours/day). In calculating PDR, the same values were used for  $C_w$  and ET as those used in calculating ADR. The ingestion rate (IR) was assumed to be 0.05 L/hr for children and 0.025L/hr for adults (USEPA, 2003).

Both STADD and SADD were calculated from ADR and PDR by dividing by default body weights of 70 kg for an adult (Thongsinthusak *et al.*, 1993) and 24 kg for a 6 year-old child (USEPA, 1997c). Exposure estimates are summarized in Table 31. Inhalation exposure was assumed to be negligible, and was not included in swimmer exposure estimates. The total exposure was calculated by summing dermal and non-dietary ingestion exposure estimates. Total STADD is 0.00027 mg/kg/day for adults and 0.00156 mg/kg/day for children.

**Table 31. Exposures to Endosulfan Estimated for Swimmers in Surface Waters <sup>a</sup>**

Exposure scenario	STADD (mg/kg/day) <sup>b</sup>	SADD (mg/kg/day) <sup>c</sup>	AADD (mg/kg/day) <sup>d</sup>
Adult Dermal <sup>e</sup>	0.00000218	0.0000000378	0.0000000103
Adult Non-Dietary Ingestion <sup>f</sup>	0.000268	0.00000464	0.00000127
Adult Total <sup>g</sup>	0.00027	0.00000468	0.00000128
Child Dermal <sup>e</sup>	0.00000299	0.0000000917	0.0000000251
Child Non-Dietary Ingestion <sup>f</sup>	0.00156	0.0000479	0.0000131
Child Total <sup>g</sup>	0.00156	0.0000480	0.0000131

- <sup>a</sup> - Exposure estimates include dermal and ingestion routes, as inhalation route assumed to be insignificant. Endosulfan concentrations used in exposure estimates are from the Department of Pesticide Regulation Surface Water Database (DPR, 2004). The 95<sup>th</sup> percentile total endosulfan concentration of 0.15 µg/L, calculated from the 95<sup>th</sup> percentile concentrations (Beauvais, 2007) was used in estimating short-term exposure. For long-term exposures, the median total endosulfan concentration of 0.010 µg/L was calculated from the 50<sup>th</sup> percentile concentrations (Beauvais, 2007).
- <sup>b</sup> - Short-term Absorbed Daily Dosage (STADD) calculation described in text. Swimmers assumed to swim for 5 hours in a day (USEPA, 2003). Body weight assumed to be 70 kg--adult (Thongsinthusak *et al.*, 1993); 24 kg child (USEPA, 1997).
- <sup>c</sup> - Seasonal Average Daily Dosage is a mean estimate of absorbed dose, calculated as described in text. Swimmers were assumed to swim for an average of 2.3 hours/day for children and 1.3 hours/day for adults (U.S. EPA, 2003).
- <sup>d</sup> - Annual Average Daily Dosage = SADD x (100 days)/(365 days in a year).
- <sup>e</sup> - Dermal exposure estimates assume a median surface area of 18,150 cm<sup>2</sup> (adult) & 8,565 cm<sup>2</sup> (child) (USEPA, 1997c).
- <sup>f</sup> - Incidental non-dietary ingestion assume ingestion rate of 0.05 L/hr for children; 0.025 L/hr for adults (USEPA, 2003).
- <sup>g</sup> - Child/Adult Dermal plus Non-dietary ingestion equals Adult/Child Total.

### c) Ground Water

DPR has a well monitoring program that samples numerous wells each year to determine the presence and geographical distribution of agriculturally applied pesticides in groundwater. Troiano et al. (2001) describe the monitoring program and the criteria used for well selection (for sampling), in addition to analytical methods. Between 1986 and 2003, a total of 2,758 well water samples collected in 48 California counties (out of 58 counties total) were tested for the presence of endosulfan and endosulfan sulfate (Schuette *et al.*, 2003). Endosulfan was detected in ten samples, at concentrations ranging 0.01-34.7 µg/L. All ten detections were classified as “unverified,” meaning that follow-up sampling failed to detect endosulfan or endosulfan sulfate. These results, along with reported non-detection of endosulfan residues in monitoring of drinking water systems (USDA, 2003; 2004; 2005), suggest that drinking water systems in California drawing from ground water are not likely to be a source of human exposure to endosulfan.

### d) Drinking Water

Nationally, there were no detections of  $\alpha$ -endosulfan,  $\beta$ -endosulfan or endosulfan sulfate in drinking water from 2001 to 2003 (USDA. Pesticide Data Program, 2003, 2004, 2005). Water testing, initiated by the USDA in 2001, was represented by several thousand samples in California and New York over the 2001 to 2003 period. California and New York were selected for initial sampling since they are both highly populated, have divergent climates and have divergent hydrogeological settings. Diversity of land uses, including metropolitan areas, agricultural regions and protected watersheds are also reflected within these two States. The non-detection of endosulfan isomers or the sulfate metabolite during this 3-year period indicates that endosulfan does not need to be included in dietary calculations.

## 7. Aggregate Exposure

### a) Overview

Aggregate exposure is the combined exposure of multiple pathways such as dermal, oral (non-dietary ingested), air, and dietary. As stated in the USEPA guidelines, aggregate exposure should link spatial (i.e., all pathways agree in age/gender/ethnicity and other demographic characteristics) characteristics of each route in effort to derive a consistent and reasonable assessment of total exposure (USEPA, 1999b and 2001e). The estimation of exposure and risk should focus on the individual with each of the individual sub-assessments “linked back to the same person and the aggregate intake should reflect the food, drinking water, and residential intakes that are for the same individual at the same time, in the same place, and under the same demographic conditions” (USEPA, 1999b). The collective exposures and risks for individuals are then used to develop those values for population subgroups and the entire population.

For endosulfan, the underlying assumption was that there is potential for aggregate exposure because endosulfan residues have been detected in air, on skin, in diet, and non-dietary ingested (swimmers in surface water) but not in drinking water. Due to insufficient exposure data, it was not possible to estimate the aggregate exposure at an individual level. Instead in this assessment, the population was broadly divided into occupational (workers who work with endosulfan) and the public

(those who don't handle endosulfan).

The exposure to endosulfan through the diet was considered in combination with the potential exposure for pesticide workers and to the public through ambient air and for bystanders and for the public (children and adults; dermal and non-dietary ingested) swimming in surface water. The aggregate occupational or exposure to the public in air and to swimmers plus dietary exposures were summarized in Tables 31 - 35. For aggregate (occupational plus dietary) exposure in occupational scenarios, the STADD, SADD and AADD exposure components were derived from the occupational exposure total of the dermal plus the inhalation values (Tables 17-22). In addition, for this combination of occupational plus dietary, the oral NOELs for acute, subchronic and chronic studies were used in the STADD, SADD and AADD determinations for occupational and swimmer in surface water scenarios. This is because for these particular “combined” exposures, the dietary and dermal routes comprise the primary routes, because an oral NOEL is used for dermal exposure (no acceptable dermal study) and because the oral route is also the dietary route. Females (13+ years), nursing (acute: 2.06 ug/kg; subchronic/chronic: 0.17 ug/kg/day) was used in the estimate of adult (occupational, ambient air, bystanders and swimmers in surface water) dietary exposure to endosulfan (TAS, 1989 - 1992; 1998 analysis date). Infants (non-nursing, < 1 year) was used for the public (infant) ambient air dietary exposure (acute: 3.18 ug/kg/day; subchronic/chronic: 0.28 ug/kg/day). Children (1 - 6 years) was used for the public (children) swimmers in surface water dietary exposure (acute: 3.30 ug/kg/day; subchronic/chronic: 0.41 ug/kg/day). In general, when the dietary exposure exceeded 2% of the aggregate occupational, bystander or non-dietary ingestion to swimmers in surface water plus dietary exposure, it was noted in Tables 31 - 35. This arbitrary cut off provided an indication of the magnitude of the dietary contribution in relation to the occupational exposure (or conversely the magnitude of the occupational exposure in relation to the dietary component). When non-dietary exposure was very low, the dietary component increased accordingly in the percentage contribution.

#### b) Occupational Aggregate Exposure

The predominant factor for mitigating human exposure to endosulfan is the occupational exposure. For example, in more than half of all aggregate occupational exposure scenarios (acute, subchronic, chronic), the dietary component comprised less than 3% (49/89 = 55%) of the aggregate exposure (data in **bold** Tables 31 - 33). The majority of the aggregate occupational exposures where diet comprised a higher percentage (3% or greater) was observed for STADD (18/35; 51%) and AADD (16/27; 59%). SADD total occupational aggregate exposures had a dietary component of 22% (6/27) (less than half that of the other scenarios) with a dietary component of greater than 2%. The highest percentages for dietary contribution of aggregate occupational exposure were re-entry scenarios where STADD was 60% (9/15), SADD was 30% (3/10) and AADD was 80% (8/10) (data in **bold** Table 34).

**Table 32. Potential Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic Exposure to Endosulfan by Either Aerial, Airblast or Groundboom Application**

Scenario <sup>a</sup>	Mean STADD (mg/kg/day)		Mean SADD (mg/kg/day)		Mean AADD (mg/kg/day)	
	Occupational <sup>b</sup>	Aggregate <sup>c</sup>	Occupational <sup>b</sup>	Aggregate <sup>c</sup>	Occupational <sup>b</sup>	Aggregate <sup>c</sup>
<b>Aerial</b>						
M/L - EC	0.225	0.23	0.034	0.034	0.011	0.011
M/L - WP	2.63	2.63	0.385	0.385	0.128	0.13
M/L - WP/WSP	0.185	0.187	0.044	0.044	0.015	0.015
Applicator	0.79	0.79	0.158	0.16	0.053	0.053
Flagger	0.373	0.375	0.057	0.057	0.019	0.019
<b>Airblast</b>						
M/L - EC	0.026	<b>0.028 (7%)</b>	0.006	<b>0.006 (3%)</b>	0.001	<b>0.001 (14%)</b>
M/L - WP	0.30	0.30	0.073	0.073	0.012	0.012
M/L - WSP	0.021	<b>0.023 (9%)</b>	0.008	0.008	0.001	<b>0.001 (14%)</b>
Applicator	0.18	0.19	0.048	0.048	0.008	0.008
<b>Groundboom</b>						
M/L - EC	0.041	<b>0.043 (5%)</b>	0.008	0.008	0.003	<b>0.003 (5%)</b>
M/L - WP	0.48	0.48	0.088	0.090	0.037	0.037
M/L - WSP	0.034	<b>0.036 (5%)</b>	0.010	0.010	0.004	<b>0.004 (4%)</b>
Applicator	0.045	<b>0.047 (4%)</b>	0.005	<b>0.005 (3%)</b>	0.002	<b>0.002 (8%)</b>

<sup>a</sup> - abbreviations: EC = emulsifiable concentrate. M/L = mixer/loader. M/L/A = mixer/loader/applicator. WP = wettable powder. WSP = water soluble packaging. Further description in Table 18.

<sup>b</sup> -The “occupational” component of this table is the total exposure for Dermal + Inhalation exposure reported in Tables 18 & 19

<sup>c</sup> - Aggregate = Occupational (dermal + inhalation) + dietary exposure: Acute dietary exposure = 2.06 ug/kg/day based on the 95<sup>th</sup> percentile of user-day exposure for Females (13+ years), nursing and chronic dietary exposure = 0.17 ug/kg/day (%CT; mean annual consumption for Females (13+ years)). Values were rounded to 2 significant figures.

**Bold indicates aggregate values where the dietary contribution comprises greater than 2%.**

(%) = Parentheses indicate the percent dietary contribution for aggregate exposure to endosulfan.

**Table 33. Potential Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic Exposure to Endosulfan for Handlers Using Handheld Equipment to Apply Endosulfan**

Scenario <sup>a</sup>	Mean STADD (mg/kg/day) <sup>b</sup>		Mean SADD (mg/kg/day) <sup>b</sup>		Mean AADD (mg/kg/day) <sup>b</sup>	
	Occupational	Aggregate <sup>c</sup>	Occupational	Aggregate <sup>c</sup>	Occupational	Aggregate <sup>c</sup>
<b>BACKPACK</b>						
M/L/A EC	0.043	<b>0.045 (4%)</b>	0.011	0.011	0.002	<b>0.004 (8%)</b>
<b>HIGH PRESSURE HAND WAND</b>						
M/L/A EC	0.511	0.512	0.153	0.153	0.026	0.026
<b>LOW PRESSURE HAND WAND</b>						
M/L/A EC	0.013	<b>0.015 (13%)</b>	0.003	<b>0.003 (5%)</b>	0.0005	<b>0.0007 (25%)</b>
M/L/A WP	0.10	0.10	0.015	0.015	0.003	<b>0.003 (5%)</b>
<b>DIP</b>						
M/L EC	0.00003	<b>0.002 (98%)</b>	--d	--	--	--
M/L WP	0.003	<b>0.005 (40%)</b>	--	--	--	--
Applicator	41.4	41.4	--	--	--	--

a - BP = backpack sprayer. EC = emulsifiable concentrate. HPHW = high pressure handwand. LPHW = low pressure handwand. M/L = mixer/loader. M/L/A = mixer/loader/applicator. WP = wettable powder. Dip = Nursery stock dip for treatment of cherry, peach and plum seedlings for peachtree borer. Handlers were assumed to wear gloves, respirator, and coveralls, as specified on product labels (see Beauvais, 2007).

b - From Tables 19 & 20

c - Aggregate mean occupational + dietary exposure: Acute dietary exposure = 2.06 ug/kg/day based on the 95<sup>th</sup> percentile of user-day exposure for Females (13+ years), nursing and chronic dietary exposure = 0.17 ug/kg/d (%CT; mean annual consumption for Females (13+ years)). Values rounded to 2 significant figures.

d - "--" = Values not available (Beauvais, 2007)

**Bold indicates aggregate values where the dietary contribution comprises greater than 2%.**

(%) = Parentheses indicate the percent dietary contribution for aggregate exposure to endosulfan.

**Table 34. Potential Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic Exposure to Endosulfan for Reentry Workers**

Exposure scenario <sup>a</sup>	STADD (mg/kg/day) <sup>b</sup>		SADD (mg/kg/day) <sup>b</sup>		AADD (mg/kg/day) <sup>b</sup>	
	Occupational	Aggregate <sup>c</sup>	Occupational	Aggregate <sup>c</sup>	Occupational	Aggregate <sup>c</sup>
Almond, Thinning	0.009	<b>0.01 (18%)</b>	-- d	--	--	--
Broccoli-Hand Harvest	0.03	<b>0.03 (6%)</b>	0.008	0.008	0.002	<b>0.002 (8%)</b>
Broccoli, Scouting	0.084	0.086	0.012	0.012	0.005	<b>0.005 (3%)</b>
Citrus, Thinning	0.055	<b>0.057 (3%)</b>	--	--	--	--
Sweet Corn-Hand Harvest	0.533	0.535	0.075	0.075	0.006	0.006
Cotton, Scouting	0.063	<b>0.065 (3%)</b>	0.009	<b>0.009 (17%)</b>	0.002	<b>0.002 (8%)</b>
Cucumber-Hand Harvest	0.053	<b>0.055 (3%)</b>	0.007	0.007	0.001	<b>0.001 (14%)</b>
Grape, Cane Turning	0.335	0.337	0.141	0.141	0.047	0.047
Lettuce, Scouting	0.162	0.164	0.004	<b>0.004 (4%)</b>	0.002	<b>0.002 (8%)</b>
Ornamental-Hand Harvest	0.009	<b>0.011 (18%)</b>	--	--	--	--
Peach, Thinning	0.055	<b>0.057 (3%)</b>	0.028	0.028	0.005	<b>0.005 (3%)</b>
Potato, Scouting	0.032	<b>0.034 (6%)</b>	0.004	<b>0.004 (4%)</b>	0.002	<b>0.002 (8%)</b>
Strawberry-Hand Harvest	0.067	0.069	--	--	--	--
Tomato-Hand Harvesting	0.021	<b>0.023 (9%)</b>	0.009	0.009	0.003	<b>0.003 (5%)</b>
Ornamental Cut Flowers, Hand Harvested	0.159	0.161	--	--	--	--

a -Reentry exposure scenario

b - From Tables 20 – 21

c - Aggregate mean occupational + dietary exposure: Acute dietary exposure = 2.06 ug/kg/day based on the 95<sup>th</sup> percentile of user-day exposure for Females (13+ years), nursing and chronic dietary exposure = 0.17 ug/kg/day (%CT; mean annual consumption for Females (13+ years)). Values were rounded to 2 significant figures.

d – “—“ = No seasonal or annual exposure estimates were prepared for workers reentering treated almond or citrus orchards or strawberry fields. Infrequent endosulfan use is reported on these crops (Beauvais, 2007).

**Bold indicates aggregate values where the dietary contribution comprises greater than 2%.**  
 (%) = Parentheses indicate the percent dietary contribution for aggregate exposure to endosulfan.

c) Aggregate Dietary and Exposure in Ambient Air and to Bystanders

For adults and children with aggregate exposure to endosulfan in ambient air or as bystanders plus diet showed that the dietary component for STADD, SADD and AADD is the major exposure. However all of the non-dietary exposure components for all air scenarios are very low and that is why the dietary contribution (while also quite low) appears to be so much greater (Table 35). The dietary percentage of exposure was lowest in SADD infant bystanders (33%; non-dietary exposure was 0.00056 mg/kg/day). The dietary exposure was highest in ambient air for adults (AADD, 94%), where the non-dietary exposure was 0.00001 mg/kg/day.

**Table 35. Aggregate<sup>a</sup> (Dietary + Ambient Air or Bystander) Exposure Estimates for Persons Exposed to Endosulfan**

Site <sup>b</sup>	Mean STADD--mg/kg/day <sup>c</sup>		Mean SADD~mg/kg/day <sup>c</sup>		Mean AADD~mg/kg/day <sup>c, e</sup>	
	Non-Diet	Aggregate	Non-Dietary	Aggregate	Non-Dietary	Aggregate
<b>Ambient Air -- Infants</b>						
Site SJ <sup>f</sup>	NA <sup>d</sup>	NA <sup>d</sup>	0.000037	<b>0.00032 (88%)</b>	0.00002	<b>0.0003 (93%)</b>
<b>Ambient Air -- Adults</b>						
Site SJ <sup>f</sup>	NA <sup>d</sup>	NA <sup>d</sup>	0.000017	<b>0.00019 (91%)</b>	0.00001	<b>0.00018 (94%)</b>
<b>Bystander -- Infants</b>						
East Station <sup>g</sup>	0.0016	<b>0.00478 (67%)</b>	0.00056	<b>0.00084 (33%)</b>	0.000047	<b>0.000327 (86%)</b>
<b>Bystander – Adults</b>						
East Station <sup>g</sup>	0.00076	<b>0.0028 (73%)</b>	0.00027	<b>0.00044 (39%)</b>	0.000022	<b>0.000192 (86%)</b>

a - Aggregate mean occupational + dietary exposure: Adult acute dietary exposure = 2.06 ug/kg/d based on the 95<sup>th</sup> percentile of user-day exposure for Females (13+ years), nursing and adult chronic dietary exposure = 0.17 ug/kg/d (%CT; mean annual consumption for Females (13+ years), nursing). Dietary for infant acute dietary exposure = 3.18 ug/kg/d based on 95<sup>th</sup> percentile of user-day exposure for infants, non-nursing, < 1 year) and chronic infant dietary exposure = 0.28 ug/kg/d (%CT; mean annual consumption for infants, non-nursing, < 1 year). Values were rounded to 2 significant figures.

b - Estimates based on total endosulfan concentrations from monitoring conducted in Fresno County (ambient air) in 1996, and San Joaquin County (application site for bystander exposure) in 1997 (ARB, 1998).

c - From Table 29

d - NA = Not applicable. Seasonal and annual exposure not anticipated for bystanders (see Beauvais, 2007)

e - Annual ADD = (Seasonal ADD) x (annual use months per year)/12. Annual exposure estimates are based on high-use period of 7 months for ambient air, based on use reported in San Joaquin County. Annual bystander exposure estimates are based on high-use period of 1 month, as repeated applications adjacent to any one individual are considered unlikely for longer intervals.

f - Site SJ = San Joaquin Elementary School, San Joaquin. This was the site with most samples above the LOQ.

g - East station was the application air monitoring site with the highest endosulfan TWA concentrations. Short-term exposure estimates were multiplied by 1.67, because the application rate used in the study (1.5 lbs AI/acre, or 1.7 kg AI/ha) was below the maximum rate allowed on apples (2.5 lbs AI/acre, or 2.8 kg AI/ha). Seasonal and annual exposure estimates were not adjusted for differences in application rate.

--- **Bold indicates aggregate values where the dietary contribution comprises greater than 2%.**

--- (%) = Parentheses indicate the percent dietary contribution for aggregate exposure to endosulfan.

d) Aggregate Dietary and Exposure to Swimmers in Surface Water

STADD for child non-diet ingestion (and total) had the lowest dietary component for aggregate exposure (68%) (Table 36). The non-dietary exposure for this was 0.00156 mg/kg/day and was the highest exposure of all scenarios. STADD for adult non-dietary ingestion (and total) was 0.00027

mg/kg/day and the dietary comprised 88% of the aggregate exposure. The SADD for child non-dietary and total there was an 89% dietary contribution. For all other groups, the non-dietary exposure was so comparatively low that the dietary comprised 97% to 100% of the aggregate exposure.

**Table 36. Endosulfan Exposure Swimmers in Surface Waters <sup>a</sup> on a Short Term, Seasonal or Chronic Basis**

Exposure scenario	STADD (mg/kg/day) <sup>b</sup>		SADD (mg/kg/day) <sup>b</sup>		AADD (mg/kg/day) <sup>b</sup>	
	Non-dietary	Aggregate <sup>c</sup>	Non-dietary	Aggregate <sup>c</sup>	Non-dietary	Aggregate <sup>c</sup>
Adult Dermal	0.00000218	<b>0.002 (100%)</b>	0.000000378	<b>0.00017 (100%)</b>	0.00000001	<b>0.00017 (100%)</b>
Adult Non-Diet Ingestion	0.000268	<b>0.002268 (88%)</b>	0.00000464	<b>0.000175 (97%)</b>	0.00000127	<b>0.000171 (99%)</b>
Adult Total	0.00027	<b>0.00227 (88%)</b>	0.00000464	<b>0.000175 (97%)</b>	0.00000128	<b>0.000171 (99%)</b>
Child Dermal	0.00000299	<b>0.0033 (100%)</b>	0.000000917	<b>0.0004 (100%)</b>	0.000000025	<b>0.0004 (100%)</b>
Child Non-Diet Ingestion	0.00156	<b>0.00486 (68%)</b>	0.000079	<b>0.000448 (89%)</b>	0.0000131	<b>0.000413 (97%)</b>
Child Total	0.00156	<b>0.00486 (68%)</b>	0.000048	<b>0.000448 (89%)</b>	0.0000131	<b>0.000413 (97%)</b>

a - See Table 30 for further description.

b - From Tables 31

c - Aggregate mean occupational + dietary exposure: Acute dietary exposure = 2.06 ug/kg/day based on the 95<sup>th</sup> percentile of user-day exposure for Females (13+ years), nursing and chronic dietary exposure = 0.17 ug/kg/day (%CT; mean annual consumption for Females (13+ years)). Acute dietary exposure = 3.30 ug/kg/day based on the 95<sup>th</sup> percentile of user-day exposure for Children (1-6 years) and chronic dietary exposure in children = 0.41 ug/kg/day (%CT; mean annual consumption for infants, non-nursing, < 1 year). Values were rounded to 2 significant figures.

-- **Bold indicates aggregate values where the dietary contribution comprises greater than 2%.**

-- (%) = Parentheses indicate the percent dietary contribution for aggregate exposure to endosulfan.

### C. RISK CHARACTERIZATION:

The acute, subchronic and chronic NOELs employed for the characterization of the risk for exposure to endosulfan were derived from studies performed on laboratory animals. Consequently a calculated MOE of 100 was considered prudent for protection against endosulfan toxicity. The benchmark of 100 includes an uncertainty factor of 10 for interspecies sensitivity and 10 for intraspecies variability.

The NOELs used for risk characterization were previously described (dermal = NOEL<sub>oral</sub>: 0.70, 1.18 and 0.57 mg/kg/day, for acute, subchronic and chronic, respectively; NOEL<sub>inhalation</sub> = 0.194, 0.194 and (ENEL) 0.0194 mg/kg/day for acute, subchronic and chronic, respectively). In addition, for the aggregate combination of occupational or inhalation plus dietary, the oral NOELs for short-term, subchronic and chronic dermal or inhalation were used in combination with dietary MOE estimations (Acute = 340, 95<sup>th</sup> percentile for females (13+ years), nursing; Chronic (used also for subchronic) = 3448 (females (13+ years), nursing).

#### 1. Risk Characterization (Margins of Exposure) for a Single Route (oral, inhalation):

In the assessment of single route of exposure, the risk for non-oncogenic effect was characterized in terms of a margin of exposure (MOE), defined as the ratio of the critical human equivalent NOEL to the estimated human exposure levels. The calculation is shown below:

$$\text{Single Route Margin of Exposure} = \frac{\text{NOEL (eg: oral, inhalation)}}{\text{Exposure Dosage (route specific: diet, dermal, inhalation)}}$$

a) **Occupational Risk (Dermal, Inhalation and Total = Dermal + Inhalation)**

i. Occupational MOE for Aerial, Airblast and Groundboom Application

**Dermal:** Aerial application dermal MOEs for all occupational scenarios (STADD, SADD and AADD) were less than 100, as were all STADD scenarios for airblast and groundboom. Airblast and groundboom dermal SADD and AADD scenarios were greater than 100 except for airblast (M/L-WP and applicator) and groundboom M/L-WP (Table 37). The lowest dermal MOEs were less than or equal to 1 for STADD aerial M/L with WP, and aerial applicators. The greatest dermal MOEs were both 570 for AADD for airblast M/L with WSP and airblast M/L with EC (Table 37).

**Inhalation:** Aerial application inhalation MOEs for STADD were all less than 100 (<1 – 97). Airblast STADD MOEs for inhalation were 194 for airblast (M/L – EC and applicator) and 194 for groundboom M/L – EC and applicator. The other scenarios for airblast and groundboom were less than 100 (3 – 97). SADD inhalation MOEs were greater than 100 except for Aerial (M/L – WP, M/L – WP/WSP), airblast (M/L – WP) and groundboom (M/L – WP). AADD showed all aerial scenarios had MOEs of less than 100 except for flaggers (388). All airblast scenarios for inhalation exposure had MOEs of greater than 100 except M/L – WP. AADD groundboom scenarios had MOEs of less than 100 for M/L – WP and M/L – WSP (5, 49, respectively) and the other inhalation scenarios were 194 (M/L – EC and applicator) (Table 37).

ii. Occupational MOE for Handlers Using Handheld Equipment.

**Dermal:** Several STADD dermal MOEs were below 100 for handlers using handheld equipment (Table 37), with dip applicator and HPHW M/L/A-EC MOEs being less than or equal to 1. Only dip M/L-EC or M/L-WP had MOEs greater than 100 (23333 and 2,333, respectively). Other occupational dermal MOEs were greater than 100 except for SADD HPHW M/L/A-EC (8) and LPHW M/L/A-WP (79) and AADD HPHW M/L/A-EC (23). (Table 38).

**Inhalation:** STADD inhalation MOEs below 100 for handlers using handheld equipment were HPHW M/L/A-EC (2), LPHW M/L/A-WP (65) and dip applicator (39). STADD backpack sprayer M/L/A-EC (1940), LPHW M/L/A-EC (1940) and Dip M/L-EC (194,000) and dip M/L-WP (4850) had inhalation MOEs greater than 100. Other occupational inhalation MOEs were greater than 100 except for SADD HPHW M/L/A-EC (65) and AADD HPHW M/L/A-EC (19). (Table 38).

**Table 37. Estimated Margins of Exposure for Occupational (both dermal and inhalation) Short Term, Seasonal and Chronic for Aerial, Airblast and Groundboom Workers with Endosulfan**

Scenario <sup>a</sup>	Mean STADD MOEs <sup>b</sup>			Mean SADD MOEs <sup>b</sup>			Mean AADD MOEs <sup>b</sup>		
	Dermal	Inhalation	Aggregate <sup>c</sup>	Dermal	Inhalation	Aggregate <sup>c</sup>	Dermal	Inhalation	Aggregate <sup>c</sup>
<b>Aerial</b>									
M/L-EC	3	32	3	36	194	30	52	65	29
M/L-WP	<1	<1	<1	3	5	2	5	2	1
M/L-WP/WSP	4	11	3	30	49	18	41	19	13
Applicator	1	49	<1	8	194	7	11	65	9
Flagger	2	97	2	21	970	20	30	388	28
<b>Airblast</b>									
M/L-EC	28	194	23	197	970	156	570	647	278
M/L-WP	3	6	2	18	28	11	52	19	14
M/L-WSP	37	97	25	169	194	88	570	194	139
Applicator	4	194	4	25	388	23	71	243	54
<b>Groundboom</b>									
M/L-EC	18	194	15	148	970	123	190	194	93
M/L-WP	2	3	1	15	24	9	17	5	4
M/L-WSP	23	65	16	131	194	77	143	49	36
Applicator	16	194	14	236	970	180	285	194	112

a EC =emulsifiable concentrate, M/L= mixer/loader, M/L/A =mixer/loader/applicator, WP =wetable powder, WSP =wate soluble pkg

b - Margin of Exposure = Critical Oral NOEL ) Exposure Dosage: Dermal: Critical Acute Oral NOEL (used for dermal MOE determination) = 0.7 mg/kg (Rabbit Developmental study: salivation, convulsions/thrashing, noisy/rapid breathing, hyperactivity salivation and nasal discharge). Subchronic (seasonal) Oral NOEL (used for dermal MOE) was 1.18 mg/kg/day based on increased relative liver and kidney weights, decreased food consumption & decreased body weights. Critical Chronic (annual) Oral NOEL (for dermal MOE) = 0.57 mg/kg/day (Chronic Dog study: premature deaths (not spontaneous), neurotoxicity). Inhalation: Critical acute and Subchronic (seasonal) Inhalation NOEL was 0.194 mg/kg/day based on 0.19 mg/kg/day based on increased clinical signs in a rat subchronic inhalation study. Chronic Inhalation NOEL (subchronic inhalation NOEL ÷ 10) = 0.0194 mg/kg/day. Exposure doses from Table 18-19; Values were rounded to whole integers.

c – Dietary MOE contribution to aggregate estimations were: Acute = 340, 95<sup>th</sup> percentile for females (13+ years), nursing; Chronic (used also for subchronic) = 3448 (females (13+ years), nursing). Aggregate MOE calculation:

$$\text{Aggregate Total MOE (MOE}_T\text{)} = \frac{1}{\frac{1}{\text{MOE dermal}} + \frac{1}{\text{MOE inhal}} + \frac{1}{\text{MOE diet}}}$$

**Bold indicates MOE of less than 100.**

**Table 38. Estimated Margins of Exposure for Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic for Handlers Using Handheld Equipment to Apply Endosulfan**

Scenario <sup>a</sup>	Mean STADD MOEs <sup>b</sup>			Mean SADD MOEs <sup>b</sup>			Mean AADD MOEs <sup>b</sup>		
	Dermal	Inhalation	Aggregate <sup>c</sup>	Dermal	Inhalation	Aggregate <sup>c</sup>	Dermal	Inhalation	Aggregate <sup>c</sup>
<b>Backpack Sprayer</b>									
M/L/A - EC	<b>16</b>	1940	15	107	9700	103	285	6467	253
<b>High Pressure Hand Wand</b>									
M/L/A EC	<b>1</b>	<b>2</b>	<1	<b>8</b>	<b>65</b>	<b>7</b>	<b>23</b>	<b>19</b>	<b>10</b>
<b>Low Pressure Hand Wand</b>									
M/L/A EC	<b>54</b>	1940	45	393	9700	341	1140	6467	757
M/L/A WP	<b>7</b>	<b>65</b>	<b>6</b>	<b>79</b>	485	66	194	194	93
<b>Dip</b>									
M/L EC	23,333	194000	335	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>
M/L WP	2,333	4850	280	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>
Applicator	<1	<b>39</b>	<1	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>

a - EC = emulsifiable concentrate, M/L = mixer/loader, M/L/A = mixer/loader/applicator, WP = wettable powder, Dip = Nursery stock dip for treating cherry, peach & plum seedlings for peach tree borer. Handlers assumed wearing gloves, respirator, coveralls; as specified on product labels (Beauvais, 2007).

b - Margin of Exposure = Critical Oral NOEL ) Exposure Dosage: Dermal: Critical Acute Oral NOEL (used for dermal MOE determination) = 0.7 mg/kg (Rabbit Developmental study: salivation, convulsions/thrashing, noisy/rapid breathing, hyperactivity, salivation and nasal discharge). Subchronic (seasonal) Oral NOEL (used for dermal MOE) was 1.18 mg/kg/day based on increased relative liver and kidney weights, decreased food consumption, and decreased body weights. Critical Chronic (annual) Oral NOEL (for dermal MOE) = 0.57 mg/kg/day (Chronic Dog study: premature deaths (not spontaneous), neurotoxicity). Inhalation: Critical Acute and Subchronic (seasonal) Inhalation NOEL was 0.194 mg/kg/day based on 0.19 mg/kg/day based on increased clinical signs in a rat subchronic inhalation study. Chronic Inhalation ENEL (subchronic inhalation NOEL ÷ 10) = 0.0194 mg/kg/day. Exposure doses from Tables 17-18

c – Dietary MOE contribution to aggregate estimations were: Acute = 340, 95<sup>th</sup> percentile for females (13+ years), nursing; Chronic (used also for subchronic) = 3448 (females (13+ years), nursing). Aggregate MOE calculation

$$\text{Aggregate Total MOE (MOE}_T\text{)} = \frac{1}{\frac{1}{\text{MOE dermal}} + \frac{1}{\text{MOE inhal}} + \frac{1}{\text{MOE diet}}}$$

e - NA = Not applicable

**Bold indicates MOE of less than 100**

iii. Occupational MOEs for Reentry Workers

All aggregate scenarios for STADD for reentry workers had occupational MOEs of less than 100, with a range of 1 for sweet corn, hand harvesting to 64 (ornamentals, hand harvesting and almond, thinning) (Table 39). The majority of aggregate occupational MOEs for SADD were greater than 100 (range = 108 to 283), except for sweet corn, hand harvesting (15), grape cane turning (8), broccoli, scouting (97) and peach thinning (42). Most aggregate MOEs for AADD were greater than 100 (range = 110 to 487), except for sweet corn, hand harvesting (95), and grape cane turning (12).

**Table 39. Estimated Margins of Exposure for Occupational and Aggregate (Occupational + Dietary) Short Term, Seasonal and Chronic Endosulfan Exposure for Reentry Workers**

Exposure scenario <sup>a</sup>	STADD MOEs <sup>b</sup>		SADD MOEs <sup>b</sup>		Mean AADD MOEs <sup>b</sup>	
	Occupational	Aggregate <sup>c</sup>	Occupational	Aggregate <sup>c</sup>	Occupational	Aggregate <sup>c</sup>
Almond, Thinning	78	64	--d	--	--	--
Broccoli, Hand Harvesting	23	22	148	144	285	263
Broccoli, Scouting	8	8	98	97	114	110
Citrus, Thinning	13	12	--	--	--	--
Sweet Corn, Hand Harvesting	1	1	16	15	95	92
Cotton, Scouting	11	11	131	108	285	263
Cucumber, Hand Harvesting	13	13	169	165	570	487
Grape, Cane Turning	2	2	8	8	12	12
Lettuce, Scouting	4	4	295	283	285	263
Ornamentals, Hand Harvesting	78	64	--	--	--	--
Peach, Thinning	13	12	42	42	114	110
Potato, Scouting	22	21	295	283	285	263
Strawberry, Hand Harvesting	10	10	--	--	--	--
Tomato, Hand Harvesting	33	33	131	129	190	180
Ornamentals, Cut Flowers, Hand Harvesting	4	4	--	--	--	--

a - Reentry exposure scenario from Tables 21-22

b - Margin of Exposure = Critical NOEL / Exposure Dosage: Critical Acute NOEL = 0.7 mg/kg/day (Rabbit Developmental study: salivation, convulsions thrashing, noisy/rapid breathing, hyperactivity, salivation and nasal discharge). Critical Subchronic (seasonal) NOEL was 1.18 mg/kg/day based on increased relative liver and kidney weights, decreased food consumption, and decreased body weights. Critical Chronic (annual) NOEL = 0.57 mg/kg/day (Chronic Dog study: premature deaths (not spontaneous), neurotoxicity); exposure doses from Table 22-23. Values were rounded to whole integers.

c - Aggregate = aggregate occupational and dietary exposure. Acute dietary exposure = 2.06 ug/kg/day based on the 95th percentile of user-day exposure for Females (13+ years), nursing and chronic dietary exposure = 0.17 ug/kg/day (%CT; mean annual consumption for Females (13+ years)). Values were rounded to 2 significant figures.

d - NA = Not applicable.

**Bold indicates MOE of less than 100.**

### **b. Risk to Persons Exposed to Endosulfan in Ambient Air and for Bystander**

To calculate MOE for endosulfan in ambient air and for bystanders, the short term and subchronic inhalation NOEL of 0.194 mg/kg/day from a rat subchronic inhalation study was used (Hollander, et al., 1984). The chronic inhalation (annual) ENEL of 0.0194 mg/kg/day was obtained from the subchronic NOEL of 0.194 mg/kg/day with a 10x uncertainty factor to extrapolate from subchronic to chronic (0.194 ÷ 10 = 0.0194 mg/kg/day). The non-dietary STADD for bystanders (infants and adults) had MOEs of greater than 100 (156 infants and 329 adults). Non-dietary SADD MOEs for ambient air and bystanders for adults and infants were all greater than 100, ranging from 121 for infant bystanders to 11,412 for adults in ambient air (Table 40). All non-dietary AADD MOEs for ambient air and bystander scenarios for infants and adults were greater than 100, ranging from 413 for infant bystanders to 1,940 for adults in ambient air.

**Table 40. Estimated Margins of Exposure for Non-Dietary and Aggregate (Non-Dietary + Dietary) Short Term, Seasonal and Chronic Endosulfan Exposure in Ambient Air and to Bystanders**

Site <sup>a</sup>	Mean STADD <sup>b</sup> MOEs		Mean SADD <sup>b</sup> MOEs		Mean AADD <sup>b</sup> MOEs	
	Inhalation	Aggregate <sup>c</sup>	Inhalation	Aggregate <sup>c</sup>	Inhalation	Aggregate <sup>c</sup>
<b>Ambient Air -- Infants</b>						
Site SJ	NA <sup>d</sup>	NA <sup>d</sup>	5243	1468	970	657
<b>Ambient Air – Adults</b>						
Site SJ	NA <sup>d</sup>	NA <sup>d</sup>	11415	2648	1940	1241
<b>Bystander -- Infants</b>						
East Station	121	<b>78</b>	346	296	413	343
<b>Bystander – Adults</b>						
East Station	255	146	719	595	882	702

a - For further description see Table 35

b - Margin of Exposure = *Inhalation*: Critical Acute and Subchronic (seasonal) Inhalation NOEL was 0.194 mg/kg/day based on 0.19 mg/kg/day based on increased clinical signs in a rat subchronic inhalation study. Chronic Inhalation NOEL (subchronic inhalation NOEL ÷ 10) = 0.0194 mg/kg/day. Exposure doses from Table 30

c – Dietary MOE contribution to aggregate estimations were: Acute = 340, 95<sup>th</sup> percentile for females (13+ years), nursing; Chronic (used also for subchronic) = 3448 (females (13+ years), nursing. Aggregate MOE = 1 ÷ (1 ÷ (MOE inhal) + 1 ÷ (MOE diet))

e - NA = Not applicable. **Bold indicates MOE of less than 100.**

### c. Risk to Swimmers Exposed to Endosulfan in Surface Water

All scenarios for swimmers in surface water had adult and child dermal and non-dietary ingestion MOEs of greater than 100 (STADD, SADD and AADD), Table 41 The lowest MOE was 449 for child non-dietary ingestion and total (dermal + non-dietary ingestion) and the greatest MOE was for adult dermal (321,101).

**Table 41. Aggregate Short Term, Seasonal and Chronic Endosulfan MOEs for Swimmers in Surface Waters<sup>a</sup>**

Exposure Scenario	STADD MOEs <sup>b</sup>		SADD MOEs <sup>b</sup>		AADD MOEs <sup>b</sup>	
	Non-Dietary <sup>a</sup>	Aggregate <sup>c</sup>	Non-Dietary <sup>a</sup>	Aggregate <sup>c</sup>	Non-Dietary <sup>a</sup>	Aggregate <sup>c</sup>
<b>Adult</b>						
Dermal	321101	350	31216931	6940	55339806	3353
Non-Diet Ingest	2612	309	254310	6757	448819	3328
Total	2593	308	252137	6755	445313	3328
<b>Children</b>						
Dermal	234114	212	12868048	2949	22709163	1425
Non-Diet Ingest	449	144	24635	2635	43511	1380
Total	449	144	24583	2634	43511	1380

a - See Table 36 for further description.

b - Margin of Exposure = Critical NOEL ) Exposure Dosage: Critical Acute NOEL = 0.7 mg/kg/day (Rabbit Developmental study: salivation, convulsions/thrashing, noisy/rapid breathing, hyperactivity, salivation and nasal discharge). Critical Subchronic (seasonal) NOEL was 1.18 mg/kg/day based on increased relative liver and kidney weights, decreased food consumption, and decreased body weights. Critical Chronic (annual) NOEL = 0.57 mg/kg/d; (Chronic Dog study: premature deaths (not spontaneous), neurotoxicity). Exposure doses from Table 36 Values were rounded to whole integers.

c - Aggregate mean Non-Dietary + Dietary exposure: Acute dietary exposure = 2.06 ug/kg/day based on the 95th percentile of user-day exposure for Females (13+ years), nursing and chronic dietary exposure = 0.17 ug/kg/day (%CT; mean annual consumption for Females (13+ years)). Values were rounded to 2 significant figures. Acute dietary exposure = 3.30 ug/kg/day based on the 95th percentile of user-day exposure for Children (1 - 6 years) and chronic dietary exposure in children = 0.41 ug/kg/day (%CT; mean annual consumption

for infants, non-nursing, < 1 year). Values were rounded to 2 significant figures.

#### **d. Dietary Exposure**

##### **i. Acute Dietary Margins of Exposure**

For acute dietary exposure, the MOEs were calculated for the various population subgroups using the NOEL for acute toxicity (0.7 mg/kg) (Table 42). MOEs ranged from 212 (Children, 1-6 years) to 513 (Males 13-19 years). Females (13+ years, nursing), selected for the acute dietary exposure group for adults in all exposure scenarios had a dietary MOE of 340, based on the 95<sup>th</sup> percentile of user-day exposure. Acute dietary MOE for infants (non-nursing, < 1 year) was 220, based on the 95<sup>th</sup> percentile of user-day. Infants were assessed for ambient air and bystander endosulfan exposure. The acute MOE for children (1 - 6 years) was 212. This group would be exposed to endosulfan by swimming in surface water. All acute dietary MOEs were greater than 100.

##### **ii. Subchronic Dietary Margins of Exposure**

Subchronic dietary MOEs were calculated using the definitive NOELs from the subchronic rat reproduction study (1.18 mg/kg/day) for all of the seasonal occupational studies and for the adult and child exposure to endosulfan in surface water. Subchronic dietary MOEs per se are not calculated for the general public; however, chronic dietary exposure data are used as a default.

##### **iii. Chronic Dietary Margins of Exposure**

Chronic dietary MOEs were calculated using the definitive, dog study for the critical NOEL (0.57 mg/kg/day). The chronic MOEs ranged from 1407 in children (1 - 6 years) to 7,421 in infants (nursing < 1 year of age) (Table 42). Percent crop treated (%CT) adjustments were used in these calculations. All MOEs in these population subgroups were greater than 100.

**Table 42. Dietary Margins of Exposure from Anticipated Endosulfan Residues on Raw Agricultural Commodities<sup>a</sup>**

<b>Population Subgroups</b>	<b>Acute Exposure 95<sup>th</sup> Percentile (MOEs)<sup>c</sup></b>	<b>Chronic Exposure<sup>b</sup> Annualized Average (MOEs) %CT<sup>d</sup></b>
US Population, all seasons	378	3001
Western Region	375	3167
Pacific Region	377	3252
Hispanics	360	3004
Non-Hispanic Whites	391	3034
Non-Hispanic Blacks	306	2838
Non-Hispanic Other	299	2847
All Infants	227	2597
Infants (nursing, < 1 year)	367	7421
<b>Infants (non-nursing, &lt; 1 year)</b>	<b>220</b>	<b>2039</b>
<b>Children (1 - 6 years)</b>	<b>212</b>	<b>1407</b>
Children (7 - 12 years)	336	1943
Females (13 - 19 years), not pregnant, not nursing	511	3187
Females (20+ years), not pregnant, not nursing)	462	4082
Females (13 - 50 years)	504	3840
Females (13+ years), pregnant, not nursing	441	3846
<b>Females (13+ years), nursing</b>	<b>340</b>	<b>3448</b>
Males (13 - 19 years)	513	2668
Males (20+ years)	508	3725
Seniors (55+ years)	425	4132

a - MOEs based on all label approved commodities. Exposure levels have been rounded off to 3 significant figures and are based on the 1989-1992 Continuing Survey of Food Intakes of Individuals (CSFII).

b - The acute and chronic residue files used anticipated residue values for the commodities.

c - Margin of Exposure = NOEL / Exposure Dosage. Acute NOEL = 0.7 mg/kg/day (salivation, convulsions/thrashing, noisy/rapid breathing, hyperactivity, salivation, and nasal discharge). Chronic (annual) NOEL = 0.57 mg/kg/day (Chronic Dog study: premature deaths, not spontaneous & neurotoxicity). Values were rounded to 2 significant figures.

d - %CT = percent crop treated adjustment was made to adjustment factor 2 in the chronic residue file.

NOTE: There are no subchronic (seasonal) dietary exposure data for endosulfan. Chronic exposure data are used for subchronic calculations.

#### **e. Aggregate (non-dietary plus dietary) Margins of Exposure for Occupational, or Public (swimmers in surface water) Scenarios**

The occupational, and public endosulfan MOEs were described above in V., C. RISK CHARACTERIZATION: a. Occupational Exposure (Tables 37-39); b. Risk to Persons Exposed to Endosulfan in Ambient Air and for Bystanders (Table 40); c. Risk to Swimmers Exposed to Endosulfan in Surface Water (Table 41) and the d. Dietary Exposure (Table 42). For the aggregate MOE, the occupational dermal or public non-dietary (swimmer in surface water) exposure were added to the dietary exposures (oral NOELs (0.7, 1.18 and 0.57 mg/kg/day for acute, subchronic and chronic, respectively) were divided by the occupational dermal or public non-dietary (swimmer in surface water) exposure plus the dietary exposure (Adult: acute dietary = 2.06 ug/kg/d--95<sup>th</sup> percentile of user-day exposure for Females (13+ yr), nursing and chronic dietary = 0.17 ug/kg/d (%CT; mean annual consumption for Females (13+ yr); Child: acute dietary exposure

= 3.30 ug/kg/d based on the 95<sup>th</sup> percentile of user-day exposure for Children (1 - 6 yr) and chronic dietary in children (1-6 yr) = 0.41 ug/kg/d (%CT)) divided into the relevant oral NOEL. This calculation assumes the uncertainty factors were equal for each route (dermal/oral) and that all NOELs were from the oral route.

$$\text{Aggregate MOE (oral route)} = \frac{\text{NOEL (oral)}}{\text{Occupational or Non-Dietary Exposure Dose} + \text{Dietary Exposure Dose}}$$

**f. Risk Characterization for Aggregate Exposure:**

The potential for health hazard associated with the use of endosulfan was considered to determine MOEs for occupational (inhalation + dermal + oral) and oral (non-dietary); bystanders and ambient air (inhalation + oral (dietary) and for swimmers in surface water (non-dietary ingested or dermal) in combination with oral dietary exposure. For aggregate exposure, the risk was determined by a total MOE approach (USEPA, 2001e). This approach is used when there is a common effect with different NOELs for the different routes of exposure but with the same uncertainty factor (UF) applied for both routes. The magnitude of the total MOE expressed only the risks for specified endpoints. The calculations are as follows:

$$\text{Aggregate Total MOE (MOE}_T\text{)} = \frac{1}{\frac{1}{\text{MOE dermal}} + \frac{1}{\text{MOE inhal}} + \frac{1}{\text{MOE diet}}}$$

For aggregate (occupational<sub>dermal/inhalation</sub> + dietary) MOE determinations in occupational scenarios the STADD, SADD and AADD exposure components were derived from the dermal and the inhalation values previously provided (Tables 17-22), and from dietary MOEs studies (0.7 mg/kg, 1.18 mg/kg/day and 0.57 mg/kg/day, respectively) were used in the STADD, SADD and AADD determinations for occupational and swimmer in surface water scenarios. This is because for these particular aggregate exposures, the dietary and dermal routes comprise the primary routes, because an oral NOEL is used for dermal exposure (no acceptable dermal study) and because the oral route is the dietary route.

For endosulfan exposure to the public in ambient air or for bystanders the same NOELs are used for calculations for short term and subchronic MOEs (0.194 mg/kg/day) from the subchronic, rat inhalation study (Hollander et al., 1984). The NOEL used for the chronic MOE calculations is also from the Hollander et al. (1984) study with an additional 10x uncertainty factor to extrapolate from subchronic to chronic (NOEL = 0.0194 mg/kg/day) is used.

The dietary MOE contribution to aggregate estimations were determined for acute MOE (340, 95<sup>th</sup> percentile for females (13+ years), nursing), and chronic (used also for subchronic; 3448 (females (13+ years), nursing) exposures.

i. Aggregate MOEs Occupational Exposure

a) Aggregate MOEs for Aerial and Ground Application

Aerial application MOEs for all aggregate STADD scenarios were less than 100, ranging from less than 1 (aerial M/L WP and applicator) to 25 (airblast M/L WSP). SADD aggregate MOEs were less than 100, except for airblast M/L EC (156), groundboom M/L EC (123) and applicator (180). AADD aggregate MOEs were less than 100, except for airblast M/L EC (156), airblast M/L WSP (139) and applicator (112) (Table 37).

b) Aggregate MOEs for Handlers Using Handheld Equipment.

All aggregate STADD MOEs were well below 100 (<1 to 45) for handlers using handheld equipment except dip M/L EC (335) and dip M/L WP (280) (Table 38). SADD and AADD aggregate MOEs less than 100 were for HPHW M/L/A-EC (7 and 10, respectively) and LPHW M/L/A-WP (66 and 93, respectively). Other MOEs for SADD and AADD were greater than 100 and ranged from 103 (SADD backpack sprayer M/L/A EC) to 757 (LPHW M/L/A EC).

c) Aggregate MOEs for Reentry Workers

All scenarios for STADD for reentry workers had aggregate MOEs that were less than 100, with a range of 1 for sweet corn, hand harvesting to 64 for hand harvesting ornamentals and for almond thinning (Table 39). For SADD, 4/10 aggregate MOEs were less than 100, however one of the MOEs was 97 (range = 8 for grape, cane turning to 97 for broccoli, scouting). The highest SADD MOE was 283 for both lettuce, scouting and for potato, scouting. The only AADD MOEs less than 100 were sweet corn, hand harvesting (92) and grape, cane turning (12). All other AADD MOEs (8/10) were greater than 100 (110 = peach, thinning to 487 for cucumber, hand harvesting).

ii. Data for Aggregate MOEs in Non-Occupational Scenarios

a) Aggregate MOEs for Ambient Air and for Bystanders

Aggregate bystander scenarios (no short-term ambient air exposure values) had STADD MOEs of 78 (infants) and 146 (adults). SADD MOEs were all greater than 100 for ambient air (infant: 1468, adult: 2648) and for bystanders (infant: 296; adult: 595). AADD aggregate scenarios were all greater than 100 for ambient air (infant: 657; adult: 1241) and for bystanders (infant: 343; adult: 702). The MOEs of less than 1000 and must be flagged for further evaluation under the Food Quality Protection Act (1996). (See the following section V. RISK APPRAISAL; E. ISSUES RELATED TO THE FOOD QUALITY PROTECTION ACT for a discussion of the 10x safety factor.)

b) Aggregate MOEs for Swimmers in Surface Water

All aggregate scenarios for swimmers in surface water had STADD, SADD and AADD MOEs of greater than 100 (Table 41). Aggregate MOEs for STADD ranged from 144 for child non-dietary ingestion and for child total (144; non-dietary ingestion and dermal) to 350 for adult dermal. Within scenarios for STADD, SADD and AADD the aggregate MOEs for adults or for

children did not much variation. For example MOEs for STADD aggregate scenarios had adult MOEs of 308 to 350 and child MOEs of 144 to 212. SADD aggregate MOEs for adults ranged from 6755 to 6940 and child MOEs or 2634 to 2949. AADD aggregate MOEs for adults ranged from 3328 to 3353 and for children ranged from 1380 to 1425.

## **V. RISK APPRAISAL**

### **A. INTRODUCTION**

Risk assessment is the process used to evaluate the potential for human exposure and the likelihood that the adverse effects observed in toxicity studies with laboratory animals will occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment and exposure assessment processes. This, in turn, results in uncertainty in the risk characterization that integrates all the information from the previous three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the degree or magnitude of the uncertainty can vary depending on the availability and quality of the data, and the types of exposure scenarios being assessed. Specific areas of uncertainty associated with this risk assessment for endosulfan are delineated in the following discussion.

### **B. HAZARD IDENTIFICATION**

#### **1) Acute Toxicity**

##### **a) Acute Oral NOELs**

Toxicity from acute oral exposure to endosulfan is primarily characterized by neurotoxicity. Some uncertainty in the acute hazard identification was due to the selection of the critical acute NOEL from a rabbit developmental study, where animals experiencing neurotoxic effects were pregnant (Nye, 1981). The acute oral NOEL of 0.7 mg/kg/day, in this developmental study, was lower compared to the NOELs obtained in the acute oral rat neurotoxicity test (12.5 mg/kg-male; 1.5 mg/kg-female; Bury, 1997) and the rat developmental study (maternal NOEL of 2 mg/kg/day, Fung, 1980b). The primary effect in all the studies was neurotoxicity. Although the rabbit developmental study involved multiple dosing, rather than a single acute dose of endosulfan, the neurotoxic effects were seen on the first day of treatment and were therefore acute effects. However, the pregnant rabbit appeared to be the most sensitive for this route of exposure, with the lowest NOEL of 0.7 mg/kg/day, and was therefore selected to calculate the endosulfan MOEs for acute dietary exposure, for potential acute single-day human occupational exposures, and for acute exposure to the public (children and adults, both dermal and non-dietary ingested) swimming in surface water.

The rat developmental study (Fung, 1980b) was not used as the definitive study for the following reasons: 1) The maternal NOEL was higher at 2 mg/kg/day, based on neurotoxicity and weight loss, 2) The highest dose tested was the dose at which effects were observed (6.0 mg/kg/day) and it was higher than the 1.8 mg/kg/day for rabbits, where severe neurotoxicity was observed, 3) The fetal NOEL was 2.0 mg/kg/day, based on decreased mean fetal weights, or increased growth retardation, and developmental anomalies and malformations. It is uncertain whether the fetal effects

were due to an acute toxicity directly on the developing fetuses, or whether they developed only after multiple treatments or whether they were secondary to maternal toxicity. The default assumption used by DPR, however, is that a developmental effect could be due to a single exposure and therefore considered an acute effect.

Selection of a developmental study for an acute endpoint may be an overestimation of risk as pregnant dams may be more sensitive to endosulfan toxicity. In addition, there may be an overestimation of risk because the rabbits received a bolus dose by gavage, rather than receiving an oral, dietary dose. USEPA used an acute NOEL of 1.5 mg/kg, based on results in female rats from the acute oral neurotoxicity test. Doses at which acute neurotoxic effects were observed in the acute rat neurotoxicity study were 3.0 mg/kg (female) and 25 mg/kg (male), whereas in the rabbit, severe neurotoxicity was seen at 1.8 mg/kg/day.

b) Acute Dermal NOEL

There were no acceptable acute dermal studies available to establish a dermal NOEL, so the procedure is to use the definitive acute oral NOEL (0.7 mg/kg/day; described above; Nye, 1981). For endosulfan, 100% oral absorption is assumed from available data (See III. TOXICOLOGY PROFILE; A. Pharmacokinetics). The dermal penetration factor of 47.3% was factored into the exposure assessments and MOE calculations for acute “dermal” occupational and for swimmers in surface water estimations.

c) Acute Inhalation NOEL

Non-occupational, non-dietary endosulfan exposure scenarios in ambient air and for bystanders are presented for infants and adults. An acute inhalation (LC<sub>50</sub>) study was performed (Hollander and Weigand, 1983), however, a NOEL was not achieved (LOEL = 0.567 mg/kg). There were several inhalation-specific scenarios both occupationally and to the public. Therefore an acceptable subchronic rat inhalation study (based on a subchronic rangefinding study with a LOEL of 0.44 mg/kg reported within Hollander et al., 1984) with a NOEL of 0.0010 mg/L (0.194 mg/kg/day; LOEL = 0.387 mg/kg/day) was used to calculate the potential for acute single-day inhalation MOEs to workers, and MOEs for endosulfan in ambient air or to bystanders (Hollander et al., 1984). While this selection involved some uncertainty, the advantages to using this study for the critical inhalation NOEL instead of the LC<sub>50</sub> a) LOELs from all three studies were similar (0.567, 0.44 and 0.387 mg/kg/day), b) more animals treated in the subchronic (15/sex/dose subchronic versus 5/sex/dose in the acute), c) the subchronic study used a 29 day recovery with 5 per sex per dose (acute 14d observation); d) the NOEL of 0.194 mg/kg/day is a reasonable selection based on the LOELs from the 3 studies; e) it is a conservative estimate for an acute NOEL, since acute NOELs are usually higher than subchronic or chronic NOELs. It is also noted that all three studies were performed at the same laboratory and in the same timeframe (12/7/83—Acute; 8/15/83--Subchronics). Both the LC<sub>50</sub> and the subchronic studies were acceptable according to FIFRA Guidelines.

d) Study Selected by the USEPA as the Definitive Study For the Critical Acute NOEL

The USEPA used the acute oral rat neurotoxicity study (Bury, 1997), with a NOEL of 1.5 mg/kg (F) as the definitive study for the acute NOEL. Acute inhalation MOEs for ambient air and bystanders were not presented in the RED (USEPA, 2002).

## 2. Subchronic Toxicity

### a) Subchronic Oral NOEL

There is uncertainty in the selection of the rat dietary study as the definitive study to characterize dietary and non-dietary exposure (non-dietary ingestion of water by swimmers in surface water) as well as occupational risk from the dermal exposure (occupational, and to adult and child swimmers in surface water). The critical oral subchronic NOEL from a 2-generation rat reproduction study with a NOEL of 1.18 mg/kg/day (LOEL of 5.40 mg/kg/day) was used for dietary exposure to endosulfan (Edwards et al., 1984). This study provided a lower NOEL than the subchronic dietary NOEL of 1.92 mg/kg/day (LOEL of 3.85 mg/kg/day) from a study that was also performed in rat (Barnard, et al., 1985). The reproduction NOEL was based on increased relative liver and kidney weights, decreased food consumption, and decreased body weights. The common endpoint for the reproduction and subchronic oral dietary study was an increase in both kidney and liver weights. These two studies were both acceptable according to FIFRA Guidelines; however, the reproduction study gave a slightly lower NOEL value. The rat reproduction NOEL was also more comprehensive because it provided an examination of effects occurring during pre-mating, mating, gestation, lactation and weaning for 2 generations. This NOEL was used for the dietary exposures.

### b) Subchronic Dermal NOEL

Occupational and non-occupational (adult and child swimmers in surface water) dermal exposure can occur on a seasonal basis. A dermal NOEL of 1.0 mg/kg/day (LOEL of 3.0 mg/kg/day), obtained from a rat dermal study (Elbert et al., 1985a), was close in value to the oral (rat) NOEL of 1.18 mg/kg/day. The effects in the two rat studies were based on similar systemic effects (clinical chemistry, clinical signs, liver effects and/or mortality). However, the dermal study was not acceptable by FIFRA Guidelines due to several critical deficiencies. Therefore, it is considered to be supplemental only and the oral subchronic NOEL (1.18 mg/kg/day) was used for dermal and dietary exposure and MOE estimates. For endosulfan, 100% oral absorption is assumed from available data (See III. TOXICOLOGY PROFILE; A. Pharmacokinetics). The dermal penetration factor of 47.3% was factored into the exposure assessments and MOE calculations for subchronic dermal occupational and for swimmers in surface water estimations.

### c) Subchronic Inhalation NOEL

Non-dietary exposure to the public occurs for infants and adults in ambient air and to bystanders on a subchronic (seasonal) basis. The definitive study was a subchronic rat inhalation study conducted for 21 treatments (6 hours/day) over 29 days (5 days/week) to obtain a critical NOEL of 0.0010 mg/L (0.194 mg/kg/day) and a LOEL of 0.0020 mg/L (0.387 mg/kg/day) (Hollander et al., 1984). The NOEL was based on clinical effects, decreased bodyweight gain and food consumption, increased water consumption, and effects on clinical chemistry parameters. Effects observed in this study, such as clinical signs, did not occur until after the first week, however the ultimate doses selected were based on a rangefinding study. The definitive study was acceptable according to FIFRA Guidelines and the NOEL was used to calculate the subchronic inhalation MOEs for public exposure to endosulfan in ambient air and to bystanders.

## d) Study Selected by the USEPA as the Definitive Study For the Critical Subchronic NOEL

USEPA selected a dermal NOEL of 12 mg/kg/day for dermal definitive (endpoint) study. The inhalation NOEL was obtained from the 21-day inhalation study (Hollander et al., 1984) with a NOEL of 0.2 mg/kg/day (USEPA, 2002). While a specific “oral NOEL” category was not specified by USEPA, the rat reproduction NOEL (1.18 mg/kg/day) was selected as a definitive study for dermal effects (45% dermal absorption).

**3. Chronic Toxicity**

## a) Chronic Oral NOEL

Neurotoxicity was the primary chronic effect observed in the dog study (increased nervous and behavioral signs and violent contractions of the abdomen) selected as the definitive study for the critical NOEL (0.57 mg/kg/day). A source of uncertainty was the incomplete characterization of the neurotoxic effects. 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 hours post-dosing, and when dosing was 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. It was decided, however, that the combined rat study, which had a similar NOEL (0.6 to 0.7 mg/kg/day) could be used in support of the critical NOEL. Major chronic effects in rats were aneurysms and kidney pathological effects but not neurotoxicity. However, the dog study was a better choice, since effects occurred at a greater percentage and were initiated earlier than were effects observed in rats. The USEPA, however, selected the rat study, with a NOEL of 0.6 mg/kg/day to use in chronic occupational exposure for human risk assessment. This value is virtually indistinguishable from the 0.57 mg/kg/day observed in dogs. The differences lie in the LOEL values and the intersex differences. For instance, the NOEL for females in the chronic rat study was 0.7 mg/kg/day, with LOELs of 2.9 mg/kg/day for males and 3.8 mg/kg/day for females. The dog, however, showed a NOEL of 0.65 for females and lower LOELs for both sexes (2.09 mg/kg/day for males and 1.98 mg/kg/day for females). Although these values are close, the dog study offers a slightly more sensitive system for assessing chronic effects of endosulfan.

The critical NOEL from the dog study (0.57 mg/kg/day) was used to calculate the MOEs for chronic oral and dermal scenarios (occupational and for swimmers in surface water), and for aggregate exposure [(dermal + inhalation) + dietary] for the same scenarios.

## b) Chronic Dermal NOEL

Due to several critical deficiencies, there was not an acceptable chronic dermal study available for occupational dermal and swimmers in surface water exposure scenarios. Therefore, the oral chronic NOEL (0.57 mg/kg/day) from the chronic dog study was used for occupational dermal and dermal estimates to swimmers in surface water and MOE estimates for these scenarios. For endosulfan, 100% oral absorption is assumed from available data (See III. TOXICOLOGY PROFILE; A. Pharmacokinetics). The dermal penetration factor of 47.3% was factored into the exposure

assessments and MOE calculations for chronic dermal occupational and for swimmers in surface water estimations.

c) Chronic Inhalation NOEL

A chronic inhalation study was not performed to obtain a NOEL for scenarios involving long-term occupational inhalation exposure or long-term exposure to the public through ambient air or to bystanders. There were several chronic inhalation-specific scenarios both occupationally and to the public, so in the absence of an acceptable chronic inhalation study, the NOEL for the subchronic rat inhalation study was used. To adjust from subchronic to chronic, a 10x uncertainty factor was added ( $0.194 \text{ mg/kg/day} \times 10 = 0.0194$ ), resulting in a chronic inhalation ENEL of 0.0194 mg/kg/day for exposure and MOE estimates.

d) Study Selected by the USEPA as the Definitive Study For the Critical Chronic NOEL

USEPA selection for the critical chronic NOEL was 0.6 mg/kg/day from the combined rat study (Barnard et al., 1985).

#### 4. Cholinesterase

Cholinesterase activity was examined in several reports: Kushwah and Dikshith, 1981; Seth et al., 1986; Ansari et al., 1987; Castillo et al., 2002. At toxic subchronic doses (27.17 mg/kg/day) female rats showed 59% decrease in plasma ChE, where brain ChE was increased at 4.59 mg/kg/day and greater (Barnard et al., 1985). This effect was not observed in the chronic studies. Emulsifiable concentrate (33%) applied dermally (4 weeks) showed decreased plasma ChE in both sexes at toxic doses in males (-13% at 27, -17% at 54 and -22% at 81 mg/kg/day) and in females (-22% at 12, -29% at 18 and -32% at 36 mg/kg/day, Thevenaz et al., 1988). Kushwah and Dikshith, 1981 found no effects on RBC or plasma ChE after up to 90 days of oral gavage to male rats. Brain ChE was decreased (no data shown) and authors suggested it was due to neuronal toxicity rather than a direct effect of endosulfan on brain ChE inhibition. Seth et al., 1986, showed no effects on brain ChE on rat adult, or neonates up to 5 weeks old. Adult male rats showed no effects on brain ChE at 40 mg/kg i.p. (single dose, Ansari et al., 1987). Adult male rats that received endosulfan subcutaneously at 25 mg/kg/day for 10 days showed no effects on plasma ChE (RBC and brain not tested; Castillo et al., 2002).

The above findings indicate that endosulfan does not have a consistent effect on ChE. In the cases where effects are observed doses used are highly toxic or have been administered in formulated product. Additionally, all NOELs used to determine MOEs are far below any that have been shown to induce an effect on ChE and therefore, ChE is not considered to be a toxicologically relevant endpoint.

### C. EXPOSURE APPRAISAL

#### 1. Occupational Exposure (Beauvais, 2007; Appendix E [Volume II])

##### a) Handler Exposure Estimates

## i. PHED

Exposure estimates for handlers were based on certain assumptions, due to lack of acceptable, chemical-specific data. For example, PHED data were used to estimate handler exposures for the various application methods, with the exception of applicators dipping nursery stock roots. PHED, though useful, has limitations that prevent the use of distributional statistics on exposure estimates. For example, PHED incorporates exposure data from many studies, each with a different minimum detection level for the analytical method used to detect residues in the sampling media. Moreover, as the detection of dermal exposure to the body regions was not standardized, some studies observed exposure to only selected body parts. Consequently, the subsets derived from the database for dermal exposure may have different numbers of observations for each body part, complicating interpretation of values taken from PHED. The UCLs calculated from PHED are statistically approximate and are intended to account for both statistical and non-statistical uncertainties. DPR believes that UCLs of PHED data provided the best exposure estimates possible.

USEPA also uses PHED to estimate handler exposure; however, USEPA approaches PHED data somewhat differently than DPR. First, as explained in USEPA's policy for use of PHED data (USEPA, 1999b): "Once the data for a given exposure scenario have been selected, the data are normalized (i.e., divided by) by the amount of pesticide handled resulting in standard unit exposures (milligrams of exposure per pound of active ingredient handled). Following normalization, the data are statistically summarized. The distribution of exposure values for each body part (i.e., chest upper arm) is categorized as normal, lognormal, or "other" (i.e., neither normal nor lognormal). A central tendency value is then selected from the distribution of the exposure values for each body part. These values are the arithmetic mean for normal distributions, the geometric mean for lognormal distributions, and the median for all "other" distributions. Once selected, the central tendency values for each body part are composited into a "best fit" exposure value representing the entire body." In other words, USEPA uses various central tendency estimates (often the geometric mean or median, as PHED data rarely follow a normal distribution), while DPR believes the arithmetic mean is the appropriate statistic regardless of the sample distribution (Powell, 2003). Secondly, DPR uses a 95<sup>th</sup> percentile upper bound estimate for short-term exposure estimates, while USEPA uses a central tendency estimate for all exposure durations. Third, as explained in the Exposure Assessment section, DPR calculates upper 90% confidence limits for both upper bound and mean exposures, while USEPA does not (note: DPR's policies for handling PHED data have been reviewed informally and are currently under formal review by a statistician at the University of California). The differences between short-term exposure estimates calculated according to DPR and USEPA policies are summarized in Table 43 for an example scenario, aerial applicator.

In Table 43, the exposure rate estimated by USEPA is 5.068  $\mu\text{g}$  AI/lb handled (USEPA, 2002b); the exposure rate calculated according to DPR policy is 133.286  $\mu\text{g}$  AI/lb handled. These values differ substantially, not only for the reasons explained above, but also because USEPA assumes use of closed cockpits in all aerial exposure estimates; if planes with open cockpits can be used, USEPA policy is to require an additional 10-fold safety factor in the risk calculation (USEPA, 1998b). If DPR were to assume a closed cockpit, the total exposure rate would be 46.7  $\mu\text{g}$  AI/lb handled; this estimate was included in Table 41 to show the extent to which assumption of an open cockpit affects DPR exposure estimates. The most recent information available about equipment used by aerial applicators shows that open cockpits are relatively rare, but may still be used (NAAA, 2004).

The STADD estimated by DPR is 0.790 mg/kg/day, and the corresponding exposure estimate calculated by USEPA is 0.1312 mg/kg/day. If closed cockpits were required, the DPR exposure estimate would only be 0.280 mg/kg/day, slightly more than twice the USEPA estimate. No chemical-specific exposure monitoring data were available for comparison with these estimates.

**Table 43. Comparison of Aerial Applicator Exposure to Endosulfan Estimated from Surrogate Data by DPR and USEPA Policy (Beauvais, 2007)**

Exposure estimate	Exposure rate ( $\mu\text{g AI/lb handled}$ ) <sup>a</sup>	STADD (mg/kg/d) <sup>b</sup>
DPR estimate used in Exposure Assessment (open cockpit) <sup>c</sup>	133	0.790
DPR's estimate if closed cockpit were required <sup>d</sup>	46.7	0.280
From PHED, according to USEPA policy (closed cockpit) <sup>e</sup>	5.068	0.1312

a - Total exposure rate, dermal plus inhalation, based on data in the Pesticide Handlers Exposure Database (PHED).

b - Short-Term Absorbed Daily Dosage (STADD) estimates assumed an 8-hour workday. Amount treated was assumed by both DPR and USEPA to be 350 acres (142 ha) treated/day (USEPA, 2001d). Body weight was assumed to be 70 kg by DPR (Thongsinthusak *et al.*, 1993) and USEPA (2002b).

c - Department of Pesticide Regulation (DPR) use of PHED data described in Beauvais (2006) Exposure Assessment section. Estimates assumed open-cockpit aerial application, with applicator wearing respirator but not wearing gloves. Assumed application rate was 2.5 lbs AI/acre (2.8 kg AI/ha), maximum rate on tree nuts in California. Dermal absorption assumed to be 47.3% (Craine, 1988), and inhalation absorption assumed to be 100%.

d - Estimate assumptions were the same as above, except that aerial applicators were assumed to use closed cockpit (no respirator use is assumed for closed cockpit). This estimate would be used by DPR if regulations or product labels specified a requirement for closed cockpits, which is not currently the case.

e - U.S. Environmental Protection Agency (USEPA) exposure estimates from Scenario 3 in revised exposure assessment (USEPA, 2002b). Estimates assumed closed-cockpit aerial application, with applicator not wearing gloves or respirator. Assumed application rate was 3.0 lbs AI/acre (3.4 kg AI/ha), maximum rate on pecans; dermal and inhalation absorption factors were not used, as route-specific toxicity data were used in USEPA's risk assessment.

Although there are differences in how DPR and USEPA calculate exposure estimates from PHED, there are also similarities. For example, although handlers are required to wear chemical-resistant headgear for overhead exposure, at the present time neither DPR nor USEPA (2002b) assigns a protection factor for exposure reduction for workers wearing such headgear. DPR is aware of a recent study that is anticipated to provide data supporting such a protection factor, and when these data have been evaluated DPR may begin applying an appropriate protection factor to handler exposure estimates.

#### ii. Nursery Stock Dipping Applicators

Dermal exposure was estimated based on the RAGS-E model, which estimates skin permeability ( $K_p$ ) to organic chemicals in aqueous solution (USEPA, 2004a). There are many assumptions and uncertainties associated with this and other models that use  $K_p$ , some of which were discussed in USEPA (2004a). Poda *et al.* (2001) discussed additional sources of uncertainty in models that were based on large and diverse data sets.

For endosulfan, an AI-specific  $K_p$  value was estimated based on an equation derived from a data set of about 200 organic compounds in aqueous solutions. The calculated  $K_p$  for endosulfan may be either over- or underestimated; there are not enough data available to be sure. As endosulfan is well within the range of MW and Log  $K_{ow}$  in which  $K_p$  estimates are considered valid, based on Equations 3.9 and 3.10 in USEPA (2004a), use of this equation is expected to result in a skin permeability

estimate that correlates reasonably well with available data.

However, use of  $K_p$  with solutions of formulated pesticide products may result in exposure being underestimated, as the formulations contain additives (e.g., solvents, emulsifiers, and surfactants) to increase water solubility of AIs. Numerous studies have shown enhanced dermal penetration of chemicals, including pesticides, when mixed with such additives, as they can alter the barrier properties of the skin (Baynes and Riviere, 1998; Brand and Mueller, 2002; Williams and Barry, 2004). Alternately, flux into the skin could be decreased by additives in the formulation as has been shown in some cases (Nielsen and Andersen, 2001; Riviere *et al.*, 2001), perhaps by altering how the chemical partitions between solution and skin (van der Merwe and Riviere, 2005). Exposure estimates could be improved if skin permeability measures were made using solutions of formulated products in concentrations that are pertinent to typical product use.

Another uncertainty from the use of  $K_p$  in estimating dermal exposure is that skin permeabilities are almost always estimated from *in vitro* rather than *in vivo* data. In an *in vitro* skin permeability test, a section of skin is clamped between two cells, called the “donor cell” and the “receptor cell.” The donor solution (in the donor cell) contains the compound of interest; as the compound crosses the membrane it appears in the receptor solution, which is sampled periodically. A known concentration of compound is initially in the donor solution; the rate at which the compound concentration increases in the receptor solution is related to the skin permeability. Extrapolation from *in vitro* data to permeability of skin *in vivo* is problematic because relationships between *in vivo* and *in vitro* test results have not been reliably established for many classes of compounds, and dermal penetration have been shown to vary for compounds that have been tested (Wester and Maibach, 2000; Zendzian and Dellarco, 2003). Nevertheless, these models rely on the assumption that *in vitro* dermal penetration is approximately the same as *in vivo*.

Other assumptions common to these models are that the chemical concentration of water in contact with skin ( $C_w$ ) is constant; and that absorbed dose is a function of solution concentration, skin permeability, and amount of exposed skin surface. These are reasonable assumptions, but have not been tested for solutions of pesticide products.

Another uncertainty existing in the RAGS-E model is related to the parameters  $\tau$  and B. Calculations for these parameters rely on many assumptions and limited, surrogate data. The RAGS-E model has undergone some validation, but not with pesticides in formulated products (additives in the pesticide formulations may affect  $\tau$  and B, as well as  $K_p$ ).

Estimates of inhalation exposure for workers dipping nursery stock were based on SWIMODEL equations. SWIMODEL estimates pesticides concentrations in air based on conditions that may not be met in the nursery stock-dipping scenario. In fact, substantial deviations occur from the assumptions on which the model is based. SWIMODEL relies on water-air partitioning to determine concentration of a chemical in air, using the Henry’s Law constant for the chemical. However, Henry’s Law constant applies to dilute, single-chemical aqueous solutions only. Staudinger and Roberts (2001) suggest 10,000 mg/L as an upper boundary defining a “dilute” solution under Henry’s Law. This concentration is approached in the endosulfan dipping solution (6,000 mg/L). Furthermore, other chemicals present in the pesticide formulation can interact with the pesticide molecules, potentially affecting the partitioning of the AI into air (Staudinger and Roberts, 2001). Because the calculated concentration of AI in air was higher than anticipated at saturation, the

estimated saturation concentration was used instead in inhalation exposure calculations; in other words, it was assumed that the AI is present at air-saturating concentrations. Therefore, because of this assumption, it is anticipated that the inhalation exposure will be overestimated. In spite of this, the inhalation exposure estimate was substantially below the dermal exposure estimate, and the inhalation contribution to total exposure is considered negligible in this scenario.

In the absence of exposure monitoring or surrogate data, the results obtained from these models are considered the best estimate of dermal and inhalation exposure.

### iii. Other Defaults

PUR data were used to estimate likely numbers of days that workers were exposed, based on the distribution of applications in high-use California counties. These high-use periods describe a recent work history of the handler population, and they probably overestimate the workdays for any single individual. However, they provide the best available data for seasonal and annual exposure estimates.

Additionally, the numbers of acres treated per day were based on defaults recommended by USEPA (2001a). These estimates are expected to be conservative but realistic; however, insufficient data exist to evaluate their accuracy.

### iv. Reentry Exposure Estimates

Acceptable monitoring data were lacking for fieldworker exposures. Exposure estimates for fieldworkers were appropriately based on chemical-specific DFR values; however, crop-specific DFR values were unavailable for most reentry scenarios. Because of this, DFR data from only four crops (grapes, lettuce, melons, and peaches) represented residues in all crops on which endosulfan may be used. The use of data from one crop to represent residues on another introduces uncertainties in exposure estimates. Residues may dissipate at different rates on different crops, due to factors such as leaf topography and physical and chemical properties of leaf surfaces.

The rate of contact with treated foliage, unlike DFR, is not chemical specific (USEPA, 2000b). Transfer coefficient values for various crop activities are readily available, based on studies using other chemicals. Where activity- and crop-specific TCs were not available, defaults based on studies with similar activities and crops were used. These defaults were likely to be health-protective (USEPA, 2000a).

Additionally, information is lacking about exposures resulting from some activities, such as weeding and roguing (removal of diseased crop plants) in cotton, and how these exposures might compare with those of scouts. Unlike other reentry workers, cotton harvesters' work with plants which have been intentionally defoliated; DFR residues therefore cannot be used to estimate harvester exposures. The best available exposure estimate for weeders, roguers and harvesters in cotton is considered to be the estimate provided for cotton scouts. However, no data are available which would allow comparison of exposures between cotton scouts and those of other reentry workers in cotton.

## 2. Public Exposure to Ambient Air and to Bystanders (infants and adults)

Public exposures to airborne endosulfan were estimated based on concentrations of endosulfan in air and assumptions about uptake of endosulfan from the air. No biomonitoring or other exposure-monitoring data were available. Exposure estimates were provided for adults for consistency with other scenarios, and for infants, as likely worst-case because infants have the greatest inhalation rate per body weight. Ambient air exposure estimates were based on monitoring conducted at five sites in Fresno County. The reported concentrations were based on limited monitoring data and must be considered as having some degree of uncertainty. The representativeness of the monitoring sites is unknown. Each site was monitored four days per week for a relatively short (5-week) period. Weekend days were not monitored. It is unknown whether weekdays and weekends differ systematically in numbers of endosulfan applications. Although ambient air monitoring sites were selected based on anticipated nearby endosulfan use, applications of endosulfan were not confirmed. Furthermore, examination of pesticide use data in Fresno County (Figure 3), suggests that while ambient air monitoring performed by ARB (1998) occurred during a high-use period, the highest-use period might not have been monitored. A total of 24,498 lbs endosulfan was used in Fresno County in July 1996, the highest use month that year. In July 2000, however, 30,614 lbs was reported used in Fresno County (DPR, 2006). Ambient air exposures, based on air monitoring conducted in July and August 1996, might be underestimated.

To decrease the likelihood of underestimating exposures, results of ambient air monitoring were corrected for  $\alpha$ - and  $\beta$ -endosulfan recoveries from field spikes. Recoveries of  $\alpha$ -endosulfan from spiked tubes were low, ranging 38 – 54%. If the low recoveries were due to a problem with the spiking solution, then sample results would not have been affected and the correction for the 44% mean field spike recovery would contribute to overestimates in concentrations and exposures based on them. As the reason for the low recovery could not be determined with certainty, however, the correction is an appropriate health-protective measure.

Concentrations of endosulfan in air might be anticipated to vary with different application methods and with different types of crops. Factors affecting drift from spray applications include type of crop, wind velocity and direction, volume and direction of sprayer air jets and nozzles, and application rate (Frank *et al.*, 1994; SDTF, 1997; Fox *et al.*, 1998; Richards *et al.*, 2001). Aerial and airblast applications typically result in greater spray drift than low-pressure boom applications, assuming similar spray droplet size and wind velocity (Frost and Ware, 1970; Frank *et al.*, 1994). To decrease the likelihood of underestimating exposures, application site results were corrected for field spike recoveries.

For bystander exposure estimates, data from the east monitoring station, 6.4 m from the application site, were used as a reasonable worst-case estimate for endosulfan concentration in air for short-term exposure estimates. For this reason, the mean endosulfan concentration at this site was used rather than the 95<sup>th</sup> percentile upper bound estimate. The mean concentration was multiplied by a factor of 1.67 to account for the application site monitoring study using an application rate that was lower than the maximum allowed rate. This adjustment assumes that endosulfan concentrations in air are directly proportional to application rate. Seasonal or annual exposure to application site airborne endosulfan levels is not expected because airborne concentrations are anticipated to reach ambient levels within a few days after the application. Frequent applications to most crops are prohibited. With the exception of celery, in which applications are not limited, most crops are prohibited. With

the exception of celery, in which applications are not limited, most crops are treated between one and three times per growing season. This limitation makes it unlikely that an individual would be subjected to bystander exposures for more than a few days each growing season. Even individuals living near one or more fields and working near others are unlikely to experience exposures above ambient for more than a few days. Airborne concentrations of active ingredients generally decrease as distance from the application site increases (MacCollom et al., 1968; Siebers et al., 2003), and it is unlikely that a person would be repeatedly exposed to elevated airborne concentrations in close succession that would result in a seasonal exposure. STADD estimates address exposures from less than one day up to 7 days. DPR believes that intermediate- and long-term exposures to endosulfan occur only at ambient concentrations (Beauvais, 2007).

### **3. Public Exposure to Swimmers in Surface Water (adults and children)**

Swimmer exposures to endosulfan in surface waters were estimated based on concentrations of endosulfan reported from surface water sampling and assumptions about uptake of endosulfan from water. No biomonitoring or other exposure-monitoring data were available. Exposure estimates were provided for adults for consistency with other scenarios, and for children, as likely worst-case because children have relatively greater surface area exposed to the water, per body weight, than adults.

Endosulfan concentrations used to calculate swimmer exposure estimates were derived from DPR's Surface Water Database. This database contains data reported from a variety of environmental monitoring studies targeting pesticides. These studies were conducted by several agencies, had different detection limits, and different study designs. Sampling frequency and sample collection site varied, and it is possible that the highest endosulfan concentrations were not reflected in the samples collected. If so, then short-term exposures may be underestimated. Some studies monitored irrigation drains, which would be anticipated to have higher concentrations than rivers, for example (although the highest reported concentrations occurred in samples collected from rivers). The collection sites chosen for environmental monitoring might also be biased toward those where pesticides are most likely to occur; if so, the median concentrations used to calculate long-term exposures might be overestimated.

The effectiveness of permit conditions instituted in 1991 by DPR, and incorporated into product labels, has not been assessed. DPR (1994) contains endosulfan data from sampling done between 1990 and 1996. No trend of decreasing endosulfan concentrations since 1991 is evident from these data (the last sample, collected July 22, 1996, had a total endosulfan concentration of 0.122 µg/L).

Swimmer exposures were estimated based on equations and defaults for swimmers in treated swimming pools (USEPA, 2003). The relevance of the assumptions underlying these calculations for swimmers in surface waters, rather than swimming pools, is unknown. No information is available for frequency or duration of swimming in surface waters (as opposed to community or residential swimming pool).

### **4. Dietary Exposure**

#### **a) Acute and Short Term Dietary Exposure**

Acute dietary MOEs were calculated for the various population subgroups using the

NOEL for acute toxicity (0.7 mg/kg) (Table 43). Estimates of exposure ranged from 1.37 ug/kg in females (13- 19 years), not pregnant, not nursing to 3.30 in Children (1-6 years). “Females (13+ years, nursing)” was selected for the acute dietary exposure group for adults (based on the 95<sup>th</sup> percentile of user-day exposure). Acute dietary exposure for infants (non-nursing, < 1 year) was 3.18 (based on the 95<sup>th</sup> percentile of user-day). For acute dietary exposure, an overestimation of some commodities may have occurred. For instance, fruit juice may be better represented by the average value, rather than the high (95<sup>th</sup> percentile), since it consists of large, pooled quantities of fruit. If average values were used for mixed commodities, the MOEs for nursing and non-nursing infants would increase from 367 and 220 (acute, 95<sup>th</sup> percentile) to 6374 and 1042 (50<sup>th</sup> percentile; See Appendices A and C), respectively. Conversely, fruit juice may be prepared from just a few pieces of fruit, and therefore the high estimate (95<sup>th</sup> percentile) for acute exposure would be appropriate. For some juices, the high value of the whole RAC was used (i.e. grape juice and strawberry juice). For others average values from juices were used (i.e. apple juice). See Table 25.

There is a potential for underestimation of exposure when using monitoring data versus field trial data. Field trial data come from 100% treated crops and therefore, detected/nondetected residues come from known starting residues. With monitoring data; the commodity may or may not have been treated. Therefore it is not known whether the values are true representatives of exposure or whether they are merely below the limit of detection.

Short-term dietary exposure may have been underestimated since the DPR and PDP monitoring program’s residue samples used in the assessment were composite rather than single serving samples. More variation would be expected with single serving samples and therefore, the commodity residue would probably be higher.

#### b) Subchronic Dietary Exposure

Subchronic dietary exposures were calculated using the definitive NOELs from the subchronic rat reproduction study (1.18 mg/kg/day) for all of the seasonal occupational studies and for the adult and child exposure to endosulfan in surface water. There were, however no subchronic (seasonal) dietary exposure data for endosulfan, therefore chronic dietary exposure data were used as a default.

#### b) Chronic Dietary Margins of Exposure

MOEs for chronic dietary exposure were calculated from data for the various population subgroups from Table 42 and the definitive NOEL from the chronic dog study (0.57 mg/kg/day). MOEs for subchronic and chronic dietary exposure were based on mean annual (chronic) dietary consumption of endosulfan by adults (Females, 13+ years, nursing = 3448), infants (infants non-nursing, < 1 year = 2039) and children (children 1 - 6 = 1407). The chronic dietary exposures ranged from 0.08 in infants (nursing, < 1 year old) to 0.041 in children (1 - 6 years). There were no percent crop treated (%CT) adjustments used in these calculations.

There were considerations that may have contributed to an overestimation from dietary exposure. For instance, all analyzed commodities had their residue values combined to represent total endosulfan. The acute and chronic commodity endosulfan values were not modified using any type of toxicological equivalency factor (TEF) method applied to the endosulfan  $\alpha$ -,  $\beta$  - or sulfate

forms separately because of their same relative toxicity. All available endosulfan raw agricultural commodity residue data, expressed as total endosulfan, used to conduct the DPR dietary analyses, are presented in Table 25.

For chronic estimations, there were percent of the crop treated (%CT) adjustments made for residues, when sufficient use data were available. Otherwise, by default, DPR assumes 100% of the crop is treated with the pesticide under consideration for chronic dietary exposure analysis. Assuming that 100% of the crop is treated with endosulfan may be an overestimation for some commodities; however, these are the only data available. The 100% CT value is a default assumption. The following commodities have reported endosulfan use at the federal and state levels: apple, broccoli, grape, peach, pear, strawberry and tomato. The %CT estimates tend to be more accurate than the assumption that people under normal eating conditions would be continuously exposed to the averaged residue level of a pesticide for every labeled commodity for 1 year (chronic). Multiple years of endosulfan use and acreage harvested data were evaluated at the federal and state levels.

Estimates of endosulfan residues derived from DPR's monitoring program may have been overestimated since residues are measured in the whole commodity, not just the edible portion. Tests included maceration of both the edible and non-edible portions of the plant. Metabolism and residue studies for endosulfan on bean and sugar beet plants (Beard and Ware, 1969) showed that under greenhouse conditions endosulfan was translocated from leaf surfaces to the whole plant (both edible and non-edible portions). The bulk of the residues were located on the surface (80-90%), with 8-20% located in the whole plant. This means that residues in crops such as watermelon and squash would be overestimated, since the outside, leaves and other non-edible plant components would not be consumed (Maier-Bode, 1968). In addition, lettuce residues are in trace amounts after the outer leaves are removed before consuming (Sances, et al., 1992).

Drinking water is likely not a source of uncertainty with regard to endosulfan dietary exposure. Surface and well water samplings have been negative for endosulfan residues since 1996. In addition, the PDP samples from 2001 to 2003 (PDP, 2003, 2004, 2005) have been negative for endosulfan in drinking water.

#### **D. RISK CHARACTERIZATION**

Generally an MOE of at least 100 is considered sufficiently protective of human health when the NOEL for an adverse systemic effect is derived from an animal study. This MOE allows for the possibility of humans being 10 times more sensitive than animals and for a 10-fold variation in sensitivity between the lower range of the normal distribution in the overall population and the sensitive subgroup (Dourson et al., 2002). However, when considering endosulfan exposure for the general public, specifically infants exposed in ambient air or as bystanders, the above MOE of 100 is insufficient. For infants and children exposed in ambient air or as bystanders, MOEs need to be at least 1000-fold or greater. MOEs of less than 1000 for these scenarios result in the consideration of listing endosulfan as a toxic air contaminant (California Food and Agricultural Code: 14021-14027) based on acute, subchronic and chronic neurotoxicity.

Exposure scenarios for the public involve both dietary and non-dietary components to infants (ambient air, bystanders) and children (swimmers in surface water).

## 1. Occupational and Public (ambient air, bystander, swimmer) Margins of Exposure

### a) Short Term Exposure MOEs

#### i. Occupational Scenarios

For dermal occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) STADD had 18 of 20 (90%) exposures with MOEs less than 100 (Tables 37-38). Of those, 25% of the dermal MOEs (5/20) were less than or equal to 1 (Aerial M/L-WP; applicator; HPHW M/L/A EC; dip applicator; sweet corn hand-harvesting).

Inhalation scenarios that were less than or equal to 1 was aerial M/L-WP. STADD MOEs dermal were greater than 100 for root dip M/L (both EC and WP), ranging from 2333 (M/L WP) to 23,333 (M/L EC). Inhalation scenarios that were greater than 100 were airblast (M/L-EC, and applicator), groundboom (M/L-EC and applicator), backpack sprayer (M/L/A), LPHW (M/L/A EC), and dip (M/L EC and M/L WP) (Tables 37-38).

All STADD re-entry worker exposure scenarios had MOEs that were less than 100. Sweet corn hand harvesting had an MOE of 1 (Table 39).

#### ii. Non-Dietary Ambient Air and Bystander Scenarios

Short term MOEs for non-dietary bystander scenarios were greater than 100 at 121 (infant) and 255 for adult bystanders (Table 40). Since both of these scenarios had MOEs of less than 1000, endosulfan may be listed as a potential toxic air contaminant (California Food and Agricultural Code: 14021-14027).

#### iii. Swimmer in Surface Water Scenarios

All short term non-dietary MOEs for swimmers in surface water were greater than 100 and ranged from 449 (child non-diet ingested and total) to 321,101 (adult dermal) (Table 41).

### b) Seasonal Exposure MOEs

#### i. Occupational Scenarios

For dermal occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) SADD had 12 of 17 (59%) exposures with MOEs less than 100 (Tables 37-38). SADD MOEs were greater than 100 for airblast M/L-EC (197), airblast M/L-WSP, all of groundboom scenarios except M/L-WP (15), backpack sprayer (107) and LPHW (M/L/A EC).

For inhalation occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) SADD had 5 of 17 (29%) exposures with MOEs less than 100 (Tables 37-38). SADD inhalation MOEs were greater than 100 were aerial (M/L-EC, applicator, flagger), airblast (M/L-EC, M/L WSP and applicator) all groundboom but M/L WP, backpack sprayer (M/L/A), and LPHW (M/L/A EC and M/L/A WP).

The SADD re-entry worker exposure scenarios had 4 of 10 MOEs of less than 100 (broccoli, scouting--98; sweet corn, hand harvesting--16; grape, cane turning--8; and peach, thinning—42) and the remainder was 131 or greater (Table 39).

ii. Ambient Air and Bystander Scenarios

All seasonal exposure MOEs for the infant and adult ambient air and bystander scenarios were greater than 100, ranging from 346 (bystander, infant) to 11415 (ambient air, adult) (Table 40). Note that since both the bystander scenarios have MOEs of less than 1000, endosulfan may be listed as a potential toxic air contaminant (California Food and Agricultural Code: 14021-14027).

iii. Swimmer in Surface Water Scenarios

All seasonal MOEs for swimmers in surface water were greater than 100 and ranged from 24,583(child: non-diet ingested + dermal) to 31,216,931 (adult dermal) (Table 41).

c) Annual Exposure MOEs

i. Occupational Scenarios

For dermal occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) AADD had 10 of 17 (59%) exposures with MOEs less than 100 (Tables 37-38). The dermal MOEs remaining that were greater than 100 ranged from 143 (groundboom M/L-WSP) to 1140 (low pressure handwand M/L/A-EC).

For inhalation occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) AADD had 8 of 17 (47%) exposures with MOEs less than 100 (Tables 37-38). AADD MOEs were greater than 100 for the remaining scenarios and they ranged from 194 (airblast M/L-WSP, groundboom M/L-EC and applicator and low pressure handwand M/L/A-WP) to 6467 for both backpack sprayer and low-pressure handwand M/L/A-EC.

The AADD re-entry worker exposure scenarios had 2 of 10 MOEs of less than 100 (sweet corn, hand harvesting--95; and grape, cane turning--12) and the remainder was 114 or greater (Table 39).

ii. Ambient Air and Bystander Scenarios

All annual exposure MOEs for the infant and adult ambient air and bystander scenarios were greater than 100, ranging from 413 (bystander, infant) to 1950 (ambient air, adult) (Table 40). Note that since both of the bystander scenarios have MOEs of less than 1000 endosulfan may be listed as a potential toxic air contaminant (California Food and Agricultural Code: 14021-14027).

iii. Swimmer in Surface Water Scenarios

All annual MOEs for swimmers in surface water were greater than 100 and ranged from 43,511 (child: non-diet ingested and total) to 55,339,806 (adult dermal) (Table 41).

## 2. Dietary Margins of Exposure

All population subgroups have MOEs (acute 95<sup>th</sup> percentile and chronic) greater than 100 (Table 43) and these dietary MOEs are based on anticipated endosulfan residues on RAC. All are greater than 1000 for chronic dietary exposure.

## 3. Aggregate (Combined) Margins of Exposure

### a) Short Term and Acute Total Occupational Aggregate (Combined) MOEs

Of total STADD occupational aggregate ( $MOE_{Total} = 1 \div (1/MOE_{dermal} + 1/MOE_{inhalation} + 1/MOE_{dietary})$ ) and aggregate ( $MOE_{oral/dermal} = NOEL_{oral/dermal} \div (\text{occupational} + \text{dietary exposure})$ ) scenarios 6% (2/35) had MOEs greater than 100 (Tables 36-38) and those were for handlers using handheld equipment (dip M/L-EC and dip M/L-WP).

Infant bystanders exposed to endosulfan via inhalation had an aggregate STADD MOE ( $MOE_{Total} = 1 \div (1/MOE_{inhalation} + 1/MOE_{dietary})$ ) of less than 100 (78), while adult bystanders MOE was 146. Under the TAC (ambient air/bystander adults and infants) an additional 10-fold UF must be added to the existing default 100-fold (10x-interspecies x 10x intraspecies UFs) uncertainty factors. Based on these additional UFs, the STADD MOEs for bystander exposure were both less than 1000 and therefore were not acceptable.

STADD aggregate ( $MOE_{oral/dermal} = NOEL_{oral} \div (\text{occupational} + \text{dietary exposure})$ ) aggregate for swimmers in surface water (children and adults) had MOEs greater than 100 (Table 41).

### b) Seasonal Aggregate (Combined) MOEs

Of total SADD occupational aggregate ( $MOE_{Total} = 1 \div (1/MOE_{dermal} + 1/MOE_{inhalation} + 1/MOE_{dietary})$ ) and aggregate ( $MOE_{oral/dermal} = NOEL_{oral/dermal} \div (\text{occupational} + \text{dietary exposure})$ ) scenarios 41% (11/27) had MOEs that were greater than 100, but the remainder ranged from 2 (aerial, M/L WP) to 97 (reentry workers, broccoli scouting). All aerial scenarios (M/L, applicator and flagger), airblast (M/L-WP, M/L WSP and applicator), groundboom (M/L WP and M/L WSP), high pressure handwand (M/L/A EC), LPHW (M/L/A WP) and reentry workers (broccoli scouting, sweet corn hand harvesting, grape cane turning and peach thinning) had MOEs of less than 100. MOEs that were more than 100 ranged from 103 (backpack sprayer M/L/A EC) to 341 for low-pressure handwand M/L/A EC.

All SADD aggregate MOEs for ambient air and bystanders exposed to endosulfan via inhalation ( $MOE_{Total} = 1 \div (1/MOE_{inhalation} + 1/MOE_{dietary})$ ) were greater than 100 (range: 296 bystander infant to 1940 ambient air adult). SADD aggregate MOEs for ambient air were 1468 (infant) and 2648 (adult) and for bystanders they were 296 (infant) and 595 (adults). Additional 10x UF for adults and

infants (California Food and Agricultural Code: 14021-14027 might be factored into MOE calculations, necessitating MOEs of 1000 fold to maximize health protection (Table 40). Therefore, the MOEs for bystander exposure scenarios were unacceptable.

SADD aggregate ( $MOE_{\text{oral/dermal}} = NOEL_{\text{oral}} \div (\text{occupational} + \text{dietary exposure})$ ) MOEs for swimmers in surface water (children and adults) were greater than 100 (range: child “total” 2634 to 6940 for dermal adult) (Table 41).

#### 5. Annual Aggregate (Combined) MOEs

Of total AADD occupational aggregate ( $MOE_{\text{Total}} = 1 \div (1/MOE_{\text{dermal}} + 1/MOE_{\text{inhalation}} + 1/MOE_{\text{dietary}})$ ) and aggregate ( $MOE_{\text{oral/dermal}} = NOEL_{\text{oral/dermal}} \div (\text{occupational} + \text{dietary exposure})$ ) scenarios 48% (13/27) had MOEs that were greater than 100, but the remainder ranged from 1 (aerial, M/L WP) to 93 (LPHW M/L/A WP and groundboom M/L EC). All aerial scenarios (M/L, applicator and flagger), airblast (M/L WP and applicator), groundboom (M/L WP, W/L EC and M/L WSP), high pressure handwand (M/L/A EC), LPHW (M/L/A WP) and reentry workers (sweet corn hand harvesting, grape cane turning) had MOEs of less than 100. MOEs that were more than 100 ranged from 110 (reentry: broccoli scouting, peach thinning) to 757 (LPHW M/L/A EC) backpack sprayer M/L/A EC) (Table 37-39).

All AADD aggregate MOEs for ambient air and bystanders exposed to endosulfan via inhalation ( $MOE_{\text{Total}} = 1 \div (1/MOE_{\text{inhalation}} + 1/MOE_{\text{dietary}})$ ) were greater than 100. AADD aggregate MOEs for ambient air were 657 (infant) and 1241 (adult) and for bystanders were 343 (infants) and 702 (adults). An additional 10x UF for adults and infants (California Food and Agricultural Code: 14021-14027) necessitates MOEs of 1000 fold to maximize health protection (Table 40). Therefore, the MOEs for ambient air (infant) and both bystander scenarios were unacceptable.

AADD aggregate ( $MOE_{\text{oral/dermal}} = NOEL_{\text{oral}} \div (\text{occupational} + \text{dietary exposure})$ ) MOEs for swimmers in surface water (children and adults) were greater than 100 (range: child “total” 1380 to 3353 for dermal adult) (Table 41).

### **E. ISSUES RELATED TO THE FOOD QUALITY PROTECTION ACT (FQPA)**

The Food Quality Protection Act (FQPA, 1996) mandated USEPA to “upgrade its risk assessment process as part of the tolerance setting procedures” (USEPA, 1997a, b). The improvements to risk assessment were based, in part, on the recommendations from the 1993 National Academy of Sciences report, “Pesticides in the Diets of Infants and Children” (NAS, 1993). The Act required an explicit finding that tolerances are safe for children. USEPA was required to use an extra 10-fold (10x) safety factor to take into account potential pre- and post-natal developmental toxicity (endocrine disruptor, ED) and the completeness of the data unless USEPA determined, based on reliable data, that a different margin would be safe. The USEPA (2002a) considered 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) the effects of *in utero* exposure; and 4) the potential for endocrine disrupting effects.

## **1. Concerns About Endosulfan as an Endocrine Disruptor (DPR/USEPA)**

There were data gaps of the Subchronic Neurotoxicity and Developmental Neurotoxicity (DNT) studies that were required by USEPA in order to fully assess adult rat neuropathological and testicular effects and neuropathological or endocrine effects in developing fetuses (USEPA, 2002). This was of concern because in earlier studies, there was a lack of evaluation of sperm parameters, as well as concerns about testicular lesions, increased pituitary and uterine weights and possible toxicity to the male rat sperm described in open literature and FIFRA Guideline studies. Based on the results of the required subchronic neurotoxicity study, the DNT study and recent studies in the open literature, use of the 10x FQPA Safety Factor (SF) by USEPA for endosulfan exposure in diet (USEPA, 2002) is in review.

### **a. Concerns Generated From FIFRA Guideline Studies:**

The USEPA required a Subchronic Neurotoxicity Study be performed on rats to test for possible neurological or endocrine effects in adults. No neuropathology, neurotoxic or endocrine effects were observed in this study (Sheets et al., 2004).

The USEPA required that sperm be examined in young adult male rats in a Developmental Neurotoxicity study (Gilmore et al., 2006) due to possible sperm toxicity. Pregnant females were treated via diet gestation day (GD) 6 through lactation day 21. Male pups were evaluated post-natal day (PND) 75. No effects were observed in testes or epididymal weights or in sperm/spermatid numbers or sperm morphology. The systemic NOEL in dams was 3.7 mg/kg/day based on decreased body weight, food intake and food efficiency. The Pup LOAEL was 3.74 mg/kg/day based on decreased pup weight on PND11, decreased weight gain on PND 4-11. There was also a possible delay in preputial separation at 29.8 mg/kg/d (HDT) but no effects occurred in sperm at the HDT. These results reduced the uncertainty of endosulfan as an endocrine disruptor.

Testicular lesions in rats were observed in a chronic dietary exposure study (Powers, 1978). No concern for testicular atrophy in male Osborne-Mendel rats was observed at 20.4 and 48 mg/kg/day endosulfan in diet for 82 wks (not FIFRA Guideline accepted by DPR toxicologists). The testicular effects were not replicated in the acceptable chronic 104 week exposure in diet to Sprague-Dawley rats (HDT = 3.8 mg/kg/d; NOEL = 0.6 mg/kg/d; Ruckman, et al., 1989). These results reduced the uncertainty of endosulfan as an endocrine disruptor.

Pituitary and uterine weight effects were observed in the 2-Generation Rat Reproductive Dietary study. However, the increased relative pituitary weights in F1a pups (not F1b pups) or the increased uterine weights in F2a pups (not F2b) were seen only at the HDT (6.2 mg/kg/d). This is at least 10 times higher than the 0.57 mg/kg/day used to derive a chronic RfD and no dose response occurred for these organ weight changes.

**b. Concerns Generated by Open Literature Reports on Endosulfan-induced Toxicity to the Male Reproductive System.**

Sinha et al. (1997) tested the effect of endosulfan (95% pure) on testicular maturation. Weanling male Druckrey rats (prepubertal sexual maturity at 3 weeks old, 5/dose) at 0 (peanut oil), 2.5, 5.0 or 10.0 mg/kg/day for 90 days (5 days/week) by gavage to investigate the possibility of permanent damage to the gonads. Results showed statistically significantly decreased sperm counts (cauda epididymis), increased sperm abnormality, decreased spermatid counts and decreased daily sperm production, as well as increased LDH, G6PDH and GGT, and decreased SDH, at all doses ( $\geq$  2.5 mg/kg/day). The LOEL for weanling rats was 2.5 mg/kg/day. Due to the small number of animals tested, the high doses used and the questionable purity, this study was considered to be supplemental.

Sinha et al., (1995) tested the effect of endosulfan (95% pure) on testes of adult male Druckrey rats (5/dose) prepubertal sexual maturity at 3 weeks old, 5/dose) at 0 (peanut oil), 2.5, 5.0 or 10.0 mg/kg/day for 90 days (5 days/week) by gavage to investigate the possibility of permanent damage to the gonads. The effects observed in the mature rats where endosulfan (95% pure) used in the same protocol were similar to those observed in weanlings; however, in mature rats most occurred at greater than or equal to 5 mg/kg/day, rather than at greater than or equal to 2.5 mg/kg/day as seen in weanlings. The effects observed in weanlings were dose-related, where they were not in the mature rats. Due to the small number of animals tested, the high doses used and the questionable purity, this study was considered to be supplemental.

Sinha et al. (2001a) used mated Druckrey rats (3/dose) that received endosulfan (95%, gavage) at 0, (peanut oil), 1 or 2 mg/kg/day in peanut oil from GD 12 through parturition (period of gonadal differentiation in rat). At birth, the pups were cross-fostered to untreated dams. Sperm parameters were evaluated on PND100 (i.e., young adults; 2/litter; 6/dose total). Both dose levels caused a decrease in sperm and spermatid counts, and decreased testes, seminal vesicle and epididymis weights. Testicular enzyme markers showed a decrease in LDH and in sorbitol dehydrogenase.

Dalsanter et al. (1999) treated mated Wistar rats with endosulfan (97%, gavage) at 0 (Tween 80), 1.5 and 3.0 mg/kg/day during GD 15 - LD 22. Male pups were examined PND 65 and 140 (pubertal and adult). Male pup effects had a decrease in daily sperm production and a decreased percent of seminiferous tubules with complete spermatogenesis (puberty/adult) at 3.0 mg/kg/day. At 1.5 mg/kg/day, daily sperm production was decreased only at puberty.

Dalsanter et al. (2003) treated mated Wistar rats with endosulfan (97%, gavage) at 0 (sunflower oil), 0.5 or 1.5 mg/kg/day for 21 days prior to mating through lactation. Male pups were examined on PND 15, 21, 33, and 140. No maternal toxicity was observed and there were no effects on sperm production; sperm count in cauda epididymis, sperm transit, sperm morphology, and serum testosterone. The results of this study addressed previous concerns about effects to the reproductive system induced by endosulfan (pre & postnatal to adult) exposure to Wistar rats.

Zhu et al., (2000) administered endosulfan (92% pure; 70:30 ratio of  $\alpha$ - and  $\beta$ -isomers) via gavage to pregnant female Wistar rats (10/dose) at 0 (corn oil-English translation; vegetable oil-Chinese), 0.5, 1.0 or 2.5 mg/kg/day throughout the entire gestation period through postnatal day (PND)

28 in order to investigate the effects of endosulfan treatment on pups (article translated from Chinese to English by L-H. Li, Office of Environmental Health Hazard Assessment, California EPA). Maternal decreased body weight gain and death (4/10) were observed at 2.5 mg/kg/day. Litter size and sex ratio were not affected, although birth weights and crown-to-rump ratios were somewhat lower at birth, but later recovered. Anogenital distances of males (measured days 1, 28 and 90, respectively) were not significantly decreased. There was no cryptorchidism or hypospadias in any male offspring. Apoptosis in testis germ cell was examined on PND 28 and was not significantly different and there were no obvious histological changes in these organs. Daily sperm production, epididymal sperm count and morphology as well as male fertility were not significantly changed. Results further indicate that endosulfan does not affect normal endocrine development in male offspring of rats after this extended duration of treatment.

Zaidi et al. (1985) treated neonatal albino rats (1 day old, 4/sex/dose/time point, strain not stated) were treated intraperitoneally with endosulfan (purity not stated) at 0 (40% polypropylene glycol), 0.5 and 1.0 mg/kg/day for 3 or 5 weeks, followed by an 8-day recovery period without endosulfan treatment. At 1.0 mg/kg/day adults showed statistically significantly increased <sup>3</sup>H-5HT binding to frontal cortical membrane and increased fighting behavior at 5 weeks (not reversed after 8 day recovery). The NOEL was 0.5 mg/kg/day. Data from this study were of limited value, however because the strain of rat was unknown and there was no information about purity of endosulfan used for dosing the animals (amount of endosulfan received by the animals versus impurities).

Seth et al. (1986) treated pregnant female rats, neonates and weanlings (ITRC breeding colony, location not specified) with endosulfan (purity not stated) via i.p. injection at 0, 0.5, 1.0 and 3.0 mg/kg/day for various lengths of time (6 group designations, see summary in NEUROTOXICITY section). By 5 weeks at 1.0 mg/kg/day, pups showed a slight increase in <sup>5</sup>HT and benzodiazepine and a decrease in dopamine binding. Footshock fighting behavior was decreased in pups treated to 5 weeks of age (1.0 mg/kg/day). These changes were observed 8 days after cessation of treatment. Adults treated at 3 mg/kg/day for 15-30 days had increased <sup>3</sup>H-5HT binding along with increased footshock fighting (continuing 8 days posttreatment). Pup NOEL = 0.5 mg/kg/day. Data from this study were of limited value, however because the strain of rat was unknown and there was no information about purity of endosulfan used for dosing the animals (amount of endosulfan received by the animals versus impurities).

### **c. Results Indicate There May be Differences in Susceptibility of the Male Reproductive System to Endosulfan Depending on:**

- **Rat Strain and Sperm Count Difference Between Rat Strains:** The same method of sperm count was used, but Druckrey rats (Sinha et al., 2001a) had a 38% lower control value than the Wistar rats (Dalsanter et al., 2003)

- **Exposure Duration, Purity of Endosulfan, Vehicle:**

#### **EFFECTS:**

**Sinha et al. (2001a):** A short exposure for Druckrey rats (GD12-parturition), with 95% endosulfan, and a vehicle of peanut oil resulted in sperm effects at 1 mg/kg/day.

**Dalsanter et al. (1999):** A gavage exposure for Wistar rats (GD15-LD22), with 97% pure endosulfan, and a vehicle of Tween 80 were used resulting in sperm effects at 1.5 mg/kg/day.

NOTE: The effects observed at PND 65 at 1.5 mg/kg/day were no longer observed PND 140.

**NO EFFECTS:**

**Dalsanter et al. (2003):** A gavage exposure for Wistar rats (21d premate-LD22), with 97% pure endosulfan, and a vehicle of sunflower oil were used, resulting in no sperm effects at 0.5 & 1.5 mg/kg/day.

**Gilmore et al, (2006):** A dietary exposure for Wistar rats (GD6-LD21), with 99.1% pure endosulfan, resulted in no sperm effects at 3.74 mg/kg/day and greater. There was an indication in males of delayed preputial separation at 10.8 mg/kg/day and greater (NOEL maternal and developmental < 3.74 mg/kg/day). This may be considered to be within normal range (plus or minus 2 days), but there were no historical controls for this effect included in the report.

**Zhu et al., (2000):** A gavage exposure for Wistar rats (GD0-LD28), 92% pure endosulfan, observation of male offspring through 90 days with a test for reproductive effectiveness), corn oil vehicle, resulted in deaths at 2.5 mg/kg/day and no effects of pups or adult males at 0.5 or 1.0 mg/kg/day.

**d. Endocrine Disruption and the FQPA Safety Factor:**

**FIFRA:** Uncertainty associated with the data gaps has been addressed with the submission of the USEPA-requested studies (subchronic neurotoxicity & DNT studies—both dietary). The concern for endosulfan-induced adverse effects in male offspring was alleviated since none was observed in sperm parameters, testes weights, or histopathology of the testes in the DNT study at doses up to 29.8 mg/kg/day.

**Open literature:** Dalsanter et al., (1999, 2003) demonstrate a NOAEL of 0.5 mg/kg/day (no effects at 1.5 mg/kg/d, HDT, Dalsanter et al., 2003) and a possible LOAEL of 1.5 mg/kg/day (LDT, Dalsanter, 1999) in Wistar rats (a common strain in toxicology testing; also used in DNT), a long exposure period (*in utero* - lactation), and a 97% pure test material. In contrast, the Sinha et al., 1997, study which showed effects at the 1 mg/kg/day used a less familiar or commonly used strain, (Druckrey), less pure test material (95%), a shorter exposure duration (GD 12 to parturition), and peanut oil as the vehicle. Zhu et al., (2000) used a gavage exposure in mated Wistar rats (GD0-LD28; male offspring observed to PND 90) at 0.5, 1.0 and 2.5 mg/kg/day. Dams died at 2.5 mg/kg/day, while no effects were shown in males at any dose.

There was no quantitative or qualitative evidence of increased susceptibility following *in utero* exposures of rats or rabbits to endosulfan during gestation or throughout reproduction.

**2. Other Studies Evaluated for Pre- and Post-natal Sensitivity**

Prepubescent human males showed effects to testosterone and LH in a preliminary epidemiology study (Saiyed et al., 2003). The only thing that can be concluded is that the children exposed to endosulfan had a higher blood level of endosulfan ( $1.37 \pm 0.23$  ppb, control;  $7.47 \pm 1.19$  ppm, exposed). Sexual maturation appeared to be delayed; however, the authors state the weakness in the study are 1) non-participation in the sexual maturation rating (SMR) evaluation performed by pediatricians (57% of the exposed and 33% of the control participants did not agree to undergo SMR examination). 2) Blood was collected only once from participants and sex hormone levels can vary depending on individual variation and time of day (personal cycle). The random variability of the

sex hormone levels was stated to weaken the power of the study. The authors conclude that a study with a larger sample size must be performed and that a long-term follow up must be done on individuals in order to understand the implications or suggestions initially identified. Further criticism was published in “Perspectives – Correspondence: Endosulfan’s Effects: Omissions and Flawed Data” (Abraham, C.C.) and “Endosulfan’s Effects: Inaccurate Data,” (Indulkar, A.S.) along with “Endosulfan’s Effects: Saiyed’s Response,” (Saiyed, H.N.); *Environmental Health Perspectives*, 112(10): A538 – A541, 2004. Information presented in this paper yields at best a suggestion of an effect by endosulfan. However, this paper cannot be used as a strong basis for effects in humans.

Studies with neonatal (3 week old) rats showed decreased intratesticular sperm counts and increased percentage of abnormal sperm at lower doses than observed in 3-month-old adults (Sinha et al., 1995 and 1997).

A study by Chitra et al. (1999) treated Wistar male prepubertal (45 day old) rats by gavage with endosulfan (35% concentration, possibly a formulation) at 0 (groundnut oil) and 1.0 mg/kg/day (6 animals/dose) for 30 days. Results at termination showed statistically significant effects in reproduction parameters (decreased testes, epididymal, ventral prostate, and seminal vesicle weights) and effects to 3- $\beta$ OH-steroid dehydrogenase among other biochemical parameters relating to testicular metabolism. However, there were major deficiencies in this study. For instance, 35% endosulfan concentrate was used and it was possibly a formulation, only 6 animals were treated per dose, only a single treatment dose was used, no individual data were shown, and there was a great deal of variation in assay results (e.g. weight of seminal vesicles in treated animals was 307 mg/100 g bwt +/- 143). Those deficiencies decrease the usefulness of this study for critical endpoint selection. More recent studies, such as the developmental neurotoxicity study reported in 2006 (Gilmore et al.), provide more reliable data for regulatory purposes.

A FIFRA Guideline acceptable study (Edwards et al., 1984) showed no effects on male reproduction at the highest doses tested (5.4 mg/kg/day - males and 6.55 mg/kg/day - females) when fed in diet for 2 generations, even though the systemic NOEL was 1.18 mg/kg/day (M) and 1.23 mg/kg/day (F), based on increases in relative liver and kidney weights at the high dose (Table 13). If effects occurred in sperm, they were not manifest in fertility or other reproductive parameters. However, at the time this study was performed, assessments for sperm were not performed. Female weanlings from the F0 (first mating) and F1b (first mating) showed increased pituitary and uterine weights that were not observed in offspring from the second matings. In this study, there was a slight, non-statistically significant decrease in pup weights at the highest dose tested (5.40 mg/kg/day for males and 6.55 mg/kg/day for females) in the absence of reproductive effects. The pup NOEL, due to the slight decrease in body weights, is considered to be equal to or greater than the parental systemic NOELs.

### 3. Endocrine Effects

Endosulfan, because of its estrogenic activity *in vitro* (ability to compete with 17 $\beta$ -estradiol at the estrogen receptor in MCF-7 cells) was considered to be a potential endocrine disruptor (Soto, et al., 1994, 1995; Andersen et al., 2002; Vanparys et al., 2006). Weak estrogenic responses were induced by endosulfan with MCF-7 cells at 10uM (Soto et al., 1994 and 1995), 1-25uM (Andersen et al., 2002) and 5.48uM (Vanparys et al., 2006). In general, it is necessary to have over 1000 fold greater concentrations of endosulfan than estradiol *in vitro* with MCF-7 cells in order to induce a like

estrogenic response. Other authors have shown that endosulfan likely will not cause reproductive toxicity related to estrogen action (Wade et al., 1997). Two studies from the same laboratory showed no endosulfan related effects on estrogenic or antiestrogenic activity in castrated or hemi-castrated females (Hiremath and Kaliwal, 2002 and 2003).

$\alpha$ -Endosulfan,  $\beta$ -endosulfan, and endosulfan sulfate technical (each 95 - 100% pure) were each used on Chinese hamster ovary (CHO-K1) cells at  $10^{-5}$  M (same for all) to test for estrogenic and androgenic agonist/antagonist properties to 2 human estrogen receptor (hER) subtypes, hER $\alpha$ - and hER $\beta$ - and a human androgen receptor (hAR) in a highly sensitive *in vitro* assay (Kojima, et al., 2004). Results showed that “ $\alpha$ -endosulfan and endosulfan sulfate were positive in the ER $\alpha$ ” and ER $\beta$  transactivation assays, while  $\beta$ -endosulfan was active in the ER $\beta$  transactivation assay only.  $\alpha$ - and  $\beta$ -Endosulfan were both inhibitors of androgenic transcriptional activity. Therefore, both the  $\alpha$ - and  $\beta$ - isomers of endosulfan demonstrated both estrogenic and antiestrogenic activities in this Tier I level *in vitro* test for endocrine disruption. These effects did not manifest in any of the reproduction or developmental studies in any of the studies performed in the open literature or according to FIFRA Guidelines.

Several studies conducted *in vivo* in adult male rats and *in vitro* using testicular tissue have implicated endosulfan in the disruption of sperm development and hormonal function in the male reproductive tract (Turner et al., 1997; Sinha et al., 2001a and b; Srivastava et al., 1991; Wilson and LeBlanc, 1998). However the doses at which effects occur are greater than those that induce neurotoxicity.

Finally, while endosulfan may have some action with the MCF-7 cells *in vitro* there has never been an association between endosulfan treatment with breast cancer or with any cancer in open literature or FIFRA-Guideline studies.

#### **4. Lack of Support for Additional Safety Factors**

The data for endocrine effects in prenatal, pup and adolescent rats do not warrant an additional safety factor at this time.

#### **5. Aggregate Exposure**

Data for aggregate endosulfan [(total occupational exposure (dermal + inhalation + dietary); (inhalation in ambient air/bystander + dietary exposure) and (dermal + non-dietary ingested/dermal for swimmers in surface water + dietary exposure)] were presented in IV. B. EXPOSURE ASSESSMENT and in IV. C. RISK CHARACTERIZATION (Tables 36-40). Exposure data were negative for endosulfan in drinking water. USEPA data from other parts of the United States found negligible endosulfan exposure in drinking water and ambient air (USEPA, 2000b).

Endosulfan mitigation processes include a 100-foot setback for ground applications between treated areas and water bodies, a 30-foot vegetative buffer between treated areas and water bodies, reductions in maximum application rates, reductions in maximal seasonal application rates and reductions in allowed number of applications in a season (USEPA, 2002).

## 6. Cumulative Exposure

Wade et al. (1997) conducted a study to examine the interaction between endosulfan and dieldrin in the activation of the estrogen receptor (ER) in or extracted from mammalian cells. There is no evidence for cumulative toxicity between endosulfan and other organochlorine compounds, based on available information in the open literature. Endosulfan has not been shown to interact synergistically with other organochlorines such as dieldrin, or other compounds (Wade, et al., 1997). Currently, the USEPA is developing the methodology to address this issue.

### a) DPR and USEPA's Comparison of Definitive Studies and Critical NOELs

The exposure scenarios, critical NOELs and endpoints for risk characterization of endosulfan in this document are listed in Table 44. For dietary exposure, DPR used acute and chronic NOELs to establish margins of exposure (Carr, 2006), as did USEPA. Rabbit dams, in the developmental study (Fung, 1989a & b) were more sensitive to endosulfan neurotoxicity (NOEL = 0.7 mg/kg/day) than the rat in the acute neurotoxicity study and, therefore, DPR used 0.7 mg/kg/day for the acute/short-term MOE estimates for occupational (dermal), swimmers in surface water and aggregate (Total Occupational + Dietary; swimmers in surface water) scenarios. The USEPA selected the acute neurotoxicity study in rat with a NOEL of 1.5 mg/kg as their acute dietary endpoint study (Bury, 1989).

For seasonal occupational (dermal), subchronic swimmer in surface water and aggregate (Total Occupational + Dietary) MOE estimates, DPR used a rat reproduction dietary study (Edwards et al., 1984) with a NOEL of 1.18 mg/kg/day based on increased kidney weights, decreased food consumption, and decreased body weights for MOE estimates. A dermal absorption of 47.3% (Craine, 1988) from a dermal rat study was used in the DPR exposure assessment (Beauvais, 2007). The USEPA did not establish a subchronic dietary endpoint study.

DPR did not establish a subchronic dermal endpoint, since there were no acceptable studies. USEPA selected a dermal NOEL of 12 mg/kg/day from a 21-Day dermal rat study for short term and subchronic intervals, based on mortality in females at 27 mg/kg/day (Ebert et al., 1985a). (Table 44).

DPR did not select a long-term dermal study since there were none available that were acceptable according to FIFRA Guidelines. USEPA selected a dermal NOEL of 12 mg/kg/day from a 21-Day dermal rat study for chronic dermal exposure based on mortality in females at 27 mg/kg/day (Ebert et al., 1985a). (Table 44).

For chronic occupational (dermal), chronic swimmer in surface water and aggregate (Total Occupational + Dietary) MOE estimates, DPR used the chronic dietary dog study (Brunk, 1989; NOEL = 0.57 mg/kg/day) with endpoints of death and neurotoxicity, where USEPA used 0.6 mg/kg/day from the 2-year combined/chronic rat study, with kidney and blood vessel pathological effects and body weight effects (Table 44). The NOELs were comparable (for calculation of chronic dietary MOEs, RfDs see VII. REFERENCE DOSES/ CONCENTRATIONS).

For determination of MOEs for acute (short-term), and subchronic occupational inhalation

exposure, public exposure to endosulfan in ambient air and to bystanders, and for aggregate (Total Occupational + Dietary) estimates DPR used the NOEL of 0.194 mg/kg/day (decreased body weight gain, increased hematology and clinical chemistry effects) from a subchronic rat inhalation study (Hollander, et al., 1984). This study was also used for short-term (1-30 days), and intermediate term (1-6 months) by USEPA using the same NOAEL and endpoints. For chronic estimates, the same study was used for the same scenarios with the addition of a 10x uncertainty factor to extrapolate from subchronic to chronic NOEL (NOEL for chronic inhalation = 0.0194 mg/kg/day). A chronic NOEL was not selected by USEPA for chronic inhalation exposure scenarios (Table 44). (see VII. REFERENCE DOSES/CONCENTRATIONS for RfC calculations).

#### **b) Safety Factors for FQPA**

USEPA has indicated that the current use of an FQPA SF of 10x is under review. Currently DPR does not use the Population Adjusted Dose ( $PAD = RfD \div FQPA\ 10x\ SF$ ) to determine MOEs for dietary exposure for infants and children and the DPR figures presented in Table 44 are for the purpose of comparison to the values of USEPA (USEPA, 2002a).

#### **c) Safety Factors for Toxic Air Contaminants**

Should endosulfan be listed as a potential Toxic Air Contaminant, then an additional 10x UF will be added when calculating MOE for infants and adults (general public) exposed to endosulfan in ambient air and as bystanders (see VII. Reference Doses/Concentrations for the calculations).

**Table 44. Comparison of critical no-observed-effect levels (NOELs) and endpoints for risk characterization between the Department of Pesticide Regulation and U.S. Environmental Protection Agency**

<b>DPR NOELs and Endpoints for Risk Characterization</b>		
<b>Exposure/Species</b>	<b>NOEL</b>	<b>Endpoint</b>
Developmental, rabbit <sup>a</sup> Acute Oral	0.7 mg/kg/day UF = 100 <sup>a</sup>	LOEL = 1.8 mg/kg; Abortions, death, convulsions, neurotoxic signs immediately after dosing, GD6 (Fung, 1981 a & b) RfD = 0.007 mg/kg/d <sup>c</sup>
21 day Inhalation, rat <sup>b</sup> For Acute Inhalation	0.194 mg/kg UF Interspecies= 10 UF Intraspecies= 10	Decreased body weight gain & lymphocyte counts in males; increased creatinine values in females at 0.4 mg/kg/day (LOAEL)(Hollander et al., 1984) RfC = 0.0033 mg/m <sup>3</sup> Infant; 0.0069 Adult <sup>d</sup>
Reproduction, rat <sup>b</sup> Subchronic Study	1.18 mg/kg/day UF Intra/Interspecies= 100	Increased kidney and liver weights; decreased food consumption and body weights (Edwards et al., 1984)
21 day Inhalation, rat <sup>b</sup> Short (1-30 d); Intermediate (1-6 mo)	0.194 mg/kg/day UF Interspecies= 10 UF Intraspecies= 10	Decreased body weight gain & lymphocyte counts in males; increased creatinine values in females at 0.4 mg/kg/day (LOAEL)(Hollander et al., 1984) RfC = 0.0033 mg/m <sup>3</sup> Infant; 0.0069 mg/m <sup>3</sup> Adult <sup>d</sup>
1 year dog <sup>c</sup> Chronic Dietary Study--all pops	0.57 mg/kg/day UF = 100	LOEL = 2.09 mg/kg/d; Premature deaths, neurotoxicity; dec bw gain & food consumption (Brunk, 1989);RfD = 0.0057
21 day Inhalation, rat <sup>c</sup> For Chronic Inhalation <sup>e</sup>	ENEL = 0.0194 mg/kg/day UF Inter/Intraspecies= 100 UF Subchron-Chronic=10 <sup>c</sup>	Dec body wt gain & lymphocyte counts in males; increased creatinine values in females at 0.04 mg/kg/day (ENEL)(Hollander et al., 1984) RfC = 0.00033 mg/m <sup>3</sup> Infant; 0.00069 mg/m <sup>3</sup> Adult <sup>d</sup> ; cPAD = 0.000033 mg/m <sup>3</sup>
<b>USEPA NOELs and Endpoints for Risk Characterization<sup>f</sup> (USEPA, 2002a)</b>		
Acute Study Neurotoxicity, rat <sup>a</sup>	1.5 mg/kg/day UF = 100 FQPA = under review	LOAEL = 3 mg/kg/day; Increased convulsions in females within 8 hrs after dosing (Bury, 1997) Acute RfD = 0.015 mg/kg/day; aPAD (under review)
21 day Dermal, rat <sup>b</sup> Short-term/Subchronic	12 mg/kg/day UF Interspecies = 10 UF Intraspecies = 10	Mortality in females at 27 mg/kg/day (Ebert et al., 1985a).
21 day Inhalation, rat <sup>b</sup> Short-term/Subchronic	0.2 mg/kg/d (0.001 mg/L) UF Interspecies = 10 UF Intraspecies = 10	Decreased body weight gain & lymphocyte counts in males; increased creatinine values in females at 0.4 mg/kg/day; LOAEL = 0.002 mg/L (0.4 mg/kg/day) (Hollander et al., 1984)
104 week dietary, rat <sup>c</sup> Chronic	0.6 mg/kg/day UF = 100 FQPA = under review	Decreased body weight gain, enlarged kidneys, increased progressive glomerulonephrosis; blood vessel aneurysms (Ruckman et al., 1989). Chronic RfD = 0.006 mg/kg/day; cPAD (under review)

a - Acute RfD = acute NOEL ÷ UF 10x (interspecies) x UF 10x (intraspecies); Population Adjusted Dose (aPAD = RfD ÷ FQPA safety factor)

b - Subchronic, seasonal (intermediate/short-term) exposure RfD= Subchronic NOEL ÷UF (10 interspecies x 10 intraspecies); RfC= Subchronic NOEL (also used for Acute inhalation NOEL ) UF (10 interspecies x 10 intraspecies)

c - Chronic RfD = Chronic NOEL ÷ (UF 10 interspecies) x (UF 10 intraspecies)); Population Adjusted Dose (cPAD = RfD) ) FQPA safety factor); A 10x UF is added to the subchronic inhalation NOEL to extrapolate to obtain a chronic inhalation NOEL. ENEL = (Subchronic ÷NOEL) ÷ UF (10 interspecies x 10 intraspecies)

d - Human inhalation NOEL (mg/m<sup>3</sup>) = animal inhalation NOEL (mg/kg/day) ) respiratory rate<sub>human</sub> (m<sup>3</sup>/kg) NOTE: The respiratory rate used for humans was for children (0.59 m<sup>3</sup>/kg) who are considered to be the highest risk group; adult 0.28 mg/m<sup>3</sup>; RfC (mg/m<sup>3</sup>) = human inhalation NOEL (mg/m<sup>3</sup>) ÷ (UF 10 interspecies x UF 10 intraspecies); RfC (ppm) = RfC (mg/m<sup>3</sup>) x (M. Vol (@ 25°C))(M.Wt. (406.9g)); Population Adjusted Dose (cPAD = RfD) ) FQPA safety factor)

e - RfC = (Subchronic NOEL ) 10 extrapolation factor) ) UF (10 interspecies x 10 intraspecies)

f – The endpoints, definitive studies and critical NOELs are those published in the REREGISTRATION ELIGIBILITY DOCUMENT (USEPA, 2002). USEPA is currently re-evaluating some of their endpoints and when DPR receives the updated information it will be included in the RCD.

Note: See Section VII. REFERENCE DOSES/CONCENTRATION

## VI. TOLERANCE ASSESSMENT

### A. INTRODUCTION

#### 1. USEPA

USEPA is responsible under the Federal Food, Drug, and Cosmetic Act (FFDCA) for setting tolerances for pesticide residues in RACs (Section 408 of FFDCA) and processed commodities (Section 409 of FFDCA). Tolerance is the legal maximum residue concentration of pesticide allowed in raw agricultural commodities and processed foods. The tolerances are established at levels necessary for the maximum application rate and frequency, and not expected to produce deleterious health effects in humans from chronic dietary exposure. The data requirements for tolerances include: 1) residue chemistry, 2) environmental fate studies, 3) toxicology studies, 4) product performance (efficacy), and 5) product chemistry (Code of Federal Regulations, 1997). Field studies must reflect the proposed use with respect to the rate and mode of application, number and timing of applications and formulations (USEPA, 2002).

In 1996, the Food Quality Protection Act amended the overall regulation of pesticide residues under FIFRA and FFDCA (USEPA, 2001c). One major change was the removal of the Delaney Clause that prohibited residues of cancer-causing pesticides in processed foods. The tolerances must be health-based and the same standards used to establish tolerances for both the raw agricultural commodities and their processed forms. FQPA required an explicit finding that tolerances are safe for children. USEPA was required to use an extra 10-fold safety factor to take into account potential pre- and post-natal developmental toxicity and the completeness of the data unless USEPA determined, based on reliable data, that a different margin would be safe. In addition, the evaluations of the tolerance must take into account: (1) aggregate exposure from all non-occupational sources, (2) effects from cumulative exposure to the pesticide and other substances with common mechanisms of toxicity, (3) effects of *in utero* exposure; and (4) potential for endocrine disrupting effects. (Discussion of these issues specific to endosulfan is in V. RISK APPRAISAL, E. Issues Related to the Food Quality Protection Act).

Under FQPA, USEPA is also required to reassess all existing tolerances and exemptions from tolerances for both active and inert ingredients by 2006 (USEPA, 2001c). Prior to FQPA, USEPA reassessed tolerances as part of its reregistration and Special Review processes. In the evaluation of tolerances, the USEPA uses the tier approach and the assessment includes all label-use commodities.

#### 2. California

In California, USEPA established tolerances are evaluated under the mandate of the Food Safety Act (Bronzan and Jones, 1989). The Act requires the DPR to “conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides.” In the situation where “any pesticide use represents a dietary risk that is deleterious to the health of humans, the DPR shall prohibit or take action to modify that use or modify the tolerance.....”

In the Food Safety Act, the tolerance for each specific commodity is evaluated individually and is discussed in the following sections. For a pesticide that is used on numerous commodities,

tolerance assessments are conducted for selected fruits and vegetables. Generally, commodities are selected from all the uses based on the potential for high levels of exposure. For endosulfan, the tolerances for the following commodities were evaluated (Carr, 2006, See Appendix C): carrot, sweet corn, lettuce, milk fat, potato, strawberry, beans, cauliflower, spinach, peas, peach, summer squash, pear, pineapple, winter squash, broccoli, apple, melon, tomato and grape. These commodities were selected because of high consumption rates, high frequency of consumption, and high tolerance levels.

## **B. ACUTE TOLERANCE ASSESSMENT**

An acute exposure assessment is conducted for each individual label-approved commodity using the residue level equal to the tolerance. The TAS Exposure-4™ software program and the 1989-1992 Continuing Survey of Food Intakes of Individuals (USDA, 1989-91) were used in this assessment. The acute tolerance assessment does not routinely address multiple commodities all at tolerance levels because the probability of consuming multiple commodities that are all at the tolerance level significantly decreases as the number of commodities included in the assessment increases.

An acute tolerance assessment was performed for endosulfan using the 1997 USEPA tolerances (CFR, 1997) [Note: See Section C on Dietary Exposure]. The endosulfan acute NOEL of 0.7 mg/kg-body wt/day was used to calculate margins of exposure based on a rabbit teratology study. There are currently more than 72 human consumption commodities that have endosulfan tolerances (CFR, 2004). A total of 20 commodities, including milk, were analyzed for tolerance level acute dietary exposure. (Table 25)

There were 15 commodities that had MOEs of less than 100 for 1 or more population subgroups when assessed using tolerance level values. Carrots, corn (sweet), lettuce, milk fat and potato were the 5 commodities with MOE values greater than 100 for all population subgroups. The RAC carrot tolerance MOE range is nursing infant; 254 (0.002751 mg/kg-bw) - male 20<sup>+</sup> years; 2,076 (0.000337 mg/kg-bw). The MOE range for the corn (sweet) tolerance is non-nursing infant; 354 (0.001977 mg/kg-bw) - female 13<sup>+</sup> years (pregnant, not nursing); 1,377 (0.000508 mg/kg-bw). The RAC lettuce tolerance MOE range is children 1-6 years; 139 (0.005027 mg/kg-bw) - non-nursing infants; 410 (0.001707 mg/kg-bw). The MOE range for the milkfat tolerance is non-nursing infant; 363 (0.001929 mg/kg-bw) - Seniors 55<sup>+</sup> years; 2,383 (0.000294 mg/kg-bw). The potato tolerance MOE range is children 1-6 years; 392 (0.001784 mg/kg-bw) - females 13<sup>+</sup> years (pregnant, not nursing); 1,029 (0.000680 mg/kg-bw).

Margins of exposure (MOE) were less than 100 for 2 or more population subgroups for 15 different commodities at tolerance when using the endosulfan acute NOEL value of 0.7 mg/kg bw. The highest acute tolerance residue contribution exposure was 0.154339 mg/kg-bw that occurred in the nursing infants less than 1 year of age subgroup from potential apple (including juice) consumption. The lowest exposure (highest MOE) was obtained from the cauliflower tolerance assessment of the population subgroup non-nursing infants with a value of 0.000174 mg/kg/day (4,019). Three commodities (apple, melon and tomato) with 19 or 20 population subgroups with less than 100 margins of exposure were listed separately (Table 45) from the remaining 15 tolerance evaluations.

The remaining 12 RACs that had 2 or more population subgroups with MOEs of less than 100

are; strawberry (2 subgroups with MOEs of < 100), beans (3), cauliflower (3), spinach (6), peas (6), peach (12), summer squash (13), pear (15), pineapple (15), winter squash (16), broccoli (17) and grape (17 population subgroups).

The RAC strawberry tolerance MOE ranges from non-Hispanic other (19 = 0.035953 mg/kg-bw) to non-nursing infants (2,239 = 0.000313 mg/kg-bw). The MOE range for the beans (all) tolerance is non-nursing infants; 40 (0.017500 mg/kg-bw) - females 13<sup>+</sup> years (pregnant, not nursing); 231 (0.003033 mg/kg-bw). The cauliflower tolerance MOE range is non-Hispanic other; 46 (0.015379 mg/kg-bw) B non-nursing infants; 4,019 (0.000174 mg/kg-bw). The MOE range for the spinach tolerance is children 1-6 years; 25 (0.028168 mg/kg-bw) - nursing infants; 1,120 (0.000625 mg/kg-bw). The peas tolerance MOE range is nursing infants; 38 (0.018446 mg/kg-bw) - females 13<sup>+</sup> years (pregnant, not nursing); 164 (0.004270 mg/kg-bw). The MOE ranges for peach tolerance is all infants <1 year (22 = 0.031145 mg/kg-bw) to females 13<sup>+</sup> years (pregnant, not nursing); 187 = 0.003748 mg/kg-bw). The summer squash tolerance MOE range is non-Hispanic other; 41 (0.017047 mg/kg-bw) - females 13<sup>+</sup> years (pregnant, not nursing); 130 (0.005381 mg/kg-bw). The MOE range for the pear tolerance is nursing infants; 9 (0.078549 mg/kg-bw) - females 13<sup>+</sup> years (pregnant, not nursing); 263 (0.002663 mg/kg-bw). The pineapple tolerance MOE range is nursing infants; 15 (0.045339 mg/kg-bw) - females 13<sup>+</sup> years (pregnant, not nursing); 189 (0.003703 mg/kg-bw). The MOE range for the winter squash tolerance is non-nursing infants; 30 (0.023609 mg/kg-bw) - females 13-19 years (not pregnant or nursing); 200 (0.003497 mg/kg-bw). The broccoli tolerance MOE range is children 1-6 years; 28 (0.025239 mg/kg-bw) - females 13<sup>+</sup> years (pregnant, not nursing); 168 (0.004170 mg/kg-bw). The MOE range for the grape tolerance is nursing infants; 12 (0.057697 mg/kg-bw) - females 13<sup>+</sup> years (pregnant, not nursing); 155 (0.004516 mg/kg-bw).

Table 45 is a summary for the 3 commodities that have MOEs of less than 100 for 19 or more of their population subgroups. The MOEs were based on tolerance levels of endosulfan. RACs (apple, melon and tomato) acute 95<sup>th</sup> percentile MOEs ranged from 5 for apples (nursing infants < 1 year) to greater than 100 for tomatoes (seniors 55+). All commodities for all population subgroups listed had acute 95<sup>th</sup> percentile MOEs less than 100 for apples, melons and tomatoes, except seniors (55+). Apples and melons are the only two commodities with endosulfan tolerances that have all 20 of their analyzed populations with MOEs less than 100. Tomatoes had 19 of the analyzed populations with MOE values of less than 100.

**Table 45. Summary of MOEs Less than 100 For Population Subgroups from Tolerance Levels of Endosulfan.**

Population Subgroups	Acute 95 <sup>th</sup> Percentile Margin of Exposure <sup>a,b,c</sup>		
	Apple	Melons	Tomato
US Population, all seasons	27	35	73
Western Region	32	36	74
Pacific Region	35	39	76
Hispanics	26	20	58
Non-Hispanic Whites	28	36	76
Non-Hispanic Blacks	25	33	67
Non-Hispanic Other	24	33	71
All Infants	8	12*	40
Infants (nursing, < 1 year)	5#	None	43
Infants (non-nursing, < 1 year)	9	12*	54
Children (1 - 6 years)	13	17	41
Children (7 - 12 years)	28	29	56
Females (13 - 19 years), not pregnant, not nursing	53	48	73
Females (20+ years), not pregnant, not nursing)	76	48	95
Females (13 - 50 years)	61	44	88
Females (13+ years), pregnant, not nursing	44	46	91
Females (13+ years), nursing	22	80	93
Males (13 - 19 years)	62	28	84
Males (20+ years)	85	52	85
Seniors (55+ years)	88	51	>100

a - MOEs based on label approved commodities. Exposure levels have been rounded off to 2 significant figures and were based on the 1989-1992 Continuing Survey of Food Intakes of Individuals.

b - The residue files used tolerance level values for the commodities. The number of user days from the 91 CSFII database generally acceptable. However, for all of the winter squash subpopulations analyzed (all <1% user days) the user days were unacceptable in the acute tolerance assessment.

c - Commodity population subgroup MOEs with “\*” appearing next to them indicate user days of 2% or less. Also, the “#” symbol indicates the lowest MOE reported in the complete acute tolerance assessment.

### C. CHRONIC TOLERANCE ASSESSMENT

A chronic exposure assessment using residues equal to the established tolerances for individual or combinations of commodities has not been conducted because it is highly improbable that an individual would chronically consume single or multiple commodities with pesticide residues at the tolerance levels. This conclusion is supported by data from both federal and DPR pesticide monitoring programs which indicate that less than one percent of all sampled commodities have residue levels at or above the established tolerance (DPR, 1994, 1995, 1997).

### D. TOLERANCE ASSESSMENT -- 2007

There were 72 commodities with human consumption that had USEPA endosulfan tolerances in 1998 (USEPA, 1999a). The same 72 commodities with USEPA tolerances were still registered in

2001 (USEPA, 2001a). However, 9 commodity tolerances have either been canceled or proposed for cancellation by the registrants of technical endosulfan. The unsupported or canceled tolerances are for artichoke, canola, mustard seed, raspberry, safflower seed, sugar beet, sugarcane, sunflower seed, and watercress (USEPA, 2001a,b, 2002). Sugarcane is the only one of the unsupported tolerances that has any significant consumption reported in the USDA Continuing Survey of Food Intake by Individuals (CSFII) consumption databases (USDA, 1989-92, 1994 - 98a). A proposed rule in the April 26<sup>th</sup> 2006 Federal Register (discussed in Section II, Carr, 2006) which would codify tolerance changes presented in the 2002 USEPA RED, was made final on September 15<sup>th</sup> 2006 (USEPA, 2002, 2006a, b). The two USEPA rules were the same except that the September version contained a nomenclature change (filbert to hazelnut) that did not appear in the April proposal. It should be noted that the September 15<sup>th</sup> 2006 final rule in the Federal Register did not contain all of the tolerance actions listed in the 2002 USEPA RED. Additional tolerance actions may appear in a future Federal Register notice. However, these results do not change the conclusions discussed in the current DPR dietary exposure addendum (Carr, 2006).

The USEPA July 2002 draft endosulfan re-registration eligibility decision (RED) document proposed that the tolerances for 5 commodities should be revoked (USEPA, 2002). Several of these commodities are sources of frequent consumption by infants and children. The proposed tolerance revocations are for beans (succulent), grape (including juice and raisin), peas (succulent), pecan, and spinach. Pecan is the only one of the 5 proposed for revocation that is not a frequently consumed commodity by infants and children. Succulent beans, succulent peas, and grapes are very frequently consumed commodities by infants and children. The USEPA concluded that the revocation of tolerances for succulent beans and peas, grapes, and spinach would mitigate acute dietary exposure concerns to acceptable levels for infants and children (USEPA, 2002). There were no chronic dietary exposure concerns cited by the USEPA RED (USEPA, 2002). There would be only 58 remaining tolerances after the registrants' voluntary cancellations and the USEPA proposed tolerance revocations are implemented (USEPA, 2001b, 2002). The USEPA proposed rule in the Federal Register finalizes the 2002 RED tolerance actions (USEPA, 2006).

The USEPA draft endosulfan RED also decreased the maximum label application rates for a number of commodities that will still have tolerances. The maximum annual application rates for pome fruits, stone fruits, and citrus will be decreased. The maximum rates will decrease from 3.0 pounds (lbs.) active ingredient (a.i.) per acre to 2.5 lbs. a.i./acre (USEPA, 2002). This represents a decrease of approximately 17%. The draft RED also lowered the maximum annual application rates for Brassica species, carrots, cucurbits, fruiting vegetables, dry beans, dry peas, nuts, and strawberries (USEPA, 2002). The revised maximum annual rates for these commodities will decrease from 3.0 lbs. a.i./acre to 2.0 lbs. a.i./acre (USEPA, 2002). These label changes will represent a decrease of approximately 33%. The reduction in maximum annual application rates for the above mentioned commodities could also result in a corresponding decrease in the magnitude of the residues detected on endosulfan treated commodities.

In 2003, the tolerances for endosulfan on previously mentioned commodities were re-evaluated (Carr, 2006) in light of the tolerance revocations and voluntary suspensions (mentioned above) described in the USEPA RED (2002).

In 2005, the USEPA received a request by endosulfan registrants to voluntarily cancel uses of certain endosulfan registrations (USEPA, 2005) on succulent peas, spinach, grapes, succulent beans,

pecans and spinach (USEPA, 2002).

In 2006, the USEPA announced in the Federal Register [Federal Register: September 15, 2006 (Volume 71, Number 179)] announced the final rule to “revoke, remove, modify and establish certain tolerances for endosulfan. As part of these processes, [USEPA] is required to determine whether each of the amended tolerances meets the safety standard of the FQPA.” The proposed tolerance changes listed in the Federal Register are exactly the same as those named in the RED for endosulfan (USEPA, 2002).

## VII. REFERENCE DOSES/CONCENTRATIONS

### A. Reference Doses

Exposure to endosulfan below the reference dose (RfD) is generally considered to be sufficiently low as to protect human health. RfDs were calculated for endosulfan for acute and chronic exposure by dividing the NOELs by an uncertainty factor (UF) of 100 when the NOEL is from an animal study to account for inter- and intraspecies variation in sensitivity. The USEPA (USEPA, 2002) previously retained the FQPA 10x safety factor for endosulfan, since there were no reliable data to address the following concerns and uncertainties: 1) evidence for an increased susceptibility to neuro- and reprotoxicity in prepubescent and neonatal rats. 2) many studies indicating endocrine disruption. 3) uncertainty of the neuroendocrine effects in young rats. 4) the request by USEPA for a DNT study. DPR reviewed the rat DNT study and found no increase in neurotoxicity in rats receiving endosulfan treatment in diet during pre- and post-natal development. Effects were decreased body weights in dams and pups at 3.74 mg/kg/day (lowest dose tested) and greater and male pups had a 4 - 5% delay in preputial separation at 10.8 mg/kg/day and greater possibly due to decreased body weight. Although endosulfan has effects in the male reproductive system as has been described in this document, doses that would protect for neurotoxicity would also protect for endocrine disruption (observed only at higher doses). The USEPA is currently evaluating their position on endosulfan as an endocrine disruptor and on the use of the FQPA SF. The data do not warrant an additional safety factor at this time.

Acute RfD was calculated by dividing the rat developmental systemic NOEL (0.7 mg/kg) by inter- and intra-species UF to account for sensitivity variation.

$$\text{Acute RfD} = \frac{\text{Acute NOEL}}{(10X \text{ UF Intraspecies})(10x \text{ UF Interspecies)}} = \frac{0.7 \text{ mg/kg/d}}{100} = 0.007 \text{ mg/kg/d}$$

Subchronic RfD was calculated by dividing the rat reproduction systemic NOEL (1.18 mg/kg/day) by an UF of 100 to account for inter- and intraspecies variation in sensitivity.

$$\text{Subchronic RfD} = \frac{\text{Subchronic NOEL}}{(10X \text{ UF Intraspecies})(10x \text{ UF Interspecies)}} = \frac{1.18 \text{ mg/kg/d}}{100} = 0.0118 \text{ mg/kg/d}$$

The chronic RfD was calculated as follows using the chronic dog study to obtain 0.57 mg/kg/day as the chronic NOEL:

$$\text{Chronic RfD} = \frac{\text{Chronic NOEL}}{(10X \text{ UF Intraspecies})(10x \text{ UF Interspecies)}} = \frac{0.57 \text{ mg/kg/d}}{100} = 0.0057 \text{ mg/kg/d}$$

## B. Reference Concentrations

An acute RfC was calculated for evaluating exposure of endosulfan to infants and adults in ambient air and to bystanders by using the subchronic NOEL from the rat inhalation study (0.194 mg/kg). The inhalation NOEL was converted to an equivalent human inhalation NOEL by dividing it by the respiratory rate for humans (NOEL = 1.19 mg/m<sup>3</sup>; 0.194 mg/kg/day)

$$\text{Human inhalation NOEL (mg/m}^3\text{)} = \frac{\text{animal inhalation NOEL (mg/kg)}}{\text{respiratory rate}_{\text{human}} \text{ (m}^3\text{/kg)}}$$

Since children have the highest respiratory rate for humans relative to their body weight, their respiratory rate was used for humans. The resulting equivalent acute human inhalation NOEL was 0.33 mg/m<sup>3</sup> assuming a default respiratory rate of 0.59 m<sup>3</sup>/kg/day for children and 0.28 m<sup>3</sup>/kg/day for adults. After dividing the equivalent human inhalation NOEL by an uncertainty factor of 100, the resultant acute RfCs (24 hrs) are 0.0033 mg/m<sup>3</sup> (Infants) and 0.0069 mg/m<sup>3</sup> (Adults).

$$\text{RfC (mg/m}^3\text{)} = \frac{\text{human inhalation NOEL (mg/m}^3\text{)}}{\text{uncertainty factor (e.g., 100)}}$$

$$\text{RfC (ppm)} = \frac{\text{M. Vol. (24.5L @ 25}^\circ\text{C)}}{\text{RfC (mg/m}^3\text{)} \times \text{M.Wt. (406.9g)}}$$

To evaluate seasonal exposure to the public (infants and adults) in ambient air and for bystanders, the NOEL of 0.194 mg/kg/day (0.001 mg/m<sup>3</sup>) from the subchronic rat inhalation study was used to calculate the RfC. To calculate the seasonal RfC, the NOEL was converted to the equivalent subchronic human inhalation NOEL of 0.33 mg/m<sup>3</sup>. The default respiratory rates of 0.59 m<sup>3</sup>/kg/day for children 0.28 m<sup>3</sup>/kg/day for adults were used. After dividing the equivalent human inhalation NOEL by an uncertainty factor of 100, the resultant subchronic RfCs are 0.0033 mg/m<sup>3</sup> (Infants; 0.2 ppb) and 0.0069 mg/m<sup>3</sup> (Adults; 0.4 ppb). To evaluate chronic occupational and exposure to the public (infants and adults) in ambient air and for bystanders, a NOEL of 0.0194 mg/kg/day (subchronic rat inhalation + a 10x UF for extrapolation from subchronic to chronic) was used to calculate the RfCs. Default respiratory rates of 0.59 m<sup>3</sup>/kg/day for children 0.28 m<sup>3</sup>/kg/day for adults were used. After dividing the equivalent human inhalation NOEL by an uncertainty factor of 100, the resultant chronic RfCs were 0.00033 mg/m<sup>3</sup> (Infants; 0.02 ppb) and 0.00069 mg/m<sup>3</sup> (Adults; 0.04 ppb).

Table 46 below shows the MOEs for the various exposure scenarios along with the percent RfC. The percentage should be approximately 10% or less in order to avoid listing as a TAC. Four scenarios do not exceed the threshold (eg. their MOEs are greater than 1000) and those are seasonal (SADD) for ambient air (infants and adults), and annual (AADD) for ambient air (adults). It is evident that the majority of scenarios do exceed the threshold for listing endosulfan as a TAC.

**Table 46. Estimated MOEs for Endosulfan in Ambient Air and to Bystanders and their Corresponding Percent Reference Concentrations.**

Exposure Scenario	Infants		Adults	
	MOE	%RfC <sup>a</sup>	MOE	%RfC
<b>AMBIENT AIR – San Joaquin Elementary School</b>				
Acute	N/A	--	N/A	--
Subchronic	5243	<b>2%</b>	11412	<b>1%</b>
Chronic	970	11%	1940	<b>5%</b>
<b>BYSTANDERS – East Station</b>				
Acute	121	82%	255	39%
Subchronic	346	29%	719	14%
Chronic	413	24%	882	11%

Bold and shaded values are those which fall within the threshold for the TAC (2001), that is, MOE > 1000.

a - % RfC = ([Exposure] respiratory rate) ÷ RfC x 100, where respiratory rate = 0.59 m<sup>3</sup>/kg/day (infant) and 0.28 m<sup>3</sup>/kg/day (adult).

## VIII. CONCLUSIONS

The risks for potential adverse human health effects with occupational, public (swimmers in surface water: dermal and non-dietary ingested), ambient air and dietary exposure to endosulfan were evaluated using margins of exposure (MOE) estimates. The MOEs for acute, subchronic and chronic exposure were calculated using no-observed-effect levels (NOELs) from the available guideline and literature toxicity studies for endosulfan. In selecting the NOELs to evaluate exposure, the greatest weight was given to studies that met FIFRA guidelines. Generally, an MOE greater than 100 is considered sufficiently protective of human health when the NOEL for an adverse effect is derived from an animal study. The MOE of 100 allows for humans being 10 times more sensitive than animals and for a 10-fold variation in sensitivity between the lower distribution of the overall human population and the sensitive subgroup.

### **Short Term Margins of Exposure (MOE):**

#### Occupational Scenarios

For dermal occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) STADD had 18 of 20 (90%) exposures with MOEs less than 100. Of those, 25% of the dermal MOEs (5/20) were less than or equal to 1 (Aerial M/L-WP; applicator; HPHW M/L/A EC; dip applicator; sweet corn hand-harvesting).

Inhalation scenarios that were less than or equal to 1 was aerial M/L-WP. STADD MOEs dermal were greater than 100 for root dip M/L (both EC and WP), ranging from 2333 (M/L WP) to 23,333 (M/L EC). Inhalation scenarios that were greater than 100 were airblast (M/L-EC, and applicator), groundboom (M/L-EC and applicator), backpack sprayer (M/L/A), LPHW (M/L/A EC), and dip (M/L EC and M/L WP).

All STADD re-entry worker exposure scenarios had MOEs that were less than 100. Sweet corn hand harvesting had an MOE of 1.

#### Non-Dietary Ambient Air and Bystander Scenarios

Short term MOEs for non-dietary infant and adult ambient air and bystander scenarios were greater than 100 at 121 for infant bystanders and 255 for adult bystanders. Since both of these scenarios had MOEs of less than 1000, endosulfan may be listed as a potential toxic air contaminant (California Food and Agricultural Code: 14021-14027).

#### Swimmer in Surface Water Scenarios

All short term non-dietary MOEs for swimmers in surface water were greater than 100 and ranged from 449 (child non-diet ingested and total) to 321,101 (adult dermal).

### **Seasonal Margins of Exposure**

For dermal occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) SADD had 12 of 17 (59%) exposures with MOEs less than 100. SADD MOEs were

greater than 100 for airblast M/L-EC (197), airblast M/L-WSP, all of groundboom scenarios except M/L-WP (15), backpack sprayer (107) and LPHW (M/L/A EC).

For inhalation occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) SADD had 5 of 17 (29%) exposures with MOEs less than 100. SADD inhalation MOEs were greater than 100 were aerial (M/L-EC, applicator, flagger), airblast (M/L-EC, M/L WSP and applicator) all groundboom but M/L WP, backpack sprayer (M/L/A), and LPHW (M/L/A EC and M//L/A WP).

The SADD re-entry worker exposure scenarios had 4 of 10 MOEs of less than 100 (broccoli, scouting--98; sweet corn, hand harvesting--16; grape, cane turning--8; and peach, thinning—42) and the remainder was 131 or greater.

#### Ambient Air and Bystander Scenarios

All seasonal exposure MOEs for the infant and adult ambient air and bystander scenarios were greater than 100, ranging from 346 (bystander, infant) to 11,415 (ambient air, adult). Note that since both the bystander scenarios have MOEs of less than 1000 endosulfan may be listed as a potential toxic air contaminant (California Food and Agricultural Code: 14021-14027).

#### Swimmer in Surface Water Scenarios

All seasonal MOEs for swimmers in surface water were greater than 100 and ranged from 24,583 (child: non-diet ingested + dermal) to 31,216,931 (adult dermal).

#### Annual Margins of Exposure

For dermal occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) AADD had 10 of 17 (59%) exposures with MOEs less than 100. The dermal MOEs remaining that were greater than 100 ranged from 143 (groundboom M/L-WSP) to 1140 (low pressure handwand M/L/A-EC).

For inhalation occupational scenarios (aerial, airblast, groundboom, handlers using handheld equipment) AADD had 8 of 17 (47%) exposures with MOEs less than 100. AADD MOEs were greater than 100 for the remaining scenarios and they ranged from 194 (airblast M/L-WSP, groundboom M/L-EC and applicator and low pressure handwand M/L/A-WP) to 6467 for both backpack sprayer and low-pressure handwand M/L/A-EC.

The AADD re-entry worker exposure scenarios had 2 of 10 MOEs of less than 100 (sweet corn, hand harvesting--95; and grape, cane turning--12) and the remainder was 114 or greater.

### Ambient Air and Bystander Scenarios

All annual exposure MOEs for the infant and adult ambient air and bystander scenarios were greater than 100, ranging from 413 (bystander, infant) to 1940 (ambient air, adult). Note that since all MOEs for AADD scenarios (except ambient air adults) are below 1000, endosulfan may be listed as a potential toxic air contaminant ( ).

### Swimmer in Surface Water Scenarios

All annual MOEs for swimmers in surface water were greater than 100 and ranged from 43,511 (child: non-diet ingested and total) to 55,339,806 (adult dermal).

## **Dietary Exposure Estimates and Margins of Exposure (MOEs)**

### Acute and Short Term Dietary Exposure

Acute dietary MOEs were calculated for the various population subgroups using the NOEL for acute toxicity (0.7 mg/kg). Estimates of exposure ranged from 1.37 ug/kg in Females (13- 19 years), not pregnant, not nursing to 3.30 in Children (1-6 years). Females (13+ years, nursing) was selected for the acute dietary exposure group for adults (based on the 95<sup>th</sup> percentile of user-day exposure). Acute dietary exposure for infants (non-nursing, < 1 year) was 3.18 (based on the 95<sup>th</sup> percentile of user-day).

All population subgroups have MOEs (acute 95<sup>th</sup> percentile) greater than 100 and these dietary MOEs are based on anticipated endosulfan residues on RAC. None of the MOEs for categories involving acute dietary exposure infants and children is greater than 1000 (all are greater than 100), as recommended under the FQPA (1996), however all are greater than 1000 for chronic dietary exposure.

The MOEs for acute dietary exposure ranged from 212 in children (1 -6 years) to 513 in males (13-19). Acute MOE for Females (13+, nursing) was 340. For infants (non-nursing, < 1 year old) it was 220 and for children (1-6 years) it was 212. All MOEs in these population subgroups were greater than 100.

### Subchronic and Chronic Dietary Exposure

The chronic dietary exposures ranged from 0.08 ug/kg/day in infants (nursing, < 1 year old) to 0.041 in children (1 - 6 years). Since there are no subchronic dietary data for endosulfan, chronic data were used for subchronic calculations. Chronic dietary exposure for infants (non-nursing, < 1 year) was 0.28 ug/kg/day; 0.41 ug/kg/day was used for children (1 B 6 years) exposed to endosulfan (dermal and non-dietary ingestion) by swimming in surface water and 0.17 ug/kg/day (Females (13+, nursing)) was used to represent adults, both occupational and in the general public. There were no percent crop treated (%CT) adjustments used in these calculations.

MOEs for chronic dietary exposure were calculated from data for the various population

subgroups and the definitive NOEL from the chronic dog study (0.57 mg/kg/day). The MOEs ranged from 1407 in children (1 - 6 years) to 7,421 in infants (nursing < 1 year of age). Percent crop treated (%CT) adjustments were used in these calculations. The chronic dietary exposures were the same as the subchronic subpopulations used for adults (Females (13+ years, nursing = 340), infants (infants non-nursing, < 1 year = 220) and children (children 1 B 6 = 212).

Drinking water is not a likely source of uncertainty with regard to endosulfan dietary exposure. Surface and well water samplings have been negative for endosulfan residues since 1996. In addition, the PDP samples from 2001 to 2003 (PDP, 2003, 2004, 2005) have been negative for endosulfan in drinking water.

### **Aggregate Margins of Exposure**

#### a) Aggregate MOEs Occupational Exposure

##### i. Aggregate MOEs for Aerial and Ground Application .

Aerial application MOEs for all aggregate STADD scenarios were less than 100, ranging from less than 1 (Aerial M/L-WP; applicator) to 25 (airblast M/L WSP). SADD aggregate MOEs were less than 100, except for airblast M/L EC (156), groundboom M/L EC (123) and applicator (180). AADD aggregate MOEs were less than 100, except for airblast M/L EC (156), airblast M/L WSP (139) and applicator (112) (Table 37).

##### ii. Aggregate MOEs for Handlers Using Handheld Equipment.

All aggregate STADD MOEs were well below 100, ranging from less than or equal to 1 (HPHW M/L/A EC; dip applicator) to 45 for handlers using handheld equipment except dip M/L EC (335) and dip M/L WP (280) (Table 38). SADD and AADD aggregate MOEs less than 100 were for HPHW M/L/A-EC (7 and 10, respectively) and LPHW M/L/A-WP (66 and 93, respectively). Other MOEs for SADD and AADD were greater than 100 and ranged from 103 (SADD backpack sprayer M/L/A EC) to 757 (LPHW M/L/A EC).

##### iii. Aggregate MOEs for Reentry Workers

All scenarios for STADD for reentry workers had aggregate MOEs that were less than 100, with a range of 1 for sweet corn, hand harvesting to 64 for hand harvesting ornamentals and for almond thinning (Table 39). For SADD, 4/10 aggregate MOEs were less than 100, however one of the MOEs was 97 (range = 8 for grape, cane turning to 97 for broccoli, scouting). The highest SADD MOE was 283 for both lettuce, scouting and for potato, scouting. The only AADD MOEs less than 100 were sweet corn, hand harvesting (92) and grape, cane turning (12). All other AADD MOEs (8/10) were greater than 100 (110 = peach, thinning to 487 for cucumber, hand harvesting).

#### d) Data for Aggregate MOEs in Non-Occupational Scenarios

##### i. Aggregate MOEs for Ambient Air and for Bystanders

Aggregate bystander scenarios (no short-term ambient air exposure values) had STADD MOEs

of 78 (infants) and 146 (adults). SADD MOEs were all greater than 100, ranging from 296 (bystander infants) to 2648 (ambient air adults). For AADD, aggregate scenarios for ambient air and for bystanders were all greater than 100, ranging from 343 (bystander infants) to 1241 (ambient air adults). However, since the majority of the MOEs were less than 1000 (all except SADD ambient air--infants and adults and AADD for infants) endosulfan should be considered for listing as a toxic air contaminant.

ii. Aggregate MOEs for Swimmers in Surface Water

All aggregate scenarios for swimmers in surface water had STADD, SADD and AADD MOEs of greater than 100. Aggregate MOEs for STADD ranged from 144 for child non-dietary ingestion and for child total (144; non-dietary ingestion and dermal) to 350 for adult dermal. Within scenarios for STADD, SADD and AADD the aggregate MOEs for adults or for children did not much variation. For example MOEs for STADD aggregate scenarios had adult MOEs of 308 to 350 and child MOEs of 144 to 212. SADD aggregate MOEs for adults ranged from 6755 to 6940 and child MOEs or 2634 to 2949. AADD aggregate MOEs for adults ranged from 3328 to 3353 and for children ranged from 1380 to 1425.

## XI. REFERENCES

- Abalis, I.M., Eldefrawi, M.E. and Eldefrawi, A.T., 1986. Effects of insecticides on GABA- induced chloride influx into rat brain microsacs. *J. of Toxicol. Environ. Health* 18:13-23.
- Adams, J.F., 1978. Mutagenicity of some environmental chemicals in Salmonella test systems without microsomal activation. *Mutation Research*, 53(2):
- Agrawal, A.K., Anand, M., Zaidi, N.F. and Seth, P.K., 1983. Involvement of serotonergic receptors in endosulfan neurotoxicity, *Biochemical Pharmacology*, 32(23):3591-3593.
- Ahmad, M.M., Maqsood Ahmad, M. and Sarvat, S., 1993. Effects of endosulfan and chlorpyrifos on the reproductive organs and sex hormones of neonatal rats. *Pakistan J. Zoology* 25(1):11B14
- Air Resources Board (ARB). 1998. Report for the Air Monitoring of Endosulfan in Fresno County (Ambient) and in San Joaquin County (Application). Project No. C96-034. Sacramento, CA: Engineering and Laboratory Branch, Air Resources Board, California Environmental Protection Agency. <http://www.cdpr.ca.gov/docs/empm/pubs/tac/endoslfm.htm>
- Aleksandrowicz, D.R. (1979). Endosulfan poisoning and chronic brain syndrome. *Arch. Toxicol.* 43:65-68.
- Anand, M., Khanna, R.N. and Misra, D., 1980a. Electrical activity of brain in endosulfan toxicity. *Indian J. Pharmac.* 12(4)229-235.
- Anand, M., Sur, R.N., Khanna, R.N., Gopal, K. and Gupta, G.S.D., 1980b. Effect of endosulfan on bioelectrical activity of brain in rats. *Indian J. Biochem. Biophys.* 17(4):74.
- Anand, M., Akveld, A.C. and Saxena, P.R., 1981. Effect of a neurotoxic pesticide, endosulfan, on tissue blood flow in cats, including regional cerebral circulation. *Vet. Hum. Toxicol.* 23:252-258.
- Anand, M., Mehrotra, S., Gopal, K., Sur, R.N. and Chandra, S.V., 1985. Role of neurotransmitter in endosulfan induced aggressive behaviour in normal and lesioned rats. *Toxicol. Lett.* 24:79-84.
- Anand, M., Gopal, K., Agrawal, C., Chandra, S.V., Ray, P.K., Verma, M. and Shanker, K., 1986. Endosulfan induced inhibition of 3H-S-hydroxytryptamine uptake in platelets. *Toxicol. Lett.* 32:203-208.
- Andersen, H.R., Vinggaard, A.M., Rasmussen, T.H., Gjermansen, I.M. and Bonefeld-Jorgensen, E.C., 2002. Effects of currently used pesticides in assays for estrogenicity, androgenicity and aromatase activity *in vitro*. *Toxicology and Applied Pharmacology*, 179:1-12.
- Andrews, C. and Patterson, G. 2000. Interim Guidance for Selecting Default Inhalation Rates for Children and Adults. Memo No. HSM-00010, dated December 1. Sacramento, CA: California Department of Pesticide Regulation, Worker Health and Safety Branch.

<http://www.cdpr.ca.gov/docs/whs/memo/hsm00010>

- Ansari, R.A. and Gupta, P.K., 1981. Influence of endosulfan on biochemical parameters of rat testes. *Indian J. Biochem. Biophys.* 18(Suppl.):160.
- Ansari, R.A., Siddiqui, M.K.J. and Gupta, P.K., 1984. Toxicity of endosulfan: Distribution of alpha- and beta- isomers of racemic endosulfan following oral administration in rats. *Toxicol. Lett.* 21:29-33.
- Ansari, R.A., Husain, K., and Gupta, P.K., 1987. Endosulfan toxicity influence on biogenic amines of rat brain. *J. Environ. Biology*, 8(3):229 - 236.
- Aprea, C., Sciarra, G., Sartorelli, P., Desideri, E., Amati, R. and Sartorelli, E. 1994. Biological monitoring of exposure to organophosphorus insecticides by assay of urinary alkylphosphates: Influence of protective measures during manual operations with treated plants. *International Archives of Occupational and Environmental Health* 66:333-338.
- ARB, 2004. TAC Application site monitoring status 4/12/04. Department of Air Resources, California Environmental Protection Agency.
- Archer, T.E., 1973. Endosulfan residues on alfalfa hay exposed to drying by sunlight, ultraviolet light and air. *Pesticide Science*, 4:59-68.
- Arnold, D., (Industrial Bio-Test Laboratories, Inc.), 1972. Mutagenic study with thiodan in Albino mice. DPR Vol. 182-042 #047309.
- Arrebola, F.J., Martinez Vidal, J.L., and Fernandez-Gutierrez, A., 1999. Excretion study of endosulfan in urine of a pest control operator. *Toxicology Letters*, 107:15-20.
- Awasthi, N., Manickan, N. and Kumar, A., 1997. Biodegradation of endosulfan by a bacterial coculture. *Bull Environ Contam Toxicol*, 59(6):928 - 934.
- Bajpayee, M., Pandey, A.,K., et al., 2006. DNA damage and mutagenicity induced by endosulfan and its metabolites. *Environ Mol Mutagen*, 47(9):682-692.
- Bakili, R.A., 1997a. Acute Dermal Toxicity Study to Rabbit. Department of Toxicology, Jai Research Foundation, Gujarat, India; Project ID#: 1026/JRF/TOX/97; 6/14/97. DPR volume/record #: 182-114 179769.
- Bakili, R.A., 1997b. Primary Eye Irritation Study. Department of Toxicology, Jai Research Foundation, Gujarat, India; Project ID#: 1028/JRF/TOX/97; 9/10/97. DPR volume/record #: 182-114 179771.
- Bakili, R.A., 1997c. Primary Dermal Irritation. Department of Toxicology, Jai Research Foundation, Gujarat, India; Project ID#: 1029/JRF/TOX/97; 4/28/97. DPR volume/record #: 182-114 179772.

- Banerjee, B.D. and Hussain, Q.Z., 1986. Effect of sub-chronic endosulfan exposure on humoral and cell-mediated immune responses in albino rats. *Archives of Tox*, 59:279 - 284.
- Banerjee, B.D. and Hussain, Q.Z., 1987. Effects of endosulfan on humoral and cell-mediated immune responses in rats. *Environ. Contam and Toxicol.*, 38:435-441.
- Barnard, A.V., Jones, D.R., Powell, L.A.J., Heywood, R., Street, A.E., Gibson, W.A., Gopenath, C., Majeed, S.K. and Almond, R. (Huntingdon Research Centre, England), 1984. 13-Week toxicity study in mice. DPR Vol. 182-042 #0472
- Barnard, A.V., Jones, D.R., Powell, L.A.J., Heywood, R., Street, A.E., Gibson, W.A., Gopenath, C., Majeed, S.K. and Almond, R. (Huntingdon Research Centre, England), 1985. 13-Week toxicity study in rats followed by a 4-week withdrawal period (final report). DPR Vol. 182-032 #035803.
- Baughner, D.G. 1989. Exposure of Mixer/Loader/Applicators to Thiodan 73EC Insecticide Applied to Fruit Trees by Airblast Equipment in California, 1987. Unpublished study submitted by Hoechst Celanese Corporation, Lab Project No. 24587. DPR Data Volume 182-060, Record No. 73677.
- Baynes, R.E. and Riviere, J.E. 1998. Influence of inert ingredients in pesticide formulations on dermal absorption of carbaryl. *American Journal of Veterinary Research* 59:168-175.
- Bayoumi, A.E., Garcia-Fernandez, A.J., Ordonez, C., Periz-Pertijo, Y., Cubria, J.C., Reguera, R.M., Balana-Fouce, R., and Ordonez, D., 2001. Cyclodiene organochlorine insecticide-induced alterations in the sulfur-redox cycle in CHO-K1 cells. *Comparative Biochemistry and Physiology Part C* 130:315-323.
- Beard, J.E. and Ware, G.W., 1969. Fate of endosulfan on plants and glass. *J. Agric. Food Chem*, 17(2):216-220.
- Beauvais, S.L. 2004. Nursery Stock Root Dips: Applicator Exposure Estimates. Memo No. HSM-04029, dated December 24. Sacramento, CA: California Department of Pesticide Regulation, Worker Health and Safety Branch.
- Beauvais, S., 2006. Human Exposure Assessment for Endosulfan. California Environmental Protection Agency, Department of Pesticide Regulation, Worker Health and Safety Branch, Sacramento, CA
- Bebe, F.N. and Panemangalore, M., 2003. Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissues of rats. *Journal of Environmental Science and Health; Part B—Pesticides, Food Contaminants and Agricultural Wastes*, B38(3):349-363.
- Beckman, H.F., 1962. Almond residue data. Gowan Corporation. Study No. 208. DPR Vol. 182-055-063963.
- Beech, JA. 1980. Estimated worst case trihalomethane body burden of a child using a swimming

- pool. *Medical Hypotheses* 6:303-307.
- Birth Defect Prevention Act, 1984. Chapter 2, Article 14, Statute #: 13121 B 13133 of the California Food and Agriculture Code, Sacramento, CA.
- Bowman, M.C., Schechter, M.S., Carter, R.L., 1965. Behavior of chlorinated insecticides in a broad spectrum of soil types. *J Agric Food Chem*, 13:360-365.
- Boyd, E.M., 1972. Protein deficiency and pesticide toxicity, Springfield, Illinois, Charles C. Thomas, Publisher, pp. 195-205.
- Boyd, E.M. and Dobos, I., 1969. Protein deficiency and tolerated oral doses of endosulfan. *Arch. Int. Pharmacodyn. Thera.*, 178:152-165.
- Boyd, E..M., Dobos, I. and Krijnen, C.J., 1970. Endosulfan toxicity and dietary protein. *Archives of Environmental Health*, 21:15-19.
- Brand, R.M. and Mueller, C. 2002. Transdermal penetration of atrazine, alachlor, and trifluralin: effect of formulation. *Toxicological Sciences* 68:18-23.
- Brandt, V.A., Moon, S., Ehlers, J., Methner, M.M., and Struttman, T., 2001. Exposure to endosulfan in farmers: two case studies. *Am. J. Indust. Med.* 39:643-649.
- Braun, H.E. and Lobb, B.T., 1976. Residues in milk and organs in a dairy herd following acute endosulfan intoxication. *Can. J. Anim. Sci.* 56:373-376.
- Brodberg, R.K. and Pollock, G.A., 1999. Prevalence of selected target chemical contaminants in sport fish from two California lakes: Public Health Designated Screening Study: Sacramento, CA: Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.
- Bronzan and Jones 1989. Chapter 2, Article 14, Statute #s: 13134 B 13135 of the California Food and Agriculture Code, Sacramento, CA.
- Brunk, R., 1989. Testing for toxicity by repeated oral administration (1-year feeding study) to Beagle dogs. DPR Vol. 182-065 #074850.
- Buch, S.A. and Gardner, J.R. (Life Sciences Research), 1983. Thionex 35 EC: Acute Inhalation Toxicity in the Rat. DPR Vol. 182-038 #052132.
- Budavari, S., 2001. *The Merck Index. An encyclopedia of chemicals, drugs and biologicals.* 13<sup>th</sup> ed. Whitehouse Station, NJ. Merck Research Laboratories Division of Merck & Co., Inc., Pubs.
- Burgoyne, T.W. and Hites, R.A., 1993. Effects of temperature and wind direction on the atmospheric concentrations of  $\alpha$ -endosulfan. *Environmental Science and Technology* 27:910-914.
- Bury, D., 1997. Neurotoxicological Screening in the Male and Female Wistar Rat; Acute Oral

- Toxicity. DPR Vol. 182-106#162457 (Hoechst Marion Roussel, Preclinical Development Germany, Drug Safety, FRG; Study #: 96.0373; Report #: 97.0149).
- Byers, R.A., Woodham, D.W. and Bowman, M.C., 1965. Residues on coastal bermudagrass, trash and soil treated with granular endosulfan. *J. Econ. Entomol.* 58:160-161.
- Caglar, Y., Kaya, M., et al., 2003. Ultrastructural evaluation of the effect of endosulfan on mice kidney. *Histology and Histopathology*, 18(3):703-708.
- California Assembly Bill 1807, 1983. AB 1807, Tanner. Air pollution: toxic air contaminants. Chapter 1047, pp 29-47
- California Food and Agriculture Code: Division 7, Chapter 3., Article 1.5 Pesticides: #14021-14027.
- Callahan, M.A., Slimak, M.W., Gabel, N.W., et al., 1979. Water-related environmental fate of 129 priority pollutants. Vol. I: Introduction and technical background, metals and inorganics, pesticides and PCB's. Washington, DC: U.S. Environmental Protection Agency, Office of Water Planning and Standards. EPA-440/4-79-029a, 27.1-27.16.
- Campoy, C., Jiménez, M., Olea-Serrano, M.F., Moreno-Frías, M., Cañabate, F., Olea, N., Bayés, R., Molina-Font, J.A., 2001. Analysis of organochlorine pesticides in human milk: preliminary results. *Early Human Development*, 65(supplement), S183-S190.
- Carmines, E.L. (1989). Thiodan 3EC Insecticide: Field monitoring of mixer/loader/applicator exposure. DPR Reg. Doc. #: 162:60.
- Carr, W.C., Jr., 1998. Endosulfan (Thiodan™) dietary exposure assessment. Health Assessment Section, Medical Toxicology Branch, Department of Pesticide Regulation, California EPA.
- Carr, W.C., Jr., 2006. Endosulfan (Thiodan™) dietary exposure assessment addendum. Health Assessment Section, Medical Toxicology Branch, Department of Pesticide Regulation, California EPA.
- Casida, J.E. and Lawrence, L.J., 1985. Structure-activity correlations for interactions of bicyclic phosphorus esters and some polychlorocycloaldane and pyrethroid insecticides with brain-specific t-butylbicyclic phosphorothionate receptor. *Environ. Health Perspect.* 61:123-132.
- Castillo, C.G., Montante, M., Dufour, L., Martinez, M.L. and Jiménez-Capdeville, M.E., 2002. Behavioral effects of exposure to endosulfan and methyl parathion in adult rats. *Neurotoxicology and Teratology* 24:797-804.
- Cerrillo, I., Granada, A., López-Espinosa, M., Olmos, B., Jiménez, M., Caño, A., Olea, N., Olea-Serrano, M., 2005. Endosulfan and its metabolites in fertile women, placenta, cord blood and human milk, *Environmental Research* 98:233-239.
- Chaisson, C.F., Sielken, R.L., Jr. and Waylett, D.K. 1999. Overestimation bias and other pitfalls

- associated with the estimated 99.9th percentile in acute dietary exposure assessments. *Regulatory Toxicology and Pharmacology* 29:102-127.
- Chan, P.L., Morisawa, S., Nakayama, A., Kawamoto, Y., Sugimoto, M. and Yoneda, M., 2005. Toxicokinetics of <sup>14</sup>C-endosulfan in male Sprague-Dawley rats following oral administration of single or repeated doses. Wiley Periodicals, Inc., Wiley InterScience ([www.interscience.wiley.com](http://www.interscience.wiley.com)) DOI 10.1002/tox.20142.
- Chaudhuri, K., Selvaraj, S. and Pal, A.K., 1999. Studies on the genotoxicity of endosulfan in bacterial systems. *Mutation Research* 439:63-67.
- Chitra, K.C., Latchoumycandane, C., Mathur, P.P., 1999. Chronic effect of endosulfan on the testicular functions of rat. *Asian J Androl*, ISSN 1008-682X, pages 203-206.
- Chugh, S.N., Dhawan, R., Agrawal, N. And Mahajan, S.K., 1998. Endosulfan poisoning in Northern India: a report of 18 cases. *International J. Clin. Pharm. and Therapeutics*, 36(9):474-477.
- Cifone, M. (Litton Bionetics, Inc.), 1984a. Mutagenicity evaluation of HOE 002671-Substance technical in the mouse lymphoma forward mutation assay. Final report. DPR Vol. 182-025 #035792.
- Cifone, M. (Litton Bionetics, Inc.), 1984b. Evaluation of HOE 002671-substance technical in the rat primary hepatocyte unscheduled DNA synthesis assay. Final Report. DPR Vol. 182-025 #035793.
- Cifone, M., 1983. Micronucleus test in male and female NMRI mice following oral administration. DPR Vol. 182-025 #035794.
- CFR, 2006. Code of Federal Regulations, 2006. Title 40, section 180.182. United States Government Printing Office, Washington, D.C.
- Cole, L.M. and Casida, J.E., 1986. Polychlorocycloalkane Insecticide-Induced Convulsions in Mice in Relation to Disruption of the GABA-Regulated Chloride Ionophore\*, *Life Sciences*, 39:1855-1862.
- Coleman, P.F. and Dolinger, P.M., 1982. Endosulfan monograph number four: Environmental health evaluations of California restricted pesticides. Prepared by Peter M. Dolinger Associates, Menlo Park, CA. Sacramento, CA: State of Department of Pesticide Regulation.
- Cotham, W.E. Jr. and Bidleman, T.F., 1989. Degradation of malathion, endosulfan and fenvalerate in seawater and seawater/sediment in microcosms. *J Agric Food Chem*, 37:824 - 828. .
- Craine, E.M. (Hoechst AG), 1988. A dermal absorption study in rats with <sup>14</sup>C-endosulfan with extended test duration. DPR Vol. 182-060 #073679.
- Dalsenter, P.R., Dallegrave, E., Mello, J.R.B., Langeloh, A., Oliveira, R.T., and Faqi, A.S., 1999. Reproductive effects of endosulfan on male offspring of rats exposed during pregnancy and

- lactation. *Human & Experimental Toxicology*, 18:583-589.
- Dalsenter, P.R., de Araújo, S.L., da Silva de Assis, H.C., Andrade, A.J.M., 2003. Pre and postnatal exposure to endosulfan in Wistar rats. *Human & Experimental Toxicology*, 22:171-175.
- Daniel, C.S., Agarwal, S. and Agarwal, S.S., 1986. Human red blood cell membrane damage by endosulfan. *Toxicology Letters* 32:113-118.
- Dashiell, O.L., 1973. Maternal toxicity, embryotoxicity and teratogenic potential of neoprene accelerators applied to skin of rats during organogenesis. E.I. Du Pont de Nemours and Co., Document I.D. 86-870001077. Haskell Laboratory Report #: 344-73.
- Das, N. and Garg, A., 1981. Effect of endosulfan in female rats growing on low- and high-protein cereal diet. *Pesticide Biochemistry and Physiology* 15:90-98.
- Dawson, J.L. 2003. *Human Health Risk Assessment: Carbaryl*. Washington, DC: Health Effects Division, Office of Pesticide Programs, U.S. Environmental Protection Agency.
- Deema, P., Thompson, E. and Ware, G.W., 1966. Metabolism, storage and excretion of C14-endosulfan in the mouse. *J. Econ. Entom.* 59:546-550.
- Demeter, J. and Heyndrickx, A., 1978. Two lethal endosulfan poisonings in man. *J. Anal. Toxicol.* 2:68-74.
- Demeter, J., Heyndrickx, A., Timperman, J., Lefevre, M. and Debeer, J., 1977. Toxicological analysis in a case of endosulfan suicide. *Bull. Environ. Contam. Toxicol.* 18:110-114.
- Dehn, P.F., Allen-Mocherie, S., Karek, J., and Thernapan, A., 2005. Organochlorine insecticides: impacts on human HepC2 cytochrome P4501A, 2B activities and glutathione levels. *Toxicology In Vitro.* 19:261-273.
- Den Tonkelaar, E.M. and Van Esch, G.J., 1974. No-effect levels of organochlorine pesticides based on induction of microsomal liver enzymes in short-term toxicity experiments. *Toxicology*, 2:371-380.
- Dikshith, T.S.S. and Datta, K.K., 1977. Cytogenetic studies of endosulfan in male rats. In: *Environmental Pollution and Human Health. Proceedings of the International Symposium on Industrial Toxicology*, November 4-7, 1975, pp. 578-589. Industrial Toxicology Research Centre, Lucknow, India.
- Dikshith, T.S.S., Nath, G. and Datta, K.K., 1978. Combined cytogenetic effects of endosulfan and metepa in male rats. *Indian J. Exp. Biol.*, 16:1000-1002.
- Dikshith, T.S.S., Raizada, R.B., Srivastava, M.K. and Kaphalia, B.S. 1984. Response of rats to repeated oral administration of endosulfan. *Ind. Health* 22:295-304.
- Dikshith, T.S.S., Raizada, R.B., Kumar, S.N., Scrivastava, M.K., Kaushal, R.A., Singh, R.P. and

- Gupta, K.P., 1988. Effect of repeated dermal application of endosulfan to rats. *Vet. Hum. Toxicol.* 30:219-224.
- Doman, I., 1971. Thiodan poisoning in sheep. *Magy. Allatorv. Lapja.* 26:342-343.
- Donaubauer, H.H., 1988. Carcinogenicity study in mice, 24 month feeding study. DPR Vol. 182-064 #075035.
- Donaubauer, H.H., et al. (Hoechst AG), 1985. 42-Tage Fütterungsstudie an Mäusen. (German version only). DPR Vol. 182-042 #047296.
- Dawson, J.L. 2003. Human Health Risk Assessment: Carbaryl. Washington, DC: Health Effects Division, Office of Pesticide Programs, U.S. Environmental Protection Agency.
- Dong, M.H. 1990. Dermal Transfer Factor for Cotton Scouts. Memo No. HSM-90001, dated June 8. Sacramento, CA: Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency.  
<http://www.cdpr.ca.gov/docs/whs/memo/hsm90001>
- Dorough, H.W., Huhtanen, K., Marshall, T.C. and Bryant, H.E., 1978. Fate of endosulfan in rats and toxicological considerations of apolar metabolites. *Pestic. Biochem. Physiol.* 8:241-252.
- Dourson, M., Charnley, G., and Scheuplein, R., 2002. Different sensitivity of children and adults to chemical toxicity. II. Risk and regulation. *Regul. Toxicol. Pharmacol.* 35:448-467.
- DPR, 1994. DPR Pesticide Residue Monitoring Program, 1993. Pesticide Enforcement Branch, Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.
- DPR, 1995. State of California Pesticide Use Report: Annual, 1993.
- DPR, 1996a. Pesticide use report, annual 1995 indexed by chemical. Sacramento, CA: California Environmental Protection Agency, Department of Pesticide Regulation (DPR). Available at [http://www.cdpr.ca.gov/docs/pur/pur95rep/95\\_pru.htm](http://www.cdpr.ca.gov/docs/pur/pur95rep/95_pru.htm).
- DPR, 1996b. Pesticide use report, annual 1994 indexed by chemical. Sacramento, CA: California Environmental Protection Agency, Department of Pesticide Regulation (DPR). Available at [http://www.cdpr.ca.gov/docs/pur/pur95rep/95\\_pru.htm](http://www.cdpr.ca.gov/docs/pur/pur95rep/95_pru.htm).
- DPR, 1997. DPR Pesticide Residue Monitoring Program, 1995. Pesticide Enforcement Branch, Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.
- DPR, 2000. Pesticide Use Report, Annual 1999 Indexed by Chemical. Sacramento, CA: Department of Pesticide Regulation, California Environmental Protection Agency.  
<http://www.cdpr.ca.gov/docs/pur/pur99rep/chmrpt99.pdf>

- DPR, 2001. Pesticide Use Report, Annual 2000 Indexed by Chemical. Sacramento, CA: Department of Pesticide Regulation, California Environmental Protection Agency.  
<http://www.cdpr.ca.gov/docs/pur/pur00rep/chmrpt00.pdf>
- DPR, 2002a. Pesticide Use Report, Annual 2001 Indexed by Chemical. Sacramento, CA: Department of Pesticide Regulation, California Environmental Protection Agency.  
<http://www.cdpr.ca.gov/docs/pur/purmain.htm>
- DPR, 2002b. DPR Dietary Exposure Assessment (12-10-02). Health Assessment Section, Medical Toxicology Branch, Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.
- DPR, 2002c. Customized search of the DPR and U.S.EPA Pesticide Label Databases (DPR homepage; [www.cdpr.ca.gov](http://www.cdpr.ca.gov)). Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.
- DPR, 2003. Pesticide Use Report, Annual 2002 Indexed by Chemical. Sacramento, CA: Department of Pesticide Regulation, California Environmental Protection Agency.
- DPR, 2004. Surface Water Database Complete Chemical Analysis Results. Downloadable data files updated January 2004, accessed June 8, 2005. Website to download files  
<http://www.cdpr.ca.gov/docs/sw/surfcont.htm>
- DPR 2005. Pesticide Use Report, Annual 2004 Indexed by Chemical. Sacramento, CA: Department of Pesticide Regulation, California Environmental Protection Agency.  
<http://www.cdpr.ca.gov/docs/pur/purmain.htm>
- DPR. 2006b. California Pesticide Information Portal (CalPIP), Pesticide Use Report database. Website accessed for database queries on several dates. Sacramento, CA: Department of Pesticide Regulation, California Environmental Protection Agency.  
<http://calpip.cdpr.ca.gov/cfdocs/calpip/prod/main.cfm>
- Dubey, R.K., Beg, M.U., and Singh, J., 1984. Effects of endosulfan and its metabolites on rat liver mitochondrial respiration and enzyme activities *in vitro*. *Biochem Pharmacol*, 33(21):3405-3410.
- Dubois, M., Pfohl-Leskowicz, A., DeWaziers, I., et al., 1996. Selective induction of the CYP3a family by endosulfan and DNA-adduct formation in different hepatic and hepatoma cells. *Environmental Toxicology and Pharmacology*, 1(4):249-256.
- Dureja, P. and Mukerjee, S.K., 1982. Photoinduced reactions: Part IV. Studies on the photochemical fate of 6,7,8,9,10,10-hexachloro-1,5,5a,6,9a -hexahydro-6,9-methano-2,4,3-benzo(e)dioxathiepin-3-oxide (endosulfan), an important insecticide. *Indian J. Chem.* 21B:411-413.
- Durham W.F. and Wolfe, H.R. 1962. Measurement of the exposure of workers to pesticides. *Bulletin of the WHO* 26:75-91.

- Dzwonkowska, A. and Hubner, H., 1986. Induction of chromosomal aberrations in the Syrian hamster by insecticides tested *in vivo*. Archives of Toxicology, 58:152-156.
- Ebert, E., Leist, K.H. and Kramer (Hoechst AG), 1985a. Testing for subchronic dermal toxicity (21 applications over 30 days) in Wistar rats (report of 2/22/85). DPR Vol. 182-030 #035081.
- Ebert, E., Weigand and Kramer (Hoechst AG), 1985b. Testing for subchronic dermal toxicity (21 applications over 30 days) in SPF Wistar rats (report of 3/11/85). DPR Vol. 182-029 #035800.
- Ebert, E. (Hoechst AG), 1987. Endosulfan - water-dispersible powder (50%) Subchronic dermal toxicity (21 treatments in 30 days) in the Wistar rat. DPR Vol. 182-062 #073681.
- Edmiston, S., Cowan, C., and Welsch, A., 1999. California Farm Worker Activity Profile: A database of farm worker activity demographics. Report no. HS-1751. Sacramento, CA: Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency. <http://www.cdpr.ca.gov/docs/whs/pdf/hs1751.pdf>.
- Edwards, J.A., Reid, Y.J., Offu, J.M., Almond, R.H. and Gibson, W.A., 1984. Effect of endosulfan-technical (Code: HOE 02671 0 I AT209) on reproductive function of multiple generations in the rat. DPR Vol. 182-022 #035789.
- El Beit, I.O.D., Wheelock, J.V., Cotton, D.E., 1981. Factors involved in the dynamics of pesticides in soils: The effect of pesticide concentration on leachability and adsorption. Int. J. Environ. Stud. 16:181-187.
- Elkins, D.R., 1989. Effect of commercial processing on pesticide residues in selected fruits and vegetables. J. Assoc. Off. Anal. Chem. 72(3):533-35.
- Elsa, J.R. (Hazleton Laboratories, U.S.A.), 1957. Acute oral, acute dermal and acute eye application. DPR Vol. 182-081 #126555.
- Elsa, J.R. (Hazleton Laboratories, USA), 1958. Acute oral toxicity in rats. DPR Vol. 182-042 #047295.
- Erdreich, L., Morimoto, L., 2007. Endosulfan critical review of epidemiological study “Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley.” Makhteshim-Agan of North America Inc., Raleigh, NC 27609. Report ID: AOTO 0807 LEO1; DPR volume/record #: 182-0128/235849.
- Ernst, W., 1977. Determination of the bioconcentration potential of marine organisms--a steady state approach. Chemosphere, 6:731.
- Exponent, 2004a. DEEM<sup>TM</sup>, Distributional Dietary Exposure Analysis Software, Version 7.87. Exponent, Inc., Washington, DC.
- Exponent, 2004b. DEEM-FCID<sup>TM</sup>, Distributional Acute Dietary Exposure Analysis Program,

Version 2.03. Exponent, Inc., Washington, DC.

- Fabacher, D.L., Kulkarni, A.P. and Hodgson, E., 1980. Pesticides as inducers of hepatic drug-metabolizing enzymes--I. Mixed function oxidase activity. *Gen. Pharmac.* 11:429-435.
- Fahrig, R., 1974. In: Nontesano, R. and Tomatis, L., eds. Comparative mutagenicity studies with pesticides. Proceedings of a workshop on approaches to assess the significance of experimental chemical carcinogenesis data for man, Brussels, Belgium, Dec. 10-12, 1973. Lyons, France, International Agency for Research on Cancer (XIV + pp. 230 Illus.) pp. 161-181 (Scientific Publications, No. 10, Chemical Carcinogenesis Essays).
- FDA, 1998. Pesticide Residue Surveillance Monitoring Program, FY1995-FY1997 (DPR requested search). Contaminants Division, U.S. Food and Drug Administration, Washington, D.C.
- Federal Register, 2005. Endosulfan: Notice of receipt of requests to voluntarily cancel uses of certain pesticide registrations. Meghan French, Special Review and Reregistration Division (7508C), Office of Pesticide Programs; USEPA. May 27, 2005 (Volume 70, #102).
- Fellers, G.M., McConnell, L.L., Pratt, D. and Datta, S. 2004. Pesticides in mountain yellow-legged frogs (*Rana muscosa*) from the Sierra Nevada Mountains of California, USA. *Environmental Toxicology and Chemistry* 23:2170-2177.
- Ferrando, M.D., Alacron, V., Fernandez-Casalderrey, A., et al., 1992. Persistence of some pesticides in the aquatic environment. *Bull Environ Contam Toxicol* 48(5):747-755.
- Ffrench-Constant, R.H., 1993. The molecular and population genetics of cyclodiene insecticide resistance. *Insect Biochem Mol Biol*, 24:335-345.
- Ffrench-Constant, R.H., Anthony, N., Aronstein, K., Rocheleau, T., Stilwell, G., 2000. Cyclodiene insecticide resistance: from molecular to population genetics. *Annu Rev Entomol* 48:449-466.
- Fleck, J., Ross, L., Tran, D., Melvin, J. and Fong, B. 1991. Off-target movement of endosulfan from artichoke fields in Monterey County. Report No. EH 91-05. Sacramento, CA: California Department of Pesticide Regulation, Environmental Monitoring and Pest Management Branch. Available at: <http://www.cdpr.ca.gov/docs/empm/pubs/ehapreps/eh9105.pdf>.
- Flodstrom, S., Warngard, L., Himming, H., Fransson, R. and Ahlborg, U.G., 1988. Tumour promotion related effects by the cyclodiene insecticide endosulfan studied in vitro and in vivo. *Pharmacology and Toxicology*, 62:230-235.
- FMC, 1983. Skin Irritation Study of Thiodan 50 WP in Rabbits. FMC Toxicology Laboratory, Somerville, NJ; Study #: A82-796, 3/10/83. DPR volume/record #: 182-016 042182.
- FMC, 1990. FMC Corporation, "Thiodan 3 EC Insecticide" and "Thiodan 50 WP Insecticide." In: MSDS Reference for Crop Protection Chemicals, pp. 622-625. Chemical and Pharmaceutical Press, a joint venture of John Wiley and Sons and Chemical and Pharmaceutical Publishing

Corp.

- Fox, R.D., Derksen, R.C. and Brasee, R.D. 1998. Airblast/Air-assisted application equipment and drift. In: Proceedings of the North American Conference on Pesticide Spray Drift Management. March 29-April 1, 1998. Holiday Inn By the Bay, Portland Maine. Sponsored by the Maine Board of Pesticide Control and the University of Maine Cooperative Extension Pest Management Office. Donna Buckley, Editor. [http://pmo.umext.maine.edu/drift/drift\\_proceedings.pdf](http://pmo.umext.maine.edu/drift/drift_proceedings.pdf)
- FQPA, 1996. Food Quality Protection Act of 1996. Public Law 104-170 (August 3, 1996).
- Frank, R., Ripley, B.D., Lampman, W., Morrow, D., Collins, H., Gammond, G.R. and McCubbin, P. 1994. Comparative spray drift studies of aerial and ground applications 1983-1985. Environ. Monit. Assess. 29:167-181.
- Fransson-Steen, R. and Warngard, L., 1992. Inhibitory effects of endosulfan on gap junctional intercellular communication in WB-F344 rat liver cells and primary rat hepatocytes. Carcinogenesis, 13(4): 657-662.
- Fransson-Steen, R., Flodstrom, S. and Warngard, L., 1992. The insecticide endosulfan and its two stereoisomers promote the growth of altered hepatic foci in rats. Carcinogenesis, 13(12):2299-2303.
- Freeman, C. 1989a. Acute oral toxicity study in rats. DPR Vol. 182-078 #124457.
- Freeman, C. 1989b. Acute dermal toxicity study in rabbits. DPR Vol. 182-078 #124458.
- Freeman, C. 1989c. Acute inhalation toxicity study in rats. DPR Vol. 182-078 #124459.
- Freeman, C. 1989d. Primary eye irritation study in rabbits. DPR Vol. 182-078 #124460.
- Freeman, C. 1989e. Primary skin irritation study in rabbits. DPR Vol. 182-078 #124461.
- Freeman, C. 1989f. Skin sensitization study in guinea pigs. DPR Vol. 182-078 #124463.
- Frost, K.R. and Ware, G.W. 1970. Pesticide drift from aerial and ground applications. Agricultural Engineering 51: 460-464.
- Fung, W.-P., 1981a. Final report. Range-finding study with FMC 5462 in pregnant rabbits. DPR Vol. 182-041 #045582.
- Fung, W.-P., 1981b. Range-finding study with FMC 5462 in pregnant rabbits (Supplier: Dutchland Laboratories, Inc.) Raltech study no. 79032. DPR Vol. 182-057 060604.
- Fung, W.-P., 1980a. Range-finding study with FMC 5462 in pregnant rats. DPR Vol. 182-057 #060605.

- Fung, W.-P., 1980b. Teratology study with FMC rats. DPR Volumes 182-026 and 057, #'s 017686 and 060606.
- Gaines, T.B., 1969. Acute toxicity of pesticides. *Toxicol. Appl. Pharmacol.*, 14:515-534.
- Gant, D.B., Eldefrawi, M.E. and Eldefrawi, A.T., 1987. Cyclodiene insecticides inhibit GABA receptor-regulated chloride transport. *Toxicol Appl Pharmacol* 88:313 - 321.
- Ganapathy, C., Nordmark, C., Bennett, K., Bradley, A., Feng, H., Hernandez, J. and White, J. 1997. Temporal Distribution of Insecticide Residues in Four California rivers. Report No. EH 97-06. Sacramento, CA: California Department of Pesticide Regulation, Environmental Monitoring and Pest Management Branch. Available at:  
<http://www.cdpr.ca.gov/docs/empm/pubs/eh976rep.pdf>.
- Garg, A., Kunwar, K., Das, N., and Gupta, P.K., 1980. Endosulfan intoxication: blood glucose electrolytes, Ca levels, ascorbic acid and glutathione in rats. *Toxicol. Lett.*, 5(2):119-123.
- Gilbert, M.E., 1992a. Proconvulsant activity of endosulfan in amygdala kindling. *Neurotox. and Teratology*, 14:143-149.
- Gilbert, M.E., 1992b. A characterization of chemical kindling with the pesticide endosulfan. *Neurotox. and Teratology*, 14:151-158.
- Gilbert, M.E., 1992c. Neurotoxicants and limbic kindling. In: *The Vulnerable Brain and Environmental Risks, Volume 1, Malnutrition and Hazard Assessment* (Isaacson, R.L., and Jensen, K.F., eds.), pp 173-192. Plenum Press, New York and London.
- Gilbert, M.E. and Mack, C.M., 1995. Seizure thresholds in kindled animals are reduced by the pesticides lindane and endosulfan. *Neurotox. and Teratology*, 17(2):143-150.
- Gildemeister, H. and Jordan, H.J., 1984. Aerobic Soil Metabolism Study of the Insecticide Hoe 002671 (Endosulfan). Hoechst Aktiengesellschaft. Study No. (B)176/84. DPR Vol. 182-046 #052984.
- Gildemeister, H., Stumpf, K., Scheinkönig, and Rockmann, S., 1988. Anerobic metabolism of endosulfan in a sandy loam and a silt loam soil. Hoechst Aktiengesellschaft. Laboratory Project ID: OE-134/04.03 E. DPR Vol. 182-058 #067070.
- Gilmore, R.G. , Sheets, L.P. and Hoss, H.E., 2006. A Developmental Neurotoxicity Study with Technical Grade Endosulfan in Wistar Rats. Bayer CropScience LP, Toxicology, Stilwell, KS; Report No. 201563; 9/26/06. DPR Volume/record #: 182-0122/228573.
- Goddard, G.V., McIntyre, D.C. and Leech, C.K., 1969. A permanent change in brain function resulting from daily electrical stimulation. *Exp. Neurol.* 25:295-330.
- Gonzalez, D., Ross, L.J., Segawa, R. and Fong, B. 1987. Variation of Endosulfan Residues in Water and Sediment Taken from the Moss Landing Drainage of Monterey County. Report EH 87-02.

- Sacramento, CA: Environmental Monitoring Branch, Department of Pesticide Regulation, California Environmental Protection Agency.  
<http://www.cdpr.ca.gov/docs/empm/pubs/ehapreps/eh8702.pdf>.
- Gorbach, S.G., Christ, O.E., Kellner, H., Kloss, G. and Borner, E., 1968. Metabolism of endosulfan in milk sheep. *J. Agr. Food Chem.* 16:950-953.
- Gorlitz, G., 1987. Adsorption/desorption in the system soil/water. DPR Vol. 182-058 #067069.
- Gowan Corporation, 1967. Gowan endosulfan 3EC residue data. Gowan Corporation. DPR Vol. 182-039-52120.
- Greve, P.A. and Wit, S.L., 1971. Endosulfan in the Rhine River. *Journal of Water Pollution Control Fed.* 43:2338 - 2348.
- Guerin, T.F. and Kennedy, I.R., 1992. Distribution and dissipation of endosulfan and related cyclodienes in sterile aqueous systems: Implications for studies on biodegradation. *J. Agric. Food Chem.* 40:2315-2323.
- Guo, F. and Spurlock, 2000. Recommendations for Priority Surface Water Monitoring Studies on Selected Pesticides. Memorandum dated August 2. Sacramento, CA: Environmental Monitoring Branch, Department of Pesticide Regulation, California Environmental Protection Agency. <http://www.cdpr.ca.gov/docs/empm/pubs/ehapreps/m080200.pdf>
- Gupta, P.K., 1976. Endosulfan-induced neurotoxicity in rats and mice. *Bull. Environ. Contamin. Toxicol.* 15(6):708-713.
- Gupta, P.K., 1978. Distribution of endosulfan in plasma and brain after repeated oral administration to rats. *Toxicology* 9:371-377.
- Gupta, P.K. and Chandra, S.V., 1975. The toxicity of endosulfan in rabbits. *Bull. Environ. Contam. Toxicol.* 14:513-519.
- Gupta, P.K. and Chandra, S.V., 1977. Toxicity of endosulfan after repeated oral administration to rats. *Bull. of Environ. Contamin. Toxicol.* 18(3):378-384.
- Gupta, P.K., Chandra, S.V. and Saxena, D.K., 1978. Teratogenic and embryotoxic effects of endosulfan in rats. Letter to the editor, *Acta. Pharmacol. et Toxicol.*, 42:150-152.
- Gupta, P.K. and Ehrnebo, M., 1979. Toxicokinetics of alpha- and beta- endosulfan (E) in rabbits [Abstract]. *Pharmacologist* 20:187.
- Gupta, P.K. and Gupta, R.C., 1977. Effect of endosulfan pretreatment on organ weights and on pentobarbital hypnosis in rats. *Toxicology* 8:283-288.
- Gupta, P.K., Murthy, R.C. and Chandra, S.V., 1981. Toxicity of endosulfan and manganese chloride: Cumulative toxicity rating. *Toxicology Letters*, 7:221-227, 1981.

- Gupta, P.K., Srivastava, S.C. and Ansari, R.A., 1981. Toxic effects of endosulfan on male reproductive organs in rats. *Indian J. Biochem. Biophys.* 18(4):159 (Suppl.).
- Hack, R., Ebert, E. and Leist, K.H., 1995. Chronic toxicity and carcinogenicity studies with the insecticide endosulfan in rats and mice. *Food Chem Toxicol* 33(11):941-950.
- Hacker, L.A., 1989. Endosulfan (Thiodan 3 EC) field dissipation study of terrestrial uses on tomatoes in Georgia/USA. DPR Vol. 182-071 #088318.
- Hahn, G.J. and Meeker, W.Q. 1991. *Statistical Intervals: A Guide for Practitioners*. New York, John Wiley & Sons, Inc.
- Hamilton, D., Ambrus, A., Dieterle, R., Felsot, A., Harris, C., Petersen, B., Racke, K., Wong, S., Gonzalez, R., Tanaka, K., Earl, M., Roberts, G., and Bhula, R., 2004. Pesticide residues in food B acute dietary exposure. *Pest Manage. Sci.*, 60:311-339.
- Hatzilazarou S.P., Charizopoulos, E.T., Papadopoulou-Mourkidou, E. and Economou, A.S. 2004. Dissipation of three organochlorine and four pyrethroid pesticides sprayed in a greenhouse environment during hydroponic cultivation of gerbera. *Pest Management Science* 60:1197-1204
- HCDB, 1986. *Hazardous chemical data book*. 2<sup>nd</sup> ed. Park Ridge, NJ. Noyes Data Corporation.
- Hincal, F., Gurbay, A., and Giray, B., 1995. Induction of lipid peroxidation and alteration of glutathione redox status by endosulfan. *Biological Trace Element Research*, 47:321-326.
- Hinstridge, P.A., 1968. Thiodan and thiodan sulphate (residues on fresh pineapple and pineapple bran). FMC Corporation. Study No. R-1097. DPR Vol. 182-019-042219.
- Hiremath, M.B. and Kaliwal, B.B. (2002). Effect of endosulfan on ovarian compensatory hypertrophy in hemicastrated albino mice. *Reproductive Toxicology* 16: 783 - 790.
- Hiremath, M.B. and Kaliwal, B.B. (2003). Evaluation of estrogenic activity and effect of endosulfan on biochemical constituents in ovariectomized (OVX) Swiss albino mice. *Bull. Environ. Contam. Toxicology*, 71:458-464.
- Hodapp, D.M. and Winterlin, W., 1989. Pesticide degradation in model soil evaporation beds. *Bull environ Contam Toxicol*, 43:36-44.
- Hoechst, 1987. Endosulfan - active ingredient technical (code, HOE 02671 OI ZD97 0003): 30-day feeding study in adult male Wistar rats. Hoechst Aktiengesellschaft, Frankfurt, Germany. Project #: 87.0129 [not available].
- Hoechst, 1990. Summary and evaluation of the toxicity data for endosulfan - substance technical. Hoechst Aktiengesellschaft, Frankfurt, Germany. Report #: 90.0848, [not available].

- Hollander, M. and Wolfe, D., 1973. *Non-Parametric Statistical Methods*. Wiley & Sons, New York.
- Hollander, H. and Weigand, W. (Hoechst AG), 1983. Testing for acute aerosol inhalation toxicity in male and female SPF Wistar rats. 4 hours - LC50. DPR Vol. 182- 089 #128477.
- Hollander, H. and Weigand, W. (Hoechst AG), 1984. Endosulfan B Active ingredient technical: Testing for subchronic inhalation toxicity (21 exposures in 29 days) in SPF Wistar Rats. Hoechst, AG, Germany; 8/15/84, Study #: 84.0103. DPR Vol. 182- 084 #126577.
- HSDB, 1999. Hazardous substance data bank. National Library of Medicine, Bethesda, MD.
- Hughes, P.C.R. and Tanner, J.M., 1970a. A longitudinal study of the measurement and rates of growth for skull, limbs, pelvis, nose-rump and tail lengths. *J. Anat.* 106:349-70.
- Hughes, P.C.R. and Tanner, J.M., 1970b. The assessment of skeletal maturity in the growing rat. *J. Anat.* 106:371-402.
- Indraningsih, McSweeney, C.S. and Ladds, P.W., 1993. Residues of endosulfan in the tissues of lactating goats. *Aust Vet J*, 70(2):59-62.
- Industria Prodotti Chimici, 1975. Primary skin irritation study in rabbits: endosulfan technical. Final report. Hazleton Laboratories America, Inc., Vienna, VA. Project #: 915-111.
- Intox Laboratories, 1985a. Acute oral toxicity study in rats of endosulfan 50WP. Microflo Company Study No. LAN-AT-010. DPR Vol. 182-050 064469.
- Intox Laboratories, 1985b. Acute dermal toxicity study in rabbits of endosulfan 50WP. Microflo Company Study No. LAN-AT-008. DPR Vol. 182-050 064467.
- Intox Laboratories, 1985c. Primary eye irritation study of endosulfan 50WP. Microflo Company Study No. LAN-AT-003. DPR Vol. 182-050 064465.
- Intox Laboratories, 1985d. Primary dermal irritation study of endosulfan 50WP. Microflo Company Study No. LAN-AT-004. DPR Vol. 182-050 064464.
- Intox Laboratories, 1985e. Guinea Pig Maximization Test Using Endosulfan 50 WP. Microflo Company Study No. LAN-AT-009. DPR Vol. 182-050 064466.
- Jamil, K., Shaik, A.P., Mahboob, M., and Krisna, D., 2004. Effect of organophosphorus and organochlorine pesticides (monochrotophos, chlorpyriphos, dimethoate, and endosulfan) on human lymphocytes in vitro. *Drug and Chemical Toxicology*, 27(2):133-144.
- Je, K.H., Kim, K.N., et al., 2005. TERT mRNA expression is up-regulated in MCF-7 cells and a mouse mammary culture (MMOC) system by endosulfan treatment. *Arch Pharm Research*, 28(3):351-357.
- Jung, R. and Weigand, W. (Hoechst AG), 1983. Testing for sensitizing properties in female

Pirbright-White guinea pigs according to the method of BUEHLER. DPR Vol. 182-089 #128478.

- Kalender, Y., Kalender, S., et al., 2004. Effects of endosulfan on B cells of Langerhans islets in rat pancreas. *Toxicology*, 200(2-3):205-11.
- Kamijima, M. and Casida, J.E., 2000. Regional modification of [<sup>3</sup>H]ethynylbicycloorthobenzoate binding in mouse brain GABA<sub>A</sub> receptor by endosulfan, fipronil and avermectin B<sub>1a</sub>. *Toxicology and Applied Pharmacology*, 163:188-194.
- Kannan, K., Ballabh, D., Shanker, R., and Bhatia, B., 1982. Effect of endosulfan on oxygen consumption of leukocytes. *Int. J. Immunopharmacology*, 4(abstract):322.
- Kannan, K., Holcombe, R.F., Jain, S.K., Alvarez-Hernandez, X., Chervenak, R., Wolf, R.E., and Glass, J., 2000. Evidence for the induction of apoptosis by endosulfan in a human T-cell leukemic line. *Molecular and Cellular Biochemistry*, 205:53-66.
- Kenne, K., Fransson-Steen, R., Honkasalo, S, et al., 1994. Two inhibitors of gap junctional intercellular communication, TPA and endosulfan: Different effects on phosphorylation of connexin 43 in the rat liver epithelial cell line, IAR 20. *Carcinogenesis* 15(6):1161-1165.
- Khanna, R.N., Misra, D., Anand, M. and Sharna, H.K., 1979. Distribution of endosulfan in cat brain. *Bull. Environ. Contam. Toxicol.* 22:72-79.
- Khan, P.K. and Sinha, S.P., 1996. Ameliorating effect of vitamin C on murine sperm toxicity induced by 3 pesticides (endosulfan, phosphamidon and mancozeb). *Mutagenesis* 11(1):33-36.
- Klonne, D.R., Fuller, R. and Howell, C. 2000. Determination of Dermal and Inhalation Exposure to Reentry Workers During Harvesting in Nursery Stock. Unpublished study submitted by the Agricultural Reentry Task Force. ARTF Study ARF044. DPR Data Volume 50366-176, Record No. 181586.
- Kojima, H., Katsura, E., Takeuchi, S., Niiyama, K., and Kobayashi, K., 2004. Screening for estrogen and androgen receptor activities in 200 pesticides by *in Vitro* reporter gene assays using Chinese Hamster ovary cells. *Environmental Health Perspectives*, 112(5):524 B 531
- Kovalkovicova, N., Sutiakova, I., et al., 2001. Chromosomal aberrations induced by the insecticide endosulfan in sheep peripheral lymphocytes in vitro. *Acta Veterinaria (Belgrade)*, 51(5-6):365-372.
- Kurinnyi, A.I., Pilinskaya, M.A., German, I.V. and L=vova, T.S., 1982. Implementation of a program of cytogenetic study of pesticides: *Tsitologiya i Genetika*, 16(1):45-49.
- Kushwah, H.S. and Dikshith, T.S.S., 1981. In vivo and in vitro response to endosulphan vis-a-vis cholinergic phenomenon. *Indian J. Biochem. Biophys.* 18:152 (Suppl.).
- Lachman, G. 1987. Dermal Absorption of <sup>14</sup>C-Endosulfan in Rhesus Monkeys. Unpublished study

submitted by Hoechst Celanese Corporation, Lab Project No. BIEV-V-66.697. DPR Data Volume 182-060, Record No. 73678.

- Lakshmana, M.K. and Raju, T.R., 1994. Endosulfan induces small but significant changes in the levels of noradrenaline, dopamine and serotonin in the developing rat brain and deficits in the operant learning performance. *Toxicology* 91(2):139-150.
- Lawrence, L.J. and Casida, J.E., 1984. Interactions of lindane, toxaphene and cyclodienes with brain specific t-butylcyclophosphorothionate receptor. *Life Sci.* 35:171-178.
- Layton, D.W. 1993. Metabolically consistent breathing rates for use in dose assessments. *Health Physics* 64:23-36.
- Lee, S., McLaughlin, R., Harnly, M., Gunier, R. and Kreuzer, R. 2002. Community exposure to airborne agricultural pesticides in California: ranking of inhalation risks. *Environmental Health Perspectives* 110:1175-1184.
- Lee, H-K., Moon, J-K., Chang, C-H., Choi, H., Park, H-W., Park, B-S., Lee, H-S., Hwang, E-C., Lee-Y-D, Liu, K-H., and Kim, J-H., 2006. Stereoselective metabolism of endosulfan by human liver microsomes and human cytochrome P450 isoforms. *Drug Metabolism and Disposition*, 34(7):1090-1095.
- Leist, K.H. and Kramer, M. (Hoechst AG), 1985. 30-day feeding study in adult male Wistar rats. DPR Vol. 182-042 #047296.
- Leung, A.M., McDonough, D.M. and West, C.D., 1998. Determination of endosulfans in soil/sediment samples from Point Mugu, Oxnard, CA, using capillary gas chromatography/mass selective detection (GC/MSD). *Environmental Monitoring and Assessment*, 50(1):85 - 94.
- Lightowler, J.E. (Life Science Research), 1978. Thionex 35: Acute Oral Toxicity Study in Rats. DPR Vol. 182-038 #052130.
- Lightowler, J.E. and Gardner, J.R. (Life Science Research), 1978. Thionex 35: Acute Percutaneous Toxicity in Rats. DPR Vol. 182-038 #052131.
- Lonsway, J.A., Byers, M.E., Dowla, H.A., Panemangalore, M. and Atonious, G.F., 1997. Dermal and respiratory exposure of mixers/sprayers to acephate, methamidophos, and endosulfan during tobacco production. *Bulletin of Environmental Contamination and Toxicology* 59:179-186.
- Lu, Y., Morimoto, K., Takeshita, T., Takeuchi, T., and Saito, T., 2000. Genotoxic effects of  $\alpha$ -endosulfan and  $\beta$ -endosulfan on human HeoG2 cells. *Environmental Health Perspectives*, 108(6):559-561.
- L'vova, T.S., 1984. Study of the Mutagenic effect of Five Promising Pesticides on Mouse Bone Marrow, Cultured Human Peripheral Blood Lymphocytes, and Saccharomycetes. *Tsitologiya i Genetika* 18(6):455-457.

- MacCollom, G.B., Johnston, D.B. and Parker, B.L., 1968. Determination and measurement of dust particles in atmospheres adjacent to orchards. *Bulletin of Environmental Contamination and Toxicology* 3:368-374.
- Maier-Bode, H., 1968. Properties, effect, residues and analytics of the insecticide endosulfan. *Residue Rev.* 22:1-44.
- Malloy, A., 1959. One year repeated oral administration of Thiodan technical in dogs. Hazleton Laboratories NCT 252.33. DPR Vol. 182-041 #045581.
- Martens, R., 1976. Degradation of (8,9-14C)endosulfan by microorganisms. *Appl. Environ. Microbiol.* 31:853-858.
- Martens, R., 1977. Degradation of endosulfan (-8,9-14C) in soils under different conditions. *Bull. Environ. Contam. Toxicol.* 17:438-446.
- Martins, J.D., Monteiro, J.P., Antunes-Madeira, M.C., Jurado, A.S. and Madeira, V.M.C., 2003. Use of the microorganism *Bacillus stearothermophilus* as a model to evaluate toxicity of the lipophilic environmental pollutant endosulfan. *Toxicology in Vitro*, 17:595-601.
- McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C. and Caspary, W.J., 1988. Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: 72 coded chemicals. *Environmental and Molecular Mutagenesis* 12:85-154.
- Menzie, C.M., 1969. Metabolism of pesticides. U.S. Department of the Interior, Bureau of Sport Fisheries and Wildlife. Washington, DC: U.S. Government Printing Office. Publication 127, 197.
- Menzie, 1978. Metabolism of pesticides, update II. Washington, D.C.: U.S. Department of the Interior, Fish and Wildlife Service. Special Scientific Report - Wildlife no. 212, pg 133.
- Meister, R.T. and Sine, C., 2006. In: Meisterpro crop protection handbook, Meister and Sine, eds. Volume 92, page D169. Meister Publishing Company, Willoughby, OH
- Mellano, D. (Istituto di Richerche Biomediche), 184. Study of the mutagenic activity “in vitro” of the compound endosulfan B technical. DPR Vol. 182-025 #035796.
- Miles, J.R.W. and Harris, C.R., 1978. Insecticide residues in organic soils of sex vegetable growing areas in southwestern Ontario, 1976. *J. Environ. Sci. Health B.* 13:199.
- Miles, J.R.W. and Moy, P., 1979. Degradation of endosulfan and its metabolites by a mixed culture of soil microorganisms. *Bull. Environ. Contam. Toxicol.* 23:13-19.
- Milone, M.F. and Hirsch, I.E. (Istituto di Ricerche Biomediche), 1984. Study of the mutagenic activity of the compound endosulfan - technical (Code HOE 002671 01 ZD97 0003) with *Saccaromyces cervisiae*. DPR Vol. 182-042 #047310.

- Milone, M.F. and Hirsch, I.E. (Istituto di Ricerche Biomediche), 1986. Endosulfan (technical): Chromosome aberration in human lymphocytes cultured "in vitro." DPR Vol. 182-042 048638.
- Moriya, M., Ohta, T., Watanabe, K., Miyazawa, T., Kato, K. and Shirasu, Y., 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutation Research*, 116:185-216.
- Murono, E.P., Derk, R.C., de Leon, J.H., 2001. Differential effects of octylphenol, 17 $\beta$  -estradiol, endosulfan, or bisphenol A on the steroidogenic competence of cultured adult rat Leydig cells. *Reproductive Toxicology*, 15:551-560.
- Musil, L.S., et al., 1990. Differential phosphorylation of the gap junction protein connexin 43 in junctional communication-competent and deficient cell lines. *J Cell Biol*, 111:2077-2088.
- NAAA. 2004. Pesticide Use Survey Report for Agricultural Aviation: A Study Conducted by the National Agricultural Aviation Association (NAAA). Unpublished report dated May 2004.
- Naqvi, S.M. and Vaishnavi, C., 1993. Mini Review. Bioaccumulative potential and toxicity of endosulfan insecticide to non-target animals; *Comp Biochem Physiol C*, 105(3):347-361.
- Narayan, S., Dani, H.M. and Misra, U.K., 1984. Effect of intratracheally administered DDT and endosulfan on pulmonary and hepatic respiratory cytochromes. *Bull. Environ. Contam. Toxicol.*, 33:193-199.
- Narayan, S., Dani, H.M. and Misra, U.K., 1990a. Changes in lipid profiles of liver microsomes of rats following intratracheal administration of DDT of endosulfan. *B25(2):243-257*.
- Narayan, S., Dani, H.M. and Misra, U.K., 1990b. Lung subcellular fractions and surfactant lipid metabolism of rats exposed with DDT or endosulfan intratracheally. *B25(2):259-272*.
- Nath, G. and Dikshith, T.S.S., 1979. Endosulfan residues in rat tissues. *Natl. Acad. Sci. Lett.* 2:278-279.
- Naqvi, S.M. and Vaishnavi, C., 1993. Bioaccumulative potential and toxicity of endosulfan insecticide to non-target animals. *Comp Biochem Physiol; C105(3):347-361*.
- Nielsen, J.B. and Andersen, H.R. 2001. Dermal in vitro penetration of methiocarb, paclobutrazol, and pirimicarb: Effect of nonylphenoethoxylate and protective gloves. *Environmental Health Perspectives* 109:129-132.
- Nicholson, S.S. and Cooper, G.W., 1977. Apparent endosulfan toxicosis in calves [clinical item]. *J. Am. Vet. Med. Assoc.* 130:319.
- Nogami, K., 1970. Acute oral toxicity in dogs. DPR Vol. 182-087 #128475.

- Novak, B., and Ahmad, N., 1989. Residues in fish exposed to sublethal doses of endosulfan and fish collected from cotton growing area. *J. Environ Sci Health B24:97-109.*
- Novigen, 2001. DEEM™, Distributional Dietary Exposure Analysis Software, Version 7.76. Novigen Sciences, Inc., Washington, D.C.
- NRC, 1993. 1993 National Academy of Sciences (NAS) report on Pesticides in the Diets of Infants and Children, by the National Research Council, National Academy Press, publishers, Washington, D.C.
- NRCC, 1975. Endosulfan: Its effects on environmental quality. Ottawa, Ontario: National Research Council Canada, Environmental Secretariat, Publication #: NRCC 14098.
- NTP, 1988. National Toxicology Program Fiscal Year 1988 Annual Plan. Department of Health and Human Services, U.S. Public Health Service, pp. 51, 59, 87, 88.
- Nye, D., 1981. Teratology study with FMC 5462 in rabbits. DPR Volumes 027 and 057, #'s 035798 and 060607.
- OEHHA. 2000. Air Toxics Hot Spots Program Part IV: Technical support document. Exposure assessment and stochastic analysis. Scientific Review Panel Draft. Sacramento, CA: Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. [http://www.oehha.ca.gov/air/hot\\_spots/finalStoc.html#download](http://www.oehha.ca.gov/air/hot_spots/finalStoc.html#download)
- Okumura, D.Y. 1992. Development of Endosulfan Regulations. Letter No. ENF 92-10 to County Agricultural Commissioners from Douglas Y. Okumura, Chief, Pesticide Enforcement Branch, DPR, dated January 31. Sacramento, CA: Department of Pesticide Regulation, California Environmental Protection Agency.
- Olea, N., Barba, A., Lardelli, P., Rivas, A., Olea-Serrano, M.F., 1999. Inadvertent exposure to xenoestrogens in children. *Toxicology and Industrial Health*, 15:151-158.
- Ozoe, Y. and Matsumura, F., 1986. Structural requirements for bridged bicyclic compounds acting on picrotoxinin receptor. *J Ag Food Chem*, 34:126 - 134.
- Paul, V., Balasubramaniam, E., Muthu, P. and Krishnamoorthy, M.S., 1992. Evidence for a hazardous interaction between ethanol and the insecticide endosulfan in rats. *Pharmacology and Toxicology*, 70:268-270.
- Paul, V. and Balasubramaniam, E., 1997. Effects of single and repeated administration of endosulfan on behaviour and its interaction with centrally acting drugs in experimental animals: a mini review. *Environmental Tox and Pharm*, 3:151-157.
- Paul, V., Balasubramaniam, E., Jayakumar, A.R., and Kazi, M., 1995. A sex-related difference in the neurobehavioral and hepatic effects following chronic endosulfan treatment in rats. *Eur J Pharmacol Env Toxicol Pharmacol Sect* 293, 355.

- Paul, V., Balasubramaniam, E. and Kazi, M., 1994a. Alteration in the tranquillizing potency of chlorpromazine in rats exposed chronically to the insecticide, endosulfan. *Bull Environ Contam Toxicol*, 53:665.
- Paul, V., Balasubramaniam, E. and Kazi, M., 1994b. The neuro-behavioral toxicity of endosulfan in rats: a serotonergic involvement in learning impairment. *Eur J Pharmacol Environ Toxicol Pharmacol*, Section 270,1.
- Pednekar, M.D., Gandhi, S.R. and Netrawali, M.S., 1987. Evaluation of mutagenic activities of endosulfan, phosalone, malathion and permethrin, before and after metabolic activation in the Ames *Salmonella* test. *Bull. Environ. Contam. Toxicol.* 38:925-933.
- Peterson, S.M. and Batley, G.E. 1993. The fate of endosulfan in aquatic ecosystems. *Environmental Pollution* 82:143-152.
- PHED, 1995. The Pesticide Handlers Exposure Database, Version 1.1. Prepared for the PHED Task Force representing Health and Welfare Canada, U.S. Environmental Protection Agency, and the National Agricultural Chemicals Association; prepared by Versar, Inc., 6850 Versar Center, Springfield, VA 22151.
- PISP (Pesticide Illness Surveillance Program), 2004. PSIP 2002: Summary of cases by pesticide and by type of illness. Illnesses and injuries reported by California physicians associated with pesticide exposure summarized by pesticide(s) and type of illness 2002. California Department of Pesticide Regulation, Pesticide Illness Surveillance Program, page 1.
- Poda, G.I., Landsittel, D.P., Brumbaugh, K., Sharp, D.S., Frasch, H.F. and Demchuk, E. 2001. Random sampling or 'random' model in skin flux measurements? [Commentary on "Investigation of the mechanism of flux across human skin in vitro by quantitative structure-permeability relationships"]. *European Journal of Pharmaceutical Sciences* 14:197-200.
- Pomes, A., Rodriguez-Farre, E. and Sunol, C., 1994. Disruption of GABA-dependent chloride flux by cyclodienes and hexachlorocyclohexanes in primary cultures of cortical neurons. *J Pharmacol Exp Ther* 271(3):1616-1623.
- Powell, 2002. Approximating confidence limits for upper bound and mean exposure estimates from the Pesticide Handlers Exposure Database (PHED V1.1). Memo #: HSM-02037, dated September 27. Sacramento, CA: Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency.
- Powell, S., 2003. Why Worker Health and Safety Branch Uses Arithmetic Means in Exposure Assessment. HSM-03022, dated September 22. Sacramento, CA: Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency.
- Powers, M.B., Voelker, R.W., Olson, W.A. and Weatherholtz, W.M., 1978. Bioassay of endosulfan for possible carcinogenicity. DPR Vol. 182-007 #921663.
- Proposition 65, 1986. Safe Drinking Water and Toxic Enforcement Act of 1986. California.

- Quinto, I., Martire, G., Vricella, G., Riccardi, F., Perfumo, A., Giulivo, R. and De Lorenzo, F., 1981. Screening of 24 pesticides by *Salmonella*/microsome assay: mutagenicity of benazolin, metoxuron and paraoxon. *Mutation Research*, 85:265.
- Raizada, R.B., Datta, K.K. and Dikshith, T.S.S., 1981. Effect of endosulphan on reproductive organs in ovariectomized albino rats. *Indian J. Biochem. Biophys.* 18:158 (Suppl.).
- Ramaneswari, K. and Rao, L.M., 2000. Bioconcentration of endosulfan and monocrotophos by *Labeo rohita* and *Channa punctata*, *Environmental Contamination and Toxicology*, 65:618-622.
- Rao, D.M.R. and Murty, A.S., 1980. Persistence of endosulfan in soils. *J. Agric. Food Chem.* 28:1099-1101.
- Reinert, J.C., Nielsen, A.P., Lunchick, C., Hernandez, O. and Mazzetta, D.M. 1986. The United States Environmental Protection Agency's guidelines for applicator exposure monitoring. *Toxicology Letters* 33:183-191.
- Reisch, D., Rossi, S.S., Mearns, A.J., Oshida, P.S., and Wildes, F.G., 1979. Marine and estuarine pollution. *Journal WPCF*, 51(6):1477 - 1516.
- Rice, C.P., Chernyak, S.M., Hapeman, C.J., et al., 1997a. Air-water distribution of the endosulfan isomers. *Journal of Environmental Quality*, 26(4):1101-1106.
- Rice, C.P., Hapeman, C.J., Chernyak, S.M., 1997b. Experimental evidence for the interconversion of endosulfan isomers. 213<sup>th</sup> National Meeting of the American Chemical Society, San Francisco, CA, USA, April 13B17, 1997; Abstract of Papers American Chemical Society 213 (1-3).
- Richards, S.M., McClure, G.Y., Lavy, T.L., Mattice, J.D., Keller, R.J. and Gandy, J. 2001. Propanil (3,4-dichloropropionanilide) particulate concentrations within and near the residences of families living adjacent to aerially sprayed rice fields. *Archives of Environmental and Contamination Toxicology* 41:112-116.
- Richardson, E.M. and Epstein, E., 1971. Retention of three insecticides on different size soil particles suspended in water. *Soil Sci. Soc. Amer. Proc.* 35:884-887.
- Robacker, K.M., Kulkarni, A.P. and Hodgson, E., 1981. Pesticide induced changes in the mouse hepatic microsomal cytochrome P-450-dependent monooxygenase system and other systems. *J. Environ. Sci. Health B16(5):*529-545.
- Roberts, D., 1972. The assimilation and chronic effects of sublethal concentrations of endosulfan on condition and spawning in the common mussel *Mytilus edulis*. *Marine Biology* 16:119-125.
- Roberts, E.M., English, P.B., Grether, J.K., Windham, G.C., Somberg, L., and Wolff, C., 2007. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. *Environmental Health Perspectives*,

115(10):1482-1489.

- Roberts, N.L. and Phillips, C.N.K., 1983. Acute delayed neurotoxicity study with endosulfan technical (Code: HOE 002671 0I Z97 0003) in the domestic hen. DPR Vol. 028 #035799.
- Rosa, R., Rodriguez-Farré and Sanfeliu, C., 1996. Cytotoxicity of hexachlorocyclohexane isomers and cyclodienes in primary cultures of cerebellar granule cells. *The Journal of Pharmacology and Experimental Therapeutics*, 278:163-169.
- Rosenfeld, G., 1985. Acute inhalation toxicity study in rats. Microflo Company Study No. 1237C. DPR Vol. 182-050 064468.
- Ross, L.J., Stein, R., Hsu, J., White, J. and Hefner, K. 1996. Distribution and Mass Loading of Insecticides in the San Joaquin River, California: Winter 1991-92 and 1992-93. Report No. EH 96-02. Sacramento, CA: Environmental Monitoring and Pest Management Branch, Department of Pesticide Regulation, California Environmental Protection Agency. <http://www.cdpr.ca.gov/docs/empm/pubs/ehapreps/eh9602.pdf>
- Ross, L.j., Stein, R., Hsu, J., White, J. and Hefner, K., 1999. Distribution and mass loading of insecticides in the San Joaquin River, California: Spring 1991 and 1992. Report No. EH 99-01. Environmental Monitoring and Pest Management Branch, Department of Pesticide Regulation, Sacramento, CA.
- Ross, L.J., Stein, R., Hsu, J., White, J. and Hefner, K. 2000. Insecticide Concentrations in the San Joaquin River, California: Summer 1991 and 1992. Report No. EH 00-09. Sacramento, CA: Environmental Monitoring and Pest Management Branch, Department of Pesticide Regulation, California Environmental Protection Agency. <http://www.cdpr.ca.gov/docs/empm/pubs/ehapreps/eh0009.pdf>
- Ruch, R.J., Fransson, R., Flodstrom, S., Warngard, L. and Klaunig, J.E., 1990. Inhibition of hepatocyte gap junctional intercellular communication by endosulfan, chlordane and heptachlor. *Carcinogenesis* 11(7):1097-1101.
- Ruckman, S.A., Waterson, L.A., Crook, D., Gopinath, C., Majeed, S.K. Anderson, A. and Chanter, D.O., 1989. Combined chronic toxicity/carcinogenicity study 104-week feeding in rats. DPR Vol. 182-066 #074851.
- Rupa, D.S., Reddy, P.P. and Reddi, O.S., 1989a. Chromosomal Aberrations in Peripheral Lymphocytes of Cotton Field Workers Exposed to Pesticides. *Environ Research* 49:1-6.
- Rupa, D.S., Reddy, P.P. and Reddi, O.S., 1989b. Frequencies of chromosomal aberrations in smokers exposed to pesticides in cotton fields. *Mutation Research* 222:37-41.
- Rutz, R. and Krieger, R.I. 1992. Exposure to pesticide mixer/loaders and applicators in California. *Reviews of Environmental Contamination and Toxicology* 129:121-139.
- Saiyed, H., Dewan, A., Bhatnagar, V., Shenoy, U., Shenoy, R., Rajmohan, H., Patel, K., Kashyap, R.,

- Kulkarni, P., Rajan, B., and Lakkad, B., 2003. Effect of endosulfan on male reproductive development. *Environ Health Perspectives*, 111(16):1958-1962.
- Sances, F.V., Toscano, N.C. and Gaston, L.K., 1992. Minimization of pesticide residues on head lettuce: Within-head residue distribution of selected insecticides. *Journal of Economic Entomology*, 85:202-207.
- Sarafin, R., 1979a. Hoe 052618 and Hoe 052619 ( $\alpha$ - and  $\beta$ -Endosulfan) solubility in water. Unpublished study submitted by FMC Corporation, Philadelphia, PA. Report #: (B)154/87. DPR Volume/record #: 182-056/063585.
- Sarafin, R. 1979b. Hoe 052618 and Hoe 052619 ( $\alpha$ - and  $\beta$ -Endosulfan) Partition Coefficient octanol/Water. Unpublished study submitted by FMC Corporation, Philadelphia, PA. Report No. (B)124/87. DPR Volume/record #: 182-056/063586.
- Sarafin, R. 1982. Hoe 002671 (Endosulfan), Hoe 052618 ( $\alpha$ -Endosulfan) and Hoe 052619 ( $\beta$ -Endosulfan) - Vapor Pressure. Unpublished study submitted by FMC Corporation, Philadelphia, PA. Report #: (B)153/87. DPR Volume/record #: 182-056/063587.
- Sava, R.J. 1985. Monterey County Residential Air Monitoring. Report No. EH 85-07. Sacramento, CA: Environmental Monitoring Branch, Department of Pesticide Regulation, California Environmental Protection Agency.  
<http://www.cdpr.ca.gov/docs/empm/pubs/ehapreps/eh8507.pdf>.
- Scholz, J. and Weigand, W. (Hoechst AG), 1971a. Acute oral toxicity to the male Sherman rat. DPR Vol. 182-086 #128472.
- Scholz, J. and Weigand, W. (Hoechst AG), 1971b. Acute oral toxicity to the female Sherman rat. DPR Vol. 182-086 #128473.
- Schimmel, S.C., Patrick, J.M., Jr. and Wilson, A.J., Jr., 1977. Acute toxicity to and bioconcentration of endosulfan by estuarine animals. In: *Aquatic toxicology and hazard evaluation*, ASTM STP 634 (Mayer, F.L. and Hamelink, J.L., eds.), pp, 241-252. American Society for Testing and Materials, Philadelphia, PA.
- Schuette, J., Weaver, D., Troiano, J., Pepple, M. and Dias, J. 2003. Sampling for Pesticide Residues in California Well Water: 2003 Well Inventory Database, Cumulative Report 1986-2003. Report No. EH03-08. Sacramento, CA: Environmental Monitoring Branch, Department of Pesticide Regulation, California Environmental Protection Agency.  
<http://www.cdpr.ca.gov/docs/empm/pubs/ehapreps/eh0308.pdf>
- SDTF. 1997. A Summary of Airblast Application Studies. Spray Drift Task Force (SDTF). Stewart Agricultural Research Services, Inc. Macon, MO.  
[http://www.agdrift.com/PDF\\_FILES/airblast.pdf](http://www.agdrift.com/PDF_FILES/airblast.pdf)
- Seshaiah, A., 1997a. Acute Oral Toxicity Study in Rat. Department of Toxicology, Jai Research Foundation, Gujarat, India; Project ID No. 1027/JRF/TOX/97; 5/1/97; DPR volume/record #:

182-114- 179768.

- Seshaiah, A., 1997b. Acute Inhalation Toxicity Study in Rat. Cosmopolitan Safety Evaluation, Inc., Lafayette, NJ; Study #S8687-9; 6/12/85; DPR volume/record #: 182-114- 179770.
- Seth, P.K., Saidi, N.F., Agrawal, A.K. and Anand, M., 1986. Neurotoxicity of endosulfan in young and adult rats. *Neurotoxicology* 7(2)623-635.
- Sharma, A.K. and Gautam, D.C., 1991. Chromosomal aberrations induced by phosphamidon and endosulfan in the bone marrow cells of mice in vivo. *Cytologia*, 56:71-78.
- Sheets, L.P., Gilmore, R.G., and Fickbohm, B.L., 2004. A Subchronic Neurotoxicity Screening Study with Technical Grade Endosulfan in Wistar Rats, Bayer CropScience LP, Toxicology, Stilwell, KS; Lab ID#: 02-N72MJ; Report #: 201069; Report ID#: B004881. DPR Volume/record #:182-0127/234702
- Shelby, M.D., Newbold, R.R., Tully, D.B., et al., 1996. Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. *Environ Health Perspectives* 104(12):1296-1300.
- Shirasu, Y., Moriya, M. and Ohta, T. (Hoechst Japan Limited--Institute of Environmental Toxicology, Japan), 1978. Microbial Mutagenicity Testing on Endosulfan. DPR Vol. 182-042 #047306.
- Shirasu, Y. Moriya, M., Tzuka, H., Teramoto, S., Ohta, T. and Inoue, T., 1982. Mutagenicity screening studies on pesticides. In: *Environmental Mutagens and Carcinogens*, Sugimura, T., Kondo, S. and Takebe, H. (editors), pp. 331-335.
- Siddiqui, M.K.J., Anjum, F. and Qadri, S.S.H., 1987. Some metabolic changes induced by endosulfan in hepatic and extra hepatic tissues of rat. *J. Environ. Sci. Health B22*:553-564.
- Siebers, J., Binner, R. and Wittich, K.P. 2003. Investigation on downwind short-range transport of pesticides after application in agricultural crops. *Chemosphere* 51:397-407.
- Singh, S.K. and Pandey, R.S., 1989a. Differential effects of chronic endosulfan exposure to male rats in relation to hepatic drug metabolism and androgen formation. *Indian J. of Exp. Biology*, 26:262-267.
- Singh, S.K. and Pandey, R.S., 1989b. Gonadal toxicity of short term chronic endosulfan exposure to male rats. *Indian J. Exp. Biology*, 27:341-346.
- Singh, S.K. and Pandey, R.S., 1989c. Toxicity of endosulfan on kidney of male rats in relation to drug metabolizing enzymes and microsomal lipid peroxidation. *Indian J. of Exp. Biology*, 27:725-728.
- Singh, S.K. and Pandey, R.S., 1990. Effect of sub-chronic endosulfan exposures on plasma gonadotrophins, testosterone, testicular testosterone and enzymes of androgen biosynthesis in rat. *Indian J. of Exp. Biology*, 28:953-956.

- Singh, S.K. and Pandey, R.S., 1991. Ethanol potentiates in vivo hepatotoxicity of endosulfan in adult male rats. *Indian Journal of Experimental Biology*, 29:1035-1038.
- Singh, N.C., Dasgupta, T.P., Roberts, E.V. and Mansingh, A., 1991. Dynamics of pesticides in tropical conditions. 1. Kinetic studies of volatilization, hydrolysis and photolysis of dieldrin and  $\alpha$ - and  $\beta$ -endosulfan. *J. Agric. Food Chem.*, 39:575-579.
- Singh, N.C., Roberts, E.V. and Dasgupta, T.P., 1984. Investigation on the dynamics of pesticide loss in tropical conditions: Dieldrin and endosulfan. Abstract No. 11, ACS Div. Pesticide Chem. 188th ACS National Meeting. Philadelphia, PA.
- Singh, N., Singh, C.P., Kumar, H. and Kaur Brar, G., 1992. Endosulfan poisoning: A study of 22 cases. *J. Assoc. Physicians India*, 40(2)87-88.
- Singhasemanon, N. 1995. Review of the Toxic Substances Monitoring Program=s 1993-1994 Preliminary Data Report. Memo dated October 16. Sacramento, CA: Environmental Monitoring and Pest Management Branch, Department of Pesticide Regulation, California Environmental Protection Agency.
- Singhasemanon, N. 1996. Review of the State Mussel Watch 1995 Survey Preliminary Data Report. Memo dated August 30. Sacramento, CA: Environmental Monitoring and Pest Management Branch, Department of Pesticide Regulation, California Environmental Protection Agency.
- Singhasemanon, N., 2003. U.S. Clean Water Act section 303(d) final list for registered pesticides 2002. Surface Water Protection Program Environmental Monitoring Branch DPR, March 26, 2003.
- Sinha, N., Narayan, R., Shanker, R., and Saxena, D.K., 1995. Endosulfan-induced biochemical changes in the testis of rats. *Vet Hum Toxicol* 37(6):547-549.
- Sinha, N., Narayan, R. and Saxena, D.K., 1997. Effect of endosulfan on the testis of growing rats. *Bull Environ Contam Toxicol* 58(1):79-86.
- Sinha, N., Adhikari, N., and Saxena, D.K., 2001a. Effect of endosulfan during fetal gonadal differentiation on spermatogenesis in rats. *Environmental Toxicology and Pharmacology*, 10:29-32.
- Sinha, N., Adhikari, N., and Saxena, D.K., 2001b. Effect of endosulfan on the enzymes of polyol pathway in rat Sertoli-germ cell coculture. *Bulletin of Environmental Contamination and Toxicology*, 67:821-827.
- Sittig, M., ed., 1980. Priority toxic pollutants: Health impacts and allowable limits. Noyes Data Corp., Park Ridge, NJ. pp. 208-213.
- Smith, L.D. 2005. Amended Report: Determination of Dermal and Inhalation Exposure to Workers During Application of a Liquid Pesticide Product by Open Cab Airblast Application to Orchard

- Crops. Study Number AHE07, dated August 23. Unpublished study submitted by Agricultural Handlers Exposure Task Force. DPR Data Volume 108-340, Record No. 219609.
- Sobti, R.C., Krishan, A. and Davies, J., 1983. Cytokinetic and cytogenetic effect of agricultural chemicals on human lymphoid cells in vitro. *Arch. Toxicol.* 52:221-231.
- Sohn, H.Y., Kwon, C.s., et al., 2004. Induction of oxidative stress by endosulfan and protective effect of lipid-soluble antioxidants against endosulfan-induced oxidative damage. *Toxicology Letters*, 151(2):357-365.
- Soto, A.M., Chung, K.L. and Sonnenschein, C., 1994. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. *Environmental Health Perspectives*, 102(4):380-383.
- Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N., and Serrano, F.O., 1995. The E-Screen assay as a tool to identify estrogens: an update on estrogen environmental pollutants. *Environmental Health Perspectives*, 103(7):113-122.
- Sparling, D.W., Fellers, G.M. and McConnell, L.L. 2001. Pesticides and amphibian population declines in California, USA. *Environmental Toxicology and Chemistry* 20:1591-1595.
- Srikanth, NS., Seth, P.K., Desai, D., 1989. Inhibition of calmodulin activated  $Ca^{+2}$ -ATPase by endosulfan in rat brain. *J. Toxicol. Environ. Health*, 28:473-481.
- Srivastava, A.K., Gupta, B.N., Mathur, A.K., Mathur, N., Mahendra, P.N. and Bharti, R.S., 1991. The clinical and biochemical study of pesticide sprayers. *Human & Experimental Toxicology*, 10:279-283.
- Staudinger, J. and Roberts, P.V. 2001. A critical compilation of Henry's law constant temperature dependence relations for organic compounds in dilute aqueous solutions. *Chemosphere* 44:561-576.
- Stewart, D.K.R. and Cairns, K.G., 1974. Endosulfan persistence in soil and uptake by potato tubers. *Journal Agric Food Chem*, 22:984 - 986. .
- Stumpf, K., 1987. Photodegradation of alpha-endosulfan (Hoe 052618) and beta-endosulfan (Hoe 052619) in water. DPR Vol. 182-058 #067071.
- Sundar, S.R., 1997. Dermal Sensitization Study-Guinea Pig Maximization Test. Department of Toxicology, Jai Research Foundation, Gujarat, India; Project ID#: 1030/JRF/TOX/97; 4/28/97. DPR volume/record #: 182-114 179773.
- Suntio, L.R., Shiu, W.Y., MacKay, D., et al., 1988. Critical review of Henry's Law constants for pesticides. *Rev. Environ. Contam. Tox.* 103:1-59.
- Sutherland, T.D., Horne, I., Weir, K.M., Russell, R.J., and Oakeshott, J.G., 2004. Toxicity and residues of endosulfan isomers. *Rev Environ Contam Toxicol*, 183:99-113.

- TAS, 1996a. EXPOSURE 4™, Detailed Distributional Dietary Exposure Analysis, Version 3.35. Technical Assessment Systems, Inc., Washington, D.C.
- TAS, 1996b. EXPOSURE 1™, Chronic Dietary Exposure Analysis, Version 3.35. Technical Assessment Systems, Inc., Washington, D.C.
- Thevenaz, Ph., Luetkemeier, H., Chevalier, H.J., Vogel, W., Terrier, Ch., 1988. Endosulfan – emulsifiable concentrate, Subchronic (4-week) repeated dose dermal toxicity study in rats. Unpublished study submitted by Hoechst Celanese Corporation, Somerville, NJ Lab Project ID No. 094590. DPR Vol. 182-061, Record No. 073680.
- Toledo, M.C.F. and Jonsson, C.M., 1992. Bioaccumulation and elimination of endosulfan in zebra fish (*Brachydanio rerio*). *Pesticide Science*, 36:207-211.
- Thongsinthusak, T., Ross, J., and Meinders, D., 1993. Guidance for the preparation of human pesticide exposure assessment documents. Report no. HS-1612. Sacramento, CA: Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency.
- Troiano, J., Weaver, D., Marade, J., Spurlock, F., Pepple, M., Nordmark, C. and Bartkowiak, D. 2001. Summary of well water sampling in California to detect pesticide residues resulting from nonpoint-source applications. *Journal of Environmental Quality* 30:448-459.
- TSCA, 1984. Health Effects Test Guidelines. Section 4A of the Toxic Substances Control Act.
- TSCA, 1985. Genetic Toxicity, 40 CFR, Part 798, Subpart F. Toxic Substances Control Act Test Guidelines, Final Rules.
- Tu, C.M., 1991. Effect of some technical and formulated insecticides on microbial activities in soil. *J. Environ. Sci. Health B26(5 & 6):557-573*.
- Turner, K.O., Syvanen, M. and Meizel, S., 1997. The human acrosome reaction is highly sensitive to inhibition by cyclodiene insecticides. *J Androl*, 18(6):571-575.
- Tyagi, S.R., Singh, Y., Srivastave, P.K., et al., 1984. Induction of hepatic mixed function oxidase system by endosulfan in rats. *Bull. Environ. Contam. Toxicol.* 32:550-556.
- University of California Cooperative Extension (UCCE). 2004. Available Cost and Return Studies. Department of Agricultural and Resource Economics. Website accessed for crop-specific information from September through November 2004.  
<http://www.agecon.ucdavis.edu/outreach/crop/cost.htm>
- USDA, 1989-92. Food and Nutrient Intake by Individuals in the United States, 1 Day, 1989-1992. Continuing Survey of Food Intakes by Individuals, 1989-1992. U.S. Department of Agriculture, Agricultural Research Service.

- USDA, 1994a. Agricultural Chemical Usage 1993 Fruits Summary. National Agricultural Statistics Service, U.S. Department of Agriculture, Washington, D.C. 146 pp.
- USDA, 1994b. Agricultural Statistics 1994. National Agricultural Statistics Service, U.S. Department of Agriculture, Washington, D.C. 485 pp.
- USDA, 1994c. Compound Evaluation and Residue Information 1994. Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C. 101 pp.
- USDA, 1996a. Agricultural Chemical Usage 1995 Fruits Summary. National Agricultural Statistics Service, U.S. Department of Agriculture, Washington, D.C. 119 pp.
- USDA, 1996b. Franks, W.I. and R.L. Epstein, eds. Pesticide Data Program (PDP) Annual Summary Calendar Year 1994. U.S. Department of Agriculture. Agricultural Marketing Service, Washington, D.C. 105 pp.
- USDA, 1996c. Vegetables Summary - Agricultural Chemical Usage 1994. National Agricultural Statistics Service, US Department of Agriculture, Washington, DC, 194 pp.
- USDA, 1994-98a. Food and Nutrient Intake by Individuals in the United States, 1994-1996 & 1998. Continuing Survey of Food Intakes by Individuals, 1994-1998. United States Department of Agriculture, Agricultural Research Service.
- USDA, 2001. Merrigan, K.A. and M. Lamont, eds. Pesticide Data Program (PDP) Annual Summary Calendar Year 1999. U.S. Department of Agriculture. Agricultural Marketing Service, Washington, D.C. 105 pp.
- USDA, 2003. Yates, A.J., ed. Pesticide Data Program (PDP) Annual Summary Calendar Year 2001, U.S. Department of Agriculture. Agricultural Marketing Service, Washington, D.C.
- USDA, 2004. Yates, A.J., ed. Pesticide Data Program (PDP) Annual Summary Calendar Year 2002, U.S. Department of Agriculture. Agricultural Marketing Service, Washington, D.C.
- USDA, 2005a. Agricultural Chemical Usage: 2004 Vegetables. U.S. Department of Agriculture. National Agricultural Statistical Service, Washington, D.C., 252 pp.
- USDA, 2005b. Clayton, K.C., ed., Pesticide Data Program (PDP) Annual Summary Calendar Year 2003, U.S. Department of Agriculture. Agricultural Marketing Service, Washington, D.C.
- USDA, 2006a. Agricultural Chemical Usage: 2005 Field Crops. U.S. Department of Agriculture. National Agricultural Statistical Service, Washington, D.C., 166 pp.
- USDA, 2006b. Agricultural Chemical Usage: 2005 Fruit. U.S. Department of Agriculture. National Agricultural Statistical Service, Washington, D.C., 295 pp.
- USDA, 2006c. Day, L.C., ed. Pesticide Data Program (PDP) Annual Summary Calendar Year 2004, U.S. Department of Agriculture. Agricultural Marketing Service, Washington, D.C.

- USEPA, 1980a. US Environmental Protection Agency Code of Fed. Regulations: 40 CFR 261.33 (e)
- USEPA, 1992. Dermal Exposure Assessment: Principles and Applications. EPA/600/8-91/011B. Washington, DC: Office of Health and Environmental Assessment, United States Environmental Protection Agency.
- USEPA, 1995. Designation, reportable quantities, and notification. List of hazardous substance and reportable quantities. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 302.4.
- USEPA, 1996. Superfund, emergency planning, and community right-to-know programs. Designation, reportable quantities, and notification. The list of extremely hazardous substances and their threshold planning quantities. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 335, Appendix A.
- USEPA, 1997a. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Food, Drug, and Cosmetic Act (FFDCA) as Amended by the Food Quality Protection Act (FQPA) of August 3, 1996. Document no. 730L97001, March 1997. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC.
- USEPA, 1997b. 1996 Food Quality Protection Act Implementation Plan. March, 1997. Office of Prevention, Pesticides and Toxic Substances (7506C), U.S. Environmental Protection Agency, Washington, DC. (<http://www.epa.gov/fedrgstr/>).
- USEPA, 1997c. Exposure Factors Handbook. EPA/600/P-95/002Fa. Washington, D.C.: Office of Research and Development, U.S. Environmental Protection Agency.
- USEPA, 1998a. Health Effects Test Guidelines, OPPTS 870.1000, Acute Toxicity Testing. EPA 712-C-98-189, Prevention, Pesticides and Toxic Substances (7101), United States Environmental Protection Agency.
- USEPA. 1998b. Health Effects Test Guidelines. Health Effects Test Guidelines: Dermal Penetration (OPPTS 870.7600). Washington, DC: Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency.  
[http://www.epa.gov/opptsfrs/publications/OPPTS\\_Harmonized/870\\_Health\\_Effects\\_Test\\_Guidelines/Series/870-7600.pdf](http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-7600.pdf)
- USEPA, 1999a. Code of Federal Regulations Title 40, section 180.182 (endosulfan). United States Government Printing Office, Washington, D.C. 723 pp.
- USEPA, 1999b. Guidance for Performing Aggregate Exposure and Risk Assessment. Item #6043; Environmental Protection Agency, Office of Pesticide Programs. November 28, 2001.
- USEPA, 2000a. Agricultural Transfer Coefficients, Policy Number 003.1 Science Advisory council for Exposure. Revised August 7.

- USEPA, 2000b. Toxicological Profile for Endosulfan. U.S. Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry.
- USEPA, 2001a. Code of Federal Regulations Title 40, section 180.182 (endosulfan). United States Government Printing Office, Washington, D.C., page 723.
- USEPA, 2001b. Endosulfan: HED risk assessment for the endosulfan re-registration eligibility decision (RED) document. Memo from D. Locke to R. Dumas dated January 31, 2001. U.S. Environmental Protection Agency, Washington, D.C.
- USEPA, 2001c. The food quality protection act (FQPA) background. Office of Pesticide Programs, U. S. Environmental Protection Agency, Washington, D.C. Available at <http://epa.gov/oppfead1/fqpa/backgrnd.htm>.
- USEPA, 2001d. Standard Values for Daily Acres Treated in Agriculture. Policy Number 009.1, Science Advisory Council for Exposure. Revised September 25.
- USEPA, 2001e. General Principles for Performing Aggregate Exposure and Risk Assessment. Item #6043; Environmental Protection Agency, Office of Pesticide Programs. November 28, 2001.
- USEPA, 2002a. Reregistration Eligibility Decision for Endosulfan. Case 0014. Washington, DC: Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. [http://www.epa.gov/oppsrrd1/REDs/endosulfan\\_red.pdf](http://www.epa.gov/oppsrrd1/REDs/endosulfan_red.pdf)
- USEPA, 2002b. Third Revision of “Occupational and Residential Exposure Assessment and Recommendations for the Reregistration Eligibility Decision Document for Endosulfan.” D-Barcode D281052, dated February 26. Washington, DC: Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. <http://www.epa.gov/oppsrrd1/reregistration/endosulfan/D281052.red.pdf>
- USEPA, 2003. User’s Manual: Swimmer Exposure Assessment Model (SWIMODEL) Version 3.0. Washington, DC: Office of Pesticide Programs, Antimicrobials Division, U.S. Environmental Protection Agency. <http://www.epa.gov/oppad001/swimodelusersguide.pdf>
- USEPA, 2004. Risk Assessment Guidance for Superfund (RAGS), Volume 1: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment). July 2004. <http://www.epa.gov/oswer/riskassessment/ragse/pdf/chapter3.pdf>
- USEPA, 2005. Code of Federal Regulations Title 40, section 180.182 (endosulfan). United States Government Printing Office, Washington, DC, 634 pp.
- USEPA, 2006a. Endosulfan, fenarimol, imazalil, oryzalin, sodium acifluorfen, trifluralin, and ziram; proposed tolerance actions (proposed rule). United States Environmental Protection Agency, Washington, DC. Proposed rules in: Federal Register 71(80):24615 - 24627.
- USEPA, 2006b. Endosulfan, fenarimol, imazalil, oryzalin, sodium acifluorfen, trifluralin, and ziram;

- proposed tolerance actions (Final rule). United States Environmental Protection Agency, Washington, DC. Final rule in: Federal Register 71(179):54423-54434.
- Usha Rani, M.V. and Reddy, P.P., 1986. Cytogenetic effects of aldrin and endosulfan in mice. *IRSC Med. Sci.* 14:1125-1126.
- Usha Rani, M.V., Reddi, O.S. and Reddy, P.P., 1980. Mutagenicity studies involving aldrin, endosulfan, dimethoate, phosphamidon, carbaryl and cerasan. *Bull. Environm. Contam. Toxicol.* 25:277-282.
- Utklev, H.E. and Westbye, C., 1971. Endosulfan poisoning. *Nor. Veterinaertidsskr.* 83:31.
- van der Merwe, D. and Riviere, J.E. 2005. Effect of vehicles and sodium lauryl sulphate on xenobiotic permeability and stratum corneum partitioning in porcine skin. *Toxicology* 206:325-335.
- Vanparys, C., Maras, M., Lenjou, M., Robbens, J., Van Bockstaele, D., Blust, R., and De Coen, W., 2006. Flow cytometric cell cycle analysis allows for rapid screening of estrogenicity in MCF-7 breast cancer cells. *Toxicology in Vitro*, 20:1238-1248.
- Vegetable Research and Information Center (VRIC). 2004. Division of Agriculture and Natural Resources, University of California. Website accessed for crop-specific information from September through November 2004. <http://vric.ucdavis.edu>.
- Velazquez, A., Creus, A., Xamena, N. And Marcos, R., 1984. Mutagenicity of the insecticide endosulfan in *Drosophila Melanogaster*. *Mutation Research*, 136:115-118.
- Verder-Carlos, M., 2001. Occupational and residential exposure and risk assessment and recommendations for the reregistration eligibility decision document for endosulfan. Second Revision, dated January 2. Washington, DC: Health Effects Division, U.S. Environmental Protection Agency.
- Versar. 1992. PHED: The Pesticide Handlers Exposure Database reference manual. Prepared for the PHED Task Force: Health and Welfare Canada, U.S. Environmental Protection Agency, National Agricultural Chemicals Association. Springfield, VA: Versar, Inc.
- Vidal, J.L.M., Arrebola, F.J., Fernandez-Gutierrez, A. and Rams, M.A., 1998. Determination of endosulfan and its metabolites in human urine using gas chromatography - tandem mass spectrometry. *J. Chromatography*, B719:71-78.
- Wade, G.M., Desaulniers, D., Leingartner, K. and Foster, W.G., 1997. Interactions between endosulfan and dieldrin on estrogen-mediated processes in vitro and in vivo. *Reproductive Toxicology*, 11(6):791-798.
- Ware, G.W., 1994. Insecticides. In: *The Pesticide Book* (Chapter 4, pp. 44-46), Thomson Publications, Fresno, CA

- Ware, G.W., 1967. Studies of pesticide residues on alfalfa using C<sup>14</sup>-labeled endosulfan. Wooster OH: Ohio Agricultural Research & Development Center. Res Circular 151.
- Ware G.W., Morgan, D.P., Estes, B.J., Cahill, W.P. and Whitacre, D.M. 1973. Establishment of reentry intervals for organophosphate-treated cotton fields based on human data: I. Ethyl- and methyl parathion. Archives of Environmental Contamination and Toxicology 1:48-59.
- Ware G.W., Morgan, D.P., Estes, B.J. and Cahill, W.P. 1974. Establishment of reentry intervals for organophosphate-treated cotton fields based on human data: II. Azodrin, ethyl and methyl parathion. Archives of Environmental Contamination and Toxicology 2:117-129.
- Ware G.W., Morgan D.P., Estes B.J. and Cahill, W.P. 1975. Establishment of reentry intervals for organophosphate-treated cotton fields based on human data: III. 12 to 72 hours post-treatment exposure to monocrotophos, ethyl- and methyl parathion. Archives of Environmental Contamination and Toxicology 3:289-306.
- Weston, D.P., You, J. and Lydy, M.J. 2004. Distribution and toxicity of sediment-associated pesticides in agriculture-dominated water bodies of California's Central Valley. Environmental Science and Technology 38:2752-2759.
- Wester, R.C. and Maibach, H.I. 2000. Understanding percutaneous absorption for occupational health and safety. International J. of Occupational and Environmental Health 6:86-92.
- White-Stevens, R., ed. 1971. Pesticides in the environment. Vol. 1, Part 2, pp. 89, 140, 214-216. Marcel Dekker, Inc., New York, NY.
- WHO, 1984. Endosulfan. International Programme on Chemical Safety. Environmental Health Criteria 40. Geneva, Switzerland: World Health Organization, 1-62.
- Wiley, J.A., Robinson, J.P., Piazza, T., Garrett, K., Cirksena, K., Cheng, Y.T. and Martin, G. 1991. Activity Patterns of California Residents. Contract No. A6-177-33. Final Report. Sacramento, CA: Air Resources Board, Research Division, California Environmental Protection Agency. <http://www.arb.ca.gov/research/abstracts/a6-177-33.htm>
- Williams, A.C. and Barry, B.W. 2004. Penetration enhancers. Advanced Drug Delivery Reviews 56:603-618.
- Willis, G.H., McDowell, L.L., Southwick, L.M., et al., 1987. Methoxychlor and endosulfan concentrations in unit-source runoff and in channel flow of a complex watershed. Transactions of the American Society of Agricultural Engineers. 30:394 - 399.
- Wilson, V.S. and LeBlanc, G.A. (1998). Endosulfan elevates testosterone biotransformation and clearance in CD-1 mice. Toxicology and Applied Pharmacology, 148:158-168.
- Worthing, C.R. and Hance, R.J., 1991. The Pesticide Manual: A World Compendium. 9<sup>th</sup> ed. BCPC, Thornton Heath, UK.

- Yadav, A.S., Vashishat, R.K. and Kakar, S.N., 1982. Testing of endosulfan and fenitrothion for genotoxicity in *Saccharomyces cerevisiae*. *Mutation Research*, 105:403-407.
- Zaidi, N.F., Agrawal, A.K., Anand, M. and Seth, P.K., 1985. Neonatal endosulfan neurotoxicity: Behavioral and biochemical changes in rat pups. *Neurobehavioral Toxicology and Teratology*, 7:439-442.
- Zendzian, R.P. and Dellarco, M. 2003. Validating in vitro dermal absorption studies: an introductory case study. Chapter 18 in: Salem, H. and Katz, S.A., editors. *Alternative Toxicological Methods*. CRC Press, Boca Raton.
- Zhu, X.-Q., Zheng, et al., 2000. Effects of endosulfan on reproductive system of male pups after gestational and lactational exposure. *Zhongguo Yaolixue Yr Dulixue Zazhi*, 14(5):352-356.
- Zhu, X.-Q., Zheng, et al., 2002. Effects of endosulfan on the spermatogenesis and oxidative damage in rats. *Zhongguo Yaolixue Yr Dulixue Zazhi*, 16(5):391-395.
- Zweig, G., Gao, R.-Y., Witt, J.M., Pependorf, W. and Bogen. K. 1984. Dermal exposure to carbaryl by strawberry harvesters. *J of Agricultural and Food Chemistry* 32:1232-1236.
- Zweig, G., Leffingwell, J.T. and Pependorf, W. (1985). The relationship between dermal pesticide exposure by fruit harvesters and dislodgeable foliar residues. *J. Environ. Sci. Health*. B20(1):27.