UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

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MEMORANDUM

SUBJECT: **ENDOSULFAN -** Supporting documentation for findings of FQPA Safety Committee on February 11, 2002

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THROUGH: Ed Zager, Assoc. Division Director
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and

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On May 2, 2002, staff from the Health Effects Division (HED) and the Special Review and Reregistration Division (SRRD) in the Office of Pesticide Programs (OPP) participated in a conference call with the registrant, Aventis, to discuss issues regarding the human health risk assessment for the organochlorine endosulfan. HED has been asked to elaborate on the rationale for the retention of the 10X FQPA Safety Factor for risk assessment purposes.

In compliance with the Food Quality Protection Act (FQPA) of 1996 requirement for the reregistration of chemicals, HED has reevaluated the toxicological database for endosulfan. The statute in the Food Quality Protection Act states that "an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and post-natal toxicity and completeness of the data with respect to exposure and toxicity to infants and children. Notwithstanding such requirement for an additional margin of safety, the Administrator may use a different margin of safety for the pesticide chemical residue only if, on the basis of reliable data, such margin will be safe for infants and children." Moreover, in the FQPA safety factor provision the Agency is required to consider, among other relevant factors, the potential for a pesticide to have an effect in humans that is similar to effects caused by naturally occurring estrogen or other endocrine effects when establishing, modifying, leaving in effect or revoking a tolerance or exemption for a pesticide chemical residue.[1]

As part of the reevaluation process for endosulfan, the FQPA Safety Factor Committee has evaluated this chemical on two occasions. On November 2, 1998 the committee evaluated the toxicological database for endosulfan as well as the potential for exposure to this compound via the diet and/or occupational/residential uses. The background materials provided to the committee members for review and consideration are included as Appendix A of this document. Based on the information provided, the committee concluded that the 10X default FQPA safety factor could be reduced to 3X. The rationale for the conclusions of the committee are provided in the Report of the FQPA Safety Factor Committee dated November 20, 1998 and included as Appendix B of this document. In response to comments provided by the Endosulfan Task Force (ETF), the Agency reconsidered the endpoints used for dermal risk assessment purposes. On February 11th, 2002 the FQPA Safety Factor Committee convened to determine the impact these changes might have on the FQPA safety factor determination as well as reconsider recent data regarding effects on the endocrine/neuroendocrine system pursuant to the Agency guidance on the evaluation and consideration of these endpoints for FQPA safety factor determination purposes. The materials provided to the committee for review are included in this document as Appendix C. Based on the submitted background material, the committee concluded that the default 10X FQPA safety factor should be retained. The committee based its conclusions on the fact that there are no reliable data to address residual uncertainties and concerns pertaining to the possible increased susceptibility of young rats to endocrine and neurotoxic effects in the young. Additionally, the Subchronic Neurotoxicity and Developmental Neurotoxicity studies requested by the Agency are still outstanding. The rationale for retaining the 10X FQPA safety factor is explained in further detail in the Report of the FQPA Safety Factor Committee dated February 14, 2002 and included as Appendix D of this document.

On April 10th 2002, OPP staff met with members of the Endosulfan Task Force (ETF) to discuss the impact of the endocrine disrupting potential of this chemical on the retention of the default 10X

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[1] 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 . Section 408 (b)(2)(C)(i)and Section 408 (b)(2)(D)(viii).

FQPA safety factor. In addition, ETF submitted a response to the FQPA Safety Factor Committee dated April 5th, 2002. This document as well as the comments provided by ETF during the April 10th meeting have been taken into consideration by the Agency. The Agency's response to the most salient issues discussed with ETF are as follows:

1) The ETF correctly pointed out that rats exposed to 1 mg/kg/day of endosulfan from postnatal days (PND) 1-35 exhibit an increase ($p < 0.05$) in binding of serotonin in the frontal cortex of the brain and an increase in aggressive behavior. A similar effect was not seen in adult rats dosed at the 1 mg/kg/day dose level. However, adult rats exposed to a higher dose, 3 mg/kg/ day, for 15 to 30 days did exhibit the increase in serotonin and aggressive behavior. **These findings reaffirm the Agency's current determination of enhanced susceptibility on a quantitative basis (e.g., effects at 1 mg/kg/day in neonates versus 3 mg/kg/day in adults).**

2) The ETF asserted that the potential endocrine disruptor effects occur at systemically toxic doses. Again, as the Agency has stated in the past, systemic toxicity does not negate the potential for endocrine disruption effects. **The effects seen in this study may reflect a perturbation of the endocrine system.**

3) The ETF claimed that sperm parameter effects are too variable to make any assessments since these endpoints may be affected by stress, circadian rhythms, etc. If this were the case, these same external influences would affect control groups, which did **not** exhibit the sperm effects noted in the treated groups. **Therefore, the Agency considers the sperm effects to be indicative of toxicity and potential endocrine perturbations.**

4) The ETF disagreed with the Agency's use of open literature studies in its evaluation claiming the literature studies have not been thoroughly reviewed. **However, all of these studies were peer reviewed.**

5) In a recent study (Sinha et al., 2001), Druckrey rats exposed *in utero* (GD12 through parturition) only and evaluated on PND 100 (i.e. as young adults) exhibited decreases in sperm parameters, as well as decreases in testes, seminal vesicle, and epidydimis weights.[2] The ETF argues that since similar results were not observed in another study in which Wistar rats were dosed from gestation day (GD) 15-PND 21, the effects seen in the Druckrey rats are invalid. It is important to note the persistence of the effects, although exposure was exclusively *in utero*, effects were seen on PND 100. Also noteworthy is the fact that Druckrey rats are very sensitive to endocrine disruption effects. **Consequently, lack of effects in the study using Wistar rats is not a valid point to disqualify the findings in another study that used a different strain and protocol.**

6) The ETF asserted that histopathology of testicular tissue is a more sensitive and reliable endpoint

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[2] Sinha, N., Adhikari, N., and Saxena, D.K. *Effect of Endosulfan During Fetal Gonadal Differentiation in Spermatogenesis in Rats.* **Environmental Toxicology and Pharmacology 10: 29-32.** (2001)

than sperm parameters. **However, in the absence of this histopathological data, the sperm effects observed in open literature studies and found at lower doses than effects observed in studies submitted by the registrant are an indication that endocrine disruption effects may be occurring.**

7) The ETF asserted that the National Cancer Institute (NCI) study demonstrating testicular atrophy should not be used since it was not a guideline study and that systemic toxicity was seen at the same doses as endocrine disruption. **The Agency can and will use open literature studies that have been subjected to peer review as part of their evaluation. As mentioned above (#2), the presence of systemic toxicity does not negate the potential for endocrine disruption effects.**

8) The ETF argued that the pituitary and uterine weight effects in the guideline multi-generation reproductive toxicity study were not seen in the remainder of the database. However, one should consider that in other studies, where these parameters are evaluated, dosing began during adulthood. While in the 2-generation reproduction toxicity study animals were dosed *in utero*, perinatally, during pubescence and perhaps most importantly, during the sexual maturation phase of the animals life span. **Therefore, there is no basis for discounting the effects.**

9) To expand upon what was said above (#8), the ETF stated that the Agency should rely on the results of the guideline studies only, which did not detect the effects discussed in the literature studies. However, the registrant submitted reproduction study did not include the assessment of endpoints such as indicative of potential endocrine effects (such as estrous cyclicity, sperm measures, and age at sexual maturation) and the developmental rat study did not dose the dams in late gestation (i.e., during a critical period of male reproductive system development) nor follow the offspring through puberty and beyond. **Therefore**, **the effects in the registrant submitted study were not detected because they were not evaluated.**

10) The ETF acknowledged "indications of potential disruption of reproductive hormones in males" (p. 12 ETF's April 5, 2002 document). **This statement by the ETF reaffirms the Agency's position of the potential for endocrine disruption by endosulfan.**

11) The ETF asserted that the Agency suggested that the sex-hormone binding globulin (SHBG) and steroidogenic acute regulatory protein (StAR) may be involved in the endocrine disruption effects caused by endosulfan and that no evidence exists to prove this "supposition." **Given the pattern of endocrinopathology seen throughout the published literature, the Agency is justified in suggesting the possible involvement of these proteins in the endocrine disruption properties exhibited by this chemical.**

12) The ETF argued that the effects on testosterone levels are the result of liver and kidney toxicity. **However, no data is available to substantiate this statement.**

13) The ETF asserted that the operant learning test, in which increases in acquisition time (learning) and decreases in pedal presses (reward -associated with memory deficits) have been reported, may be the outcome of a decrease in appetite or lethargy. **No evidence to substantiate this statement is available.**

14) **A DNT and subchronic neurotoxicity studies are still outstanding.** The Agency had requested, and still has not received, the subchronic neurotoxicity and the DNT studies to address the uncertainty concerning a thorough characterization of the neurotoxicity properties of endosulfan.

15) Both the Agency and the ETF recognize the conclusion of the ATSDR [*Toxicological Profile for Endosulfan (Update), September 2000*], stating that "There is no conclusive evidence to suggest that young animals are more susceptible than older ones." The EPA, however, does not interpret this ATSDR statement to signify a dismissal of the possibility of enhanced susceptibility of the young. Moreover, the EPA has evaluated additional data that reaffirms the Agency's position regarding enhanced susceptibility of the young to endosulfan exposure. **Therefore**, **the conclusion of the ATSDR does not support removing the default 10X FQPA safety factor.**

RESIDUAL UNCERTAINTIES

Evaluation by the Agency of the currently available toxicological data for endosulfan has revealed two distinct aspects of residual uncertainty namely, the lack of adequate endpoint characterization for the potential of this chemical to elicit neurotoxic effects, and the lack of both endpoint and developmental stage assessments for endocrine effects. The Agency has concerns for increased susceptibility of the young given the effects seen in studies in the published literature indicative of endocrine disruption in both adults and immature animals.

There is sufficient evidence of neurotoxicity reported in the currently available guideline studies, thus the Agency required the submission of a Subchronic and Developmental Neurotoxicity Studies. In the absence of these data, the impact of endosulfan exposure on the function of the nervous system of adults and the development and function of the nervous system in the young have not been adequately assessed.

Residual uncertainty regarding the potential effect of endosulfan exposure on the endocrine system and the endpoint currently used for regulatory purposes remains. While the current acute reference dose (aRfD) is based on a NOAEL of 1.5 mg/kg/day from the Acute Neurotoxicity Study in rats, published literature data describe effects on sperm parameters, lactate dehydrogenase and sorbitol dehydrogenase activity, as well as testicular, epididymal, and seminal vesicle weights at a dose level of 1.0 and 1.5 mg/kg/day (the lowest doses tested in the studies, i.e. no NOAEL for these effects has been identified). In a 1999 study by Dalsenter *et al.*, exposure to endosulfan from gestation day 15 through post-natal day 21 at the lowest dose tested (1.5 mg/kg/day) elicited a 21% decrease in daily sperm production. Additionally, histopathological assessments demonstrated that the percentage of seminiferous tubules with complete spermatogenesis was significantly decreased at puberty by 16%. The persistence of these effects is noteworthy since dosing ceased on PND21 yet effects were noted on PND 65 (i.e. puberty) and PND 100 (young adults). Similar results were reported by Sinha *et al.* in 2001. Sinha and his coworkers exposed pregnant rats to endosulfan (1 or 2 mg/kg/day) beginning on GD12 through parturition. At that point, the offspring were fostered to naive (i.e. untreated) dams. Evaluation of offspring on PND100 revealed a 37 and 53% decrease in spermatid count in the testes and sperm counts in the cauda epididymis, respectively, at a dose of 1 mg/kg/day.

Also noted at this dose level, were decreases in testicular weight (16%), epidydimal weight (35%), and seminal vesicle weight (32%). Finally, a 32% increase in lactate dehydrogenase (LDH) and a 29% decrease in sorbitol dehydrogenase (SDH) were also noted after endosulfan exposure at a dose level of 1 mg/kg/day. The effects on these two enzymes indicate a possible perturbation of germinal epithelial cell function. Since exposure to endosulfan occurred solely during gestation, these findings suggest that exposure during gonadal differentiation has a lasting effect on germ cells at sexual maturity. Given the published literature data effects at the dose used to establish the aRfD (1.5 mg/kg/day), the Agency has valid concerns that the current aRfD may not be adequately protective; thereby prompting the need to retain the 10X FQPA factor. The 10X FQPA factor would also be applicable to the chronic reference dose (cRfD) since a NOAEL for effects on sperm parameters, testicular histopathology, and reproductive organ weights has not been identified. Furthermore, a thorough endpoint and developmental stages evaluation of potential endocrine and neurotoxic effects in the young is not available.

In conclusion, given effects on sperm parameters and testicular histopathology seen in the published literature at doses currently used for regulatory purposes, the lack of evaluation of sperm parameters in the submitted guideline multi-generation study, the data gaps for a Subchronic Neurotoxicity and Developmental Neurotoxicity Study, the increased susceptibility of the young, as well as the significant uncertainty associated with the effects of endosulfan on the endocrine/neuroendocrine system, the Agency does not have reliable data that would warrant reduction of the default 10X FQPA safety factor.

APPENDIX A

Endosulfan

(PC Code: 079401)
(dsl 10/23/98)

David Liem
10/23/98

I. Toxicological Considerations for FQPA Safety Factor Selection

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 51, 6, 9, 9a - hexahydro - 6, 9 - methano - 2, 4, 3-benzodioxathiepin-3-oxide) is a chlorinated hydrocarbon. Technical grade endosulfan is a mixture of two geometric isomers of a synthetic chlorinated cyclodiene, the alpha and beta isomers. These isomers are in concentration of 70% and 30%, respectively. Endosulfan was introduced in 1956 as an experimental broad spectrum pesticide. This chemical is an insecticide and acaricide of the cyclodiene subgroup which acts as a poison to a wide variety of insects and mites on contact. Endosulfan is used primarily on a wide variety of food crops including tea, coffee, fruits, and vegetables, as well as on rice, cereals, maize, sorghum, or other grains; it may also be used as a wood preservative. Formulations of endosulfan include emulsifiable concentrate, wettable powder, ultra-low volume (ULV) liquid, and smoke tablets. Many of the formulations are intended for homeowner use on vegetables, fruit trees, and ornamentals. Formulations for greenhouses uses are applied as smoke. Applications of the emulsifiable concentrate and wettable powder formulations are generally foliar, using aircraft or ground equipment.

Endosulfan is a Restricted Use Pesticide (RUP), and is a highly toxic pesticide and is classified as toxicity class I (oral route). Labels for products containing endosulfan must bear the Signal Words DANGER - POISON, depending on formulation.

Endosulfan is highly toxic via the oral route, with oral LD50 values ranging from 9.58 to 40.38 mg/kg in rats. It is practically non-toxic via the dermal route, with a reported dermal LD50 value of 2000 mg/kg in rats. Endosulfan is highly toxic via inhalation, with a reported inhalation LC50 range between 0.16-0.5 mg/L for 4 hours exposure. It does not cause severe eye irritation in animals. Endosulfan is not a dermal sensitizer and it is a slight skin irritant. The alpha-isomer is considered to be more toxic than the beta-isomer. Animal data indicate that toxicity may also be species-specific and also influenced by level of protein in the diet. Toxic effects of endosulfan are nearly twice as severe for rats which are deprived of protein. Solvents and/or emulsifiers used with endosulfan in formulated products may influence its absorption into the system via all routes; technical endosulfan is slowly and incompletely absorbed into the body whereas absorption is more rapid in the presence of alcohols, oils, and emulsifiers. Stimulation of the central nervous system is the major characteristic of endosulfan poisoning. Symptoms noted in acutely exposed humans include those common to the other cyclodienes, e.g., incoordination, imbalance, difficulty breathing, gagging, vomiting, diarrhea, agitation, convulsions, and loss of consciousness. Reversible blindness has been documented for cows that grazed in a field sprayed with the compound. The animals completely recovered after a month following the exposure. In an accidental

exposure, sheep and pigs grazing on a sprayed field suffered a lack of muscle coordination and blindness.

Chronic toxicity: Oral doses of endosulfan at 3.8 mg/kg/day in rats and of 2.6 mg/kg/day in mice caused body weights decrease in females and increased incidences of mortality. Rats dosed at 3.8 mg/kg/day resulted in increased incidences of marked progressive glomerulonephrosis in the kidneys in males and females and blood vessel aneurysms in males. Oral dose at 1.75 mg/kg/day in dogs caused decrease body weight gain in males and increase incidences of neurological effects in males and females.

Reproductive and Developmental effects: Rats fed endosulfan up to 6.18 mg/kg/day in a two-generation reproduction study showed no observable reproductive effects. At 6.18 mg/kg/day dose level, increased pituitary and uterine weights were noted. In the developmental toxicity studies, increased incidences of deaths, tonoclonic convulsions, salivations and hyperactivity were noted in the dams dosed at 6 mg/kg/day and in does dosed at 1.8 mg/kg/day.

Mutagenic effects: Endosulfan did not show mutagenic activities in the primary rat hepatocyte UDS and mouse lymphoma forward mutation assays.

Carcinogenic effects: High mortality rates of males were noted in long-term toxicity studies in rats and mice. Chronic feeding of endosulfan to rats, up to 3.8 mg/kg/day and to mice, up to about 2.6 mg/kg/day, did not cause an increased incidence of tumors in both species. It appears that endosulfan is not carcinogenic.

Fate in humans and animals: Endosulfan is rapidly degraded into mainly water-soluble compounds and eliminated in mammals with very little absorption in the gastrointestinal tract. In rabbits, the beta-isomer is cleared from blood plasma more quickly than the alpha-isomer, with reported blood half-lives of approximately 6 hours and 10 days, respectively, which may account in part for the observed differences in toxicity. The metabolites are dependent on the mixture of isomers and the route of exposure. Most of the endosulfan seems to leave the body within a few days to a few weeks.

1. Has the scientific quality of the toxicology data base and the confidence in the hazard endpoints and dose-response assessments been completely characterized?

Answer: The Hazard Characterization of all submitted endosulfan studies has been fully assessed. A data gap of a subchronic neurotoxicity study for this food use chemical was identified. Based on the weight-of-the-evidence considerations, the Hazard ID Committee reserved the requirement for developmental neurotoxicity study in rats, pending submission of a subchronic neurotoxicity study with endosulfan.

2. Do we have adequate hazard studies for evaluation of risk to infants and children? These include, but are not limited to, developmental studies in 2 species; multi generation reproduction studies; neurotoxicity and developmental neurotoxicity studies as required for chemicals which affect the nervous system. Are additional studies being required?

Answer: The data base is complete and there are no data gaps pertaining to developmental or reproductive toxicity. A developmental neurotoxicity study in rats was reserved, pending submission of a subchronic neurotoxicity study with endosulfan.

3. Do these studies show enhanced susceptibility to infants and children? That is, do the effects in the young occur at doses not causing effects in the adults? Are the effects in the young at the same level but more severe? Completely describe the spectrum of effects in both adult and young animals (include the shape of the dose response curve, the reversibility of effects if known, etc.).

Answer: The data provided no indication of increased sensitivity of rats or rabbits to *in utero* and post-natal exposure to endosulfan. Two prenatal developmental toxicity studies, one in rats and one in rabbits, failed to show evidence of developmental toxicity in the absence of maternal toxicity. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity.

4. Have other studies (e.g., literature reviews) been considered which might influence an FQPA safety factor finding? Is there mode of action studies which may provide information on precursor effects at lower doses? Are there comparative metabolism and pharmacokinetics studies evaluating the dose at the target site or the duration of effect?

Answer: There are no additional acceptable studies available in the published literature which might influence a FQPA safety factor.

Endosulfan /John Punzi / 10/26/98
II. DIETARY EXPOSURE CONSIDERATIONS

1. Describe (semi-quantitatively) the typical use rates and frequency of application. [There is no need to reproduce the labels. We are trying to determine whether there is a likelihood of quantifiable residues in the food.]

This Chemical is used on many crops. FDA/USDA surveys find this chemical in/on crops.

Are there Codex MRL's for the compound?

Table D. Codex MRLs and applicable U.S. tolerances for endosulfan. Recommendations for compatibility are based on conclusions following reassessment of U.S. tolerances (see Table C).

Codex			Reassessed U.S. Tolerance (ppm)	Comments
Commodity, As Defined	MRL (mg/kg)	Step		
Alfalfa forage (green)	1	5/8	Revoke	Endosulfan use on alfalfa grown in the U.S. is not likely to be supported.
Broccoli	0.5	5	3.0	
Cabbages, Head	1	5	4.0	
Cabbages, Savoy	2	5	4.0	
Carrot	0.2	CXL	0.2	Compatibity exists.
Cauliflower	0.5	5	2.0	
Celery	2	5/8	8.0	
Chard	2	5	--	No U.S. registrations.
Cherries	1	5/8	2.0	
Chicory leaves	1	5	--	No U.S. registrations.
Clover	1	5/8	--	No U.S. registrations.
Common bean (pods and/or immature seeds)	0.5	5	2.0	
Cotton seed	1	CXL	1.0	Compatibility exists.
Cotton seed oil, crude	0.5	CXL	--	
Endive	1	5	--	No U.S. registrations.
Fruits	2	CXL	2.0 each for apricots, grapes, nectarines, peaches, pears, plums, prunes, and strawberries	Compatibility exists for some fruit crops.
Garden peas (young pods)	0.5	5/8	--	No U.S. registrations.

Codex			Reassessed U.S. Tolerance (ppm)	Comments
Commodity, As Defined	MRL (mg/kg)	Step		
Kale	1	5/8	2.0	
Lettuce, Head	1	5/8	11.0	
Lettuce, Leaf	1	5/8	6.0	
Meat	0.2 (carcass fat)	CXL	0.2	Compatibility exists.
Milks	0.02 [1]	CXL	0.5	
Onion, Bulb	0.2	CXL	--	No U.S. registrations.
Plums (including Prunes)	1	5/8	2.0	
Pome fruits	1	5/8	1.0	Compatibility exists.
Potato	0.2	CXL	0.2	Compatibility exists.
Rice	0.1	CXL	--	No U.S. registrations.
Spinach	2	5/8	2.0	Compatibity exists.
Sugar beet	0.1	5/8	Revoke	Endosulfan use on sugar beets grown in the U.S. is not likely to be supported.
Sugar beet leaves or tops	1	5/8	--	
Sweet potato	0.2	CXL	0.2	Compatibility exists.
Tea, Green, Black	30	CXL	24 (reflecting <0.1 ppm residues in beverage tea)	
Trefoil	1	5/8	--	No U.S. registrations.

[1] The residue is fat-soluble and MRLs for milk and milk products are derived as explained in the introductions to this Part of the Guide and to Volume XIII of Codex Alimentarius.

2. What metabolites require regulation? Are the residues systemic? That is, are they distributed throughout the plant or likely to be removed by preparation (washing, peeling, etc.)? Is information available about the dissipation or half-life of the pesticide?

The chemical is a mixture of isomers. The tol expression includes the alpha, beta isomers plus a sulfate metabolite. This is a persistent chemical and some plant uptake may be found.

3. State and characterize the available residue databases for each crop (i.e field study data, sources of available monitoring data such as PDP, FDA, etc.). What are the limits of quantitation used ? Describe semi-quantitatively the results of residue testing (ranges, frequency of positive findings, etc.).

Field trial data for most crops are poor. Circa 1960's would not even come close to adhering to todays standards. PDP and FDA have found this chemical in a variety of crops for example in 1995 endosulfan was monitored for in APPLes (7%), carrots (4%), Grape (4%), green beans (24%), ORANGES (2%) There is no tolerance on oranges!!! Peaches (8%), Potatoes (20), Spinach (14), corn (0.1) peas (0.3) Number in parent means percent of samples with detects. LOQ ~0.01 ppm.

4. Is there information available on % crop treated? If so, what is the source of the information and the uncertainties around the number? What is the likely maximum % crop treated for each crop (based on potential market)?

I don't know

5. Based on the Consumption Database used by DRES, which crops contribute significantly to the human diet for adults? Which contribute significantly to the diet of infants and children? Is there likelihood of transfer of residues to meat and/or milk? Describe the degree of refinement of the DRES analyses for acute and chronic exposure.

See above

Endosulfan / Nelson Thurman / 10/26/98

Drinking Water Exposure Considerations for FQPA Safety Factor Selection

The following environmental fate considerations for endosulfan are based on an incomplete fate database. We are in the process of reviewing new studies and pulling together other sources of data.

1) Is the environmental fate database complete enough to characterize drinking water exposure?

The environmental fate database is not complete -- the registrant has submitted several studies to fulfill data requirements. At this point, these studies are in review. Once the reviews are completed, then a quantitative fate assessment sufficient for assessing drinking water exposure can be developed.

A) Provide a brief summary of the environmental fate assessment for this compound and any metabolite that may potentially get into drinking water based on metabolite fate characteristics.

Supplemental studies listed in the EFGWB One-Liner Database suggest that endosulfan may be moderately persistent in soils, with half-lives in the lab on the order of one to several months, and half-lives in the field of several months. It appears to have a high affinity to sorb to soil particles, reducing its susceptibility to leaching. It is likely to move to surface waters via runoff attached to soil particles. This assessment will be refined after the additional studies have been reviewed.

B) Is the compound or any of its metabolites mobile and persistent? (A bottom line summary statement on drinking water exposure potential should be included.)

As noted above, endosulfan does not appear to be highly mobile, but may be persistent enough in some instances to move to ground water (we have reported detects in the EPA Pesticides in Ground Water Database, although these have not been thoroughly evaluated). Movement to surface water sources of drinking water is likely to occur via spray drift and runoff adsorbed to soil particles. We are aware of several studies which have reported endosulfan detects in surface water. However, these studies have not been evaluated for their quality or extent of applicability to drinking water exposure assessments.

Endosulfan consists of isomers which appear to have some differences in persistence. The extent to which these differences will affect the fate assessment, if any, is not yet known. We suspect endosulfan sulfate will be a degradate of concern. We will be trying to pull together as much information on the fate of this degradate as possible.

2) Discuss method for drinking water exposure assessment (ex. monitoring data, modeling, combination).

No exposure assessments have been made at this point. The method will consist of:
(1) completing the fate assessment to get as quantitative and evaluation as possible
(2) modeling exposures for high use crops and other potentially vulnerable sites (which will be determined after the Smart meeting with the registrant)
(3) if needed, evaluating the extent and quality of existing monitoring data

A) If models are used, discuss which models, describe estimated environmental concentrations (EECs) and scenarios used..

B) If monitoring data are used (ground water or surface water), describe the monitoring data and state if the data were collected from vulnerable areas at maximum label rates.

3) Please discuss extent of population potentially exposed to the pesticide via drinking water based on extent of usage and based on if chemical characteristics indicate a likelihood of drinking water contamination.

Unable to determine at this point.

Endosulfan
Jack Arthur
10/27/98

IV. RESIDENTIAL EXPOSURE CONSIDERATIONS

1. Is the compound used around the home in such a way that children and infants may be exposed? What is the frequency and rate of application ?

Endosulfan is applied on home gardens as a liquid spray (* times per season, at a maximum rate of one pound per acre) and as a dust (* times per season, at a maximum rate of 3.5 pounds per acre). Toddlers could be exposed from incidental soil ingestion by hand-to-mouth activity in garden plots.

*** Will supply this ASAP.**

2. Have Pesticide Handler Exposure Database (PHED) data been used in estimating the exposure? How well does the PHED scenario reflect the actual use pattern? Rate the data used based on the PHED grading criteria (high quality, medium quality, or low quality). If chemical-specific or other non-PHED data have been used, describe the scope of the study, resulting exposure values, and general quality of the study.

The PHED has been used to estimate residential application of endosulfan to ornamentals, shade trees and vegetable/small fruit gardens as a liquid spray using:

- **low pressure handwand (low confidence data for dermal; medium confidence for inhalation)**

*** backpack sprayer (low confidence data for dermal and inhalation)**
*** hose end sprayer (very low to low confidence for dermal and low confidence for inhalation)**

There are no data for estimating exposure to the use of the dust product.

3. For residential post application exposure scenarios, have the *Draft Standard Operating Procedures for Residential Exposure Assessments* been used as the basis for all calculations? Describe any deviations from SOP calculations and the impact on the assessment results (e.g., assessment reflects a less conservative approach by altering transfer coefficient value for dermal exposure).

The Draft SOPs will be used.

4. Is chemical-specific product use information available through BEAD or some other source? Has the assessment been developed to reflect this information? Has this information been used as a basis for characterizing the populations considered in the assessment?

Use information was provided via the LUIS report from BEAD. A large sample of labels are also being used to ascertain use, exposure and exposed populations (i.e., handlers, residential, agricultural post-application, etc.)

5. Are reliable biologically-based exposure data or epidemiology data available to support the results of the assessment (e.g., incident report, CDC monitoring data, etc.)?

No biologically-based exposure data are available, and the incidence data section has not been researched yet (from informal consultation with Jerry Blondell).

6. Have models other than PHED or those presented in the Residential SOPs been used to calculate dose in any aspect of the assessment (e.g., CONSEXPO, TherDbase, etc)? Summarize how these are integrated into the assessment.

NO.

7. Is 100% dermal absorption assumed (when dermal endpoints are derived from oral studies)?

This will be the case.

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To: Brenda Tarplee/DC/USEPA/US@EPA, Steve Devito/DC/USEPA/US@EPA
cc:
Subject: Endosulfan ORE FQPA additions

A couple of additional points for the FQPA residential exposure questions for endosulfan that I missed: (1) frequency of application for homeowner uses (small ornamentals and vegetable/fruit gardens) varies by crop type, probably ranging from 1 to 5 applications per year (during the growing season); and, (2) according to the Hazard ID Report, a 45% absorption factor will be used
for intermediate and chronic dermal dosage calculation using the oral endpoint (not the default of 100%). -- Jack.

HED Doc. No. 012901

DATE: October 7, 1998

MEMORANDUM

SUBJECT: ***ENDOSULFAN*** - Report of the Hazard Identification Assessment Review Committee.

FROM: David S. Liem, Ph.D
Reregistration Branch II
Health Effects Division (7509C)
and
Jess Rowland, Executive Secretary
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Chairman,
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Steve DeVito, Risk Assessor
Reregistration Branch II
Health Effects Division (7509C)

PC Code: 079401

On September 1, 1998 the Health Effects Division's Hazard Identification Assessment Review Committee evaluated the toxicology data base of **Endosulfan,** selected the toxicological endpoints for acute and chronic dietary as well as short, intermediate and long-term occupational/residential exposure risk assessments, evaluated the carcinogenic potential, and addressed the potential sensitivity of infants and children as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

Committee Members in Attendance

Members present were: Karl Baetcke, William Burnam, Karen Hamernik, Susan Makris, John Redden, Robert Fricke, and Jess Rowland (Executive Secretary). Member in absentia was Clarke Swentzel (Chairperson). Data was presented by David Liem of Reregistration Branch II.

Also in attendance were Paula Deschamp, Steve Devito, Kelly O'Rourke, John Punzi, Brenda Tarplee, and Pauline Wagner.

Data Presentation:
and
Report Presentation _____
David S. Liem
Toxicologist

Report Concurrence: _____
Jess Rowland
Executive Secretary

I. INTRODUCTION

On September 1, 1998 the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base of **endosulfan,** established acute and chronic Reference Doses (RfDs), evaluated the carcinogenic and mutagenic potential and selected the toxicological endpoints for occupational as well as residential exposure risk assessments. The HIARC also addressed the potential sensitivity of infants and children from exposure to Endosulfan as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 51, 6, 9, 9a - hexahydro - 6, 9 - methano - 2, 4, 3-benzodioxathiepin-3-oxide) is a chlorinated hydrocarbon. Technical grade endosulfan is a mixture of two geometric isomers of a synthetic chlorinated cyclodiene, the alpha and beta isomers. These isomers are in concentration of 70% and 30%, respectively. Endosulfan was introduced in 1956 as an experimental broad spectrum pesticide. Endosulfan is an insecticide and acaricide of the cyclodiene subgroup which acts as a poison to a wide variety of insects and mites on contact. Endosulfan is used primarily on a wide variety of food crops including tea, coffee, fruits, and vegetables, as well as on rice, cereals, maize, sorghum, or other grains; it may also be used as a wood preservative. Formulations of endosulfan include emulsifiable concentrate, wettable powder, ultra-low volume (ULV) liquid, and smoke tablets. Many of the formulations are intended for homeowner use on vegetables, fruit trees, and ornamentals. Formulations for greenhouses uses are applied as smoke. Applications of the emulsifiable concentrate and wettable powder formulations are generally foliar, using aircraft or ground equipment.

II. HAZARD IDENTIFICATION

A. Acute Reference Dose (RfD)

Study Selected: Acute Neurotoxicity Study in Rats §81-8
MRID No.: 44403101

Executive Summary: In a neurotoxicity study, male and female Wistar rats (10/sex/dose) were fasted overnight and then orally gavaged once with endosulfan (98.6%) suspended in 2% starch mucilage at a constant volume of 10 ml/kg body weights. Two separate control groups of 10 rats/sex were used in the study. One control group was assigned to males, dosed at 25, 50 and 100 mg/kg and females dosed at 3, 6 and 12 mg/kg. The other control group was assigned to males, dosed at 6.25 and 12.5 mg/kg and females at 0.75 and 1.5 mg/kg. Rats were observed for 15 days and survivors were sacrificed on week three. The treated groups were dosed at levels of 0 (vehicle), 6.25, 12.5, 25, 50 and 100 mg/kg for the males and 0 (vehicle), 0.75, 1.5, 6 and 12 mg/kg for the females. The study animals were evaluated for neuro-behavioral effects (FOB and motor activity) on day 7 prior to dosing, and days 1 (within 8 hours after dosing), 8 and 15 of post-dosing.

Neuropathological examinations were carried out at terminal sacrifice (on week 3) on ten rats/sex of controls and four 100 mg/kg male rats and five 12 mg/kg female rats. Six males dosed at 100 mg/kg and one female dosed at 12 mg/kg died or were found dead at the day of dose administration.
Treatment-related clinical signs were noted within 8 hours after dosing on day one (peak-time of effects) in males at 50 and 100 mg/kg and females dosed at 6 and 12 mg/kg. These symptoms were not observed after day 2 in all survivors. Clinical signs noted included tonoclonic convulsions, decreased spontaneous activities, stilted gait, stupor, prone position, squatting posture, straddled hindlimbs, bristle coat, palpebral fissure narrow, and irregular respiration and panting in males dosed at 50 and 100 mg/kg and females dosed at 6 and 12 mg/kg. In addition, increased incidences of the following signs, stilted gait, squatting posture, irregular respiration and decreased spontaneous activities in males dosed at 25 mg/kg; increased incidences of squatting posture, straddled hindlimbs, decreased spontaneous activities, bristle coat, irregular respiration and panting were also noted in females dosed at 3 mg/kg/day. Animals with "drawn in flanks" were only noted in females dosed at 3, 6, 12 mg/kg. Tremors were noted in three and four females dosed at 6 mg/kg and 12 mg/kg, respectively and in four males dosed at 50 mg/kg. Salivation was noted in one male dosed at 100 mg/kg, and in one female each dosed at 6 and 12 mg/kg. According to the study, the clinical effects observed were due to interaction of endosulfan with the brain γ-amino-butyric acid (GABA) receptors. No compound-related effects on motor activity were noted for rats that survived. No treatment-related effects were seen on: the rearing frequency, fore and hind-limb grip strength, and on landing foot-spread; body weight and food consumption; organ weight; gross pathology; or histo(neuro) pathology. The NOAEL was 12.5 mg/kg for males and 1.5 mg/kg for females. The LOAEL was 25 mg/kg for males based increased incidences of stilted gait, squatting posture, and irregular respiration, as well as decreased spontaneous activity. The LOAEL was 3 mg/kg for females, based on an increased incidence of stilted gait, squatting posture, straddled hindlimbs, irregular respirations, panting and bristled coat and decreased spontaneous activity.
This study is classified as **acceptable** and satisfies the Subdivision F guideline requirements for a neurotoxicity screening study in rats (§81-8).

Dose and Endpoint for Risk Assessment: NOAEL= 1.5 mg/kg based on increased incidences of convulsions seen within 8 hours after dosing in females at 3 mg/kg.

Comments about Study/Endpoint: The database included a lower NOAEL (maternal) of 0.7 mg/kg/day in the rabbit developmental toxicity study (MRID#: 00094837), based on salivation, convulsions, rapid breathing, and hyperactivity seen at 1.8 mg/kg/day. The Committee, however, decided not to use this NOAEL for this (acute) scenario because the clinical signs in the dams were seen on day 10 of gestation (i.e., after 4 treatment) whereas in the acute neurotoxicity study, convulsions were seen 8 hours after a single oral dose, thus making this endpoint more appropriate for this risk assessment.

Uncertainty Factor (UF): 100 (10x for inter-species variation and 10x for intra-species extrapolation).

ACUTE RfD: NOAEL 1.5 mg/kg/ = **0.015 mg/kg**
UF 100

This risk assessment is required.

B. Chronic RfD

Study Selected: Combined Chronic/Carcinogenicity Study in Rats §83-5
MRID No.: 41099502

Executive Summary: In a combined chronic/oncogenicity study (MRID# 41099502), groups of 50 Sprague-Dawley rats/sex/group were fed (in the diet) with technical endosulfan (97.1% purity) at 0, 3.0, 7.5, 15.0, and 75.0 ppm (≈ 0, 0.1, 0.3, 0.6, and 2.9 mg/kg/day for males and 0, 0.1, 0.4, 0.7, and 3.8 mg/kg/day for females) for 104 weeks. A satellite group of twenty rats/sex was dosed in a similar fashion and was used for hematology and clinical chemistry evaluations. No treatment-related effects on clinical signs, mortality, food consumption and urinalysis were observed. Mean body weights of the males and females dosed at 75.0 ppm were statistically significantly decreased ($p<0.01$; 17.6%) as compared to their respective controls. Grossly, enlarged kidneys were noted in females in the satellite group dosed at 75.0 ppm (8/20 *versus* 2/20 in the controls).

No treatment-related changes were noted in the clinical chemistry and hematology parameters evaluated. Marginal decreases of leukocyte (at week 26) and lymphocyte counts (at weeks 26 and 52) were noted in the males dosed at 75.0 ppm. At week 13, RBC counts and MCV values were decreased in all treated females as compared to the controls. Since dose related trends were not evident and since no changes were noted at other intervals, these changes were not judged to be related to treatment. Increased incidences of blood vessel aneurysms (18/70 *versus* 10/70 in controls) and enlarged lumbar lymph nodes (19/70 *versus* 14/70 in controls) were noted in the male rats dosed at 75.0 ppm as compared to the controls. Increased incidences of enlarged kidneys were seen in females dosed at 75 ppm (30/70 *versus 2*1/70 in controls) as compared to the controls. Other organ weights were not affected by dosing. Although slightly decreased testes weights were observed in males dosed at 15 and 75 ppm, these changes were not considered toxicologically significant.

Histopathologically, increased incidences of blood vessel aneurysms (18/70 *versus* 9/70 in controls) were noted in male rats dosed at 75.0 ppm. Also, a significant increased incidence of marked progressive glomerulonephrosis in the kidneys was seen in male (30/70 *versus* 20/70 in controls) and in female (8/70 *versus* 1/70 in controls) rats dosed at 75.0 ppm. The incidence of the glomerulonephrosis in the kidneys in the high-dose

males (43%) was higher than that observed in the historical controls data (reported at 19.7%). This data was re-evaluated because of some concerns expressed by one member of the RfD/RfC Work Group (Memorandum: L Taylor to G. Ghali, March 19, 1993). It was stated in this memo that the increase in the severity of progressive glomerulonephrosis in rats of both sexes at the high-dose level was regarded as an adverse effect and that the spontaneously occurring renal disease was exacerbated by exposure to the test material.

No treatment-related neoplastic lesions were evident in this study. A slight increased incidences of pituitary adenoma in males and females dosed at 75 ppm and fibroma/ adenoma of the mammary glands females dosed at 75 ppm were not judged to be related to treatment, because dose-related trends were not evident The doses used in this study appear to be adequate to test the carcinogenic potential of the test compound, as evidence by the compound-related systemic effects noted above.

Based on the results of this study, the systemic NOAEL is 15.0 ppm (0.6 and 0.7 mg/kg/day for males and females, respectively) and the systemic LOAEL is 75.0 ppm (2.9 and 3.8 mg/kg /day for males and females, respectively) based on decreased body weight gain in male and female rats, and/or increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in males.

Dose and Endpoint for Risk Assessment: NOAEL= 0.6 mg/kg/day. The LOAEL= 2.9 mg/kg/day, based on reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats.

Comments about Study/Endpoint: The RfD/Peer Review considered the chronic toxicity study in dogs (MRID#41099501) with a NOAEL of 0.65 mg/kg/day to be a co-critical study. In this dog study, the LOAEL of 1.75 mg/kg/day was based on decreased body weight gain in males and increased incidences of neurologic findings in males and females (loss or weakening of placing and righting reactions, tonic contractions of abdominal muscle and masticatory muscles a few hours after feeding. The HIARC concurred with the conclusions reached by the RfD/Peer Review Committee with regard to the study, dose and endpoint used in establishing the RfD.

Uncertainty Factor (UF): 100 (10x for inter-species variation and 10x for intra-species extrapolation).

CHRONIC RfD: $\frac{\text{NOAEL 0.6 mg/kg/day}}{\text{UF 100}}$ = **0.006 mg/kg/day**

This risk assessment is required.

C. Occupational/Residential Exposure

1. Dermal Absorption

Two dermal absorption studies were available.

Study Selected: Dermal Absorption studies in rats §85-3
MRID No.: 40223601 and 41048504

Executive Summary: In a dermal absorption study (MRID#40223601), three groups of 24 male Crl: CD(SD) Br rats/group were dosed topically with radio labeled endosulfan dosing suspension (94.6% purity) at nominal doses of 0.1, 1, and 10 mg/kg and exposed for 0.5, 1, 2, 4, 10 and 24 hours. The application site was shaved and then cleaned with acetone to remove surface fats and oils and to extract some lipoid from the skin, 5 hours before dosing. Then the compound was applied onto the application site (3.7 cm in diameter = 10.8 cm2). After exposure, the application sites were washed with 5 ml of mild soap solution and three 5 ml portions of water for further analysis. The animals were sacrificed and the application sites were washed with 5 ml of 1% liquid ivory soap and three 5 ml portions of water. The skin wash, filter paper, rubber ring, application site and adjacent skin, untreated skin, liver, kidney, brain and fat were analyzed for the presence of radio labeled compound. The percent doses absorbed over a 24-hour period were 2.2-21.6, 0.32-21.52, and 0.08-8.38 for the 0.1, 1, and 10 mg/kg dose groups, respectively. The percentages of endosulfan absorbed at 1, 10 and 24 hours intervals, were 1.8, 7.6 and 21.6% for rats dosed at 0.1 mg/kg, 0.57, 5.77 and 21.52%, for rats dosed at 1.0 mg/kg, and 0.29, 3.86, and 8.38% for rats dosed at 10 mg/kg. The percent doses remaining in/on the skin after soap and water washes over a 24-hour period were 62.1-56.5, 78.1-57.7, and 80.2-66.7 for the 0.1, 1, and 10 mg/kg dose groups, respectively. This data showed that significant portions of the dose remained on the skin of male rats following soap and water wash was performed. At 24-hour interval, the data showed that endosulfan bioaccumulate in the body of the rats.
This study is classified as **acceptable** and satisfies the data requirements for a dermal absorption study in rats (85-2).

In another dermal absorption study (MRID#41048504), three groups of 16 female Crl:CD(SD)BR rats/group were applied topically with radiolabeled endosulfan (purity 94.6%) at nominal doses of 0.1, 1, and 10 mg/kg (1.9, 21.9, and 231.4 mg/cm2) to determine the fate of the residue that was left in/on the skin following 10 hours of exposure. The application sites were shaved one day before dosing. Thirty minutes before dosing the sites were cleaned with acetone to remove surface fats and oils and to extract some lipoid from the skin. A rubber ring was glued on the shaved application site, then the compound was applied onto an application site within the rubber ring, and afterwards a filter paper was cemented on the rubber ring. Ten hours after dosing, the application sites were washed with 1% liquid Ivory soap and rinsed with water. The skin wash, filter paper, rubber ring, application site and adjacent skin, untreated skin, liver, kidney, brain, fat, muscle, blood, urine, feces, and carcass were analyzed for the presence of radio labeled compound. The radioactive labeled endosulfan presence was analyzed in four live rats/group at 24, 48, 72 and 168 hours after dosing. The percent doses absorbed at 24 hours were 22.1, 16.1 and 3.8% and at 168 hours were 44.8, 46.4

and 20.3% for the 0.1, 1, and 10 mg/kg dose groups, respectively. The percentages of the doses remaining on/in the skins at 168 hours were 41.4, 56.2 and 72.8% for the 0.1, 1, and 10 mg/kg dose groups, respectively. The data showed that endosulfan bioaccumulate in the body of the rats.

Dermal Absorption Factor: The HIARC selected the dermal absorption factors of 45 % (rounded of 44.8%) at 168 hours post exposure.

Comments about Dermal Absorption: The Committee selected the dermal absorption rate based on the following weight-of-evidence considerations: 1) at 24 hours, the percent absorption was comparable between males (21.6%) and females (22.1%); 2) in female rats, even after washing at 10 hours, the percent absorption increased with time, the final measurement was 44.8% at 168 hours; 3) the concern that the test material continued to be absorbed even after washing at 10 hours; 4) substantial dermal absorption is demonstrated in the 21-day dermal toxicity study with a NOAEL of 3 mg/kg/day and systemic toxicity (increased mortality, and increased liver abnormalities) evident at 9 mg/kg/day (LOAEL). In addition, this dermal absorption factor is supported by comparing the results of the oral and dermal studies in the same species. The ratio of the oral LOAEL of 6 mg/kg/day in the developmental toxicity study in rabbits and the dermal LOAEL of 9 mg/kg/day in the 21-day dermal toxicity study in rabbit with the same endpoint (increased mortality) indicate a dermal absorption rate of 67% [(6 ÷9] X 100 = 67%) as compared to the amount absorbed orally.

2. Short-Term Dermal - (1-7 days)

Study Selected: 21-Day Dermal Toxicity Study in Rats §82-2

MRID No. (ACC.No): 257684/257685

Executive Summary: In a 21-Day Dermal Toxicity Study, endosulfan (97.2% w/w) was applied onto the skin of five groups of six male and female Wistar rats at doses of 0, 1, 3, 9, and 27 mg/kg/day and onto six males only at 81 mg/kg/day, for 21 applications (5 days a week) over 30 days. Five of the six (83%) high-dose (27 mg/kg/day) females died on days 2 and 6 of the study. Three of the six (50%) high-dose (81 mg/kg/day) males died on days 2 and 3 of study (females were not tested at this dose). Two of the three 81 mg/kg/day males that died showed tonoclonic convulsions, increased salivation and respiration. Although no deaths occurred in males dosed at 27 mg/kg/day, 2 of the 6 (33%) males dosed at 9 mg/kg/day died on days 5 and 8. Prior to death, one male rat showed salivation, blood-encrusted nose, dyspnea and staggered gait and these symptoms are related to treatment. Also, these deaths are significantly increased over the controls which showed no mortality. Increased incidences of mortality in males dosed at 9 and 81 mg/kg/day and females dosed at 27 mg/kg/day appear to be a compound-related effect. The cause of death as described in the pathology report was a study-related toxic shock, and females appeared to be more sensitive than males. No changes of clinical

chemistry and hematology parameters can be attributed to treatment. Changes that occurred were small and they are not judged to be dose-related. Changes in liver cells were found at 9 mg/kg/day dose levels and above. Liver abnormalities included enlargement of parenchymal cells in peripheral sections, together with a loss of cytoplasmic basophilia, isolated cell necroses, and frequent mitoses. Females dosed at 9 mg/kg/day showed significantly increased absolute and relative spleen and absolute adrenal weights, as compared to controls. Significant dermal irritation was not produced by the test compound. Dermal irritation for all groups was very slight at all evaluation intervals. It appears that dermal irritation was more persistent in females at 3 and 9 mg/kg/day dose groups, as evidenced by greater dermal irritation scores (2-3 times) than that of controls. There was no difference between the average scores of the treated males as compared to the controls at any dose level. Although dermal irritation scores were zero at the end of the study, and although the pathology report described that dermal effects were similar in treated and control animals, there appears to be an increase in severity or prolongation of irritation found in females dosed at 3 and 9 mg/kg/day.

For systemic toxicity, the NOAEL was 3 mg/kg/day and the LOAEL was 9 mg/kg/day based on increased mortality, and increased liver abnormalities (enlargement of parenchymal cells, loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes. Increased absolute spleen weight and deaths also occurred in the 27 mg/kg/day female rats.

Dose and Endpoint Selected for Risk Assessment: Dermal NOAEL= 3 mg/kg/day based on increased mortality, and increased liver abnormalities (enlargement of parenchymal cells, loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes at 9 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The dermal NOAEL of 3 mg/kg/day is supported by the dermal equivalent dose of 4 mg/kg/day obtained by the use of the dermal absorption factor (45%) in conjunction with the oral NOAEL (2.0 mg/kg/day) established in the developmental toxicity study in rats (2 mg/kg/day÷0.45%= 4 mg/kg/day).

This risk assessment is required.

3. Intermediate-Term Dermal (7 Days to Several Months)

Study Selected: Combined Chronic/Carcinogenicity Study in Rats **(**§83-5)

MRID No.: 41099502

Executive Summary: See Chronic Dietary

Dose and Endpoint for Risk Assessment: NOAEL = 0.6 mg/kg/day based on reduced body weight gain seen during weeks 6 through 18 in male rats at 2.9 mg/kg/day. Also increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats at 2.9 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The Committee selected this dose and endpoint because: 1) the decrease in body weight gain which began at study week 18 continued throughout the study which is appropriate for this exposure period of concern (one week to several months); 2) the NOAEL in this study is comparable to the parental/systemic toxicity NOAEL of 1.23 mg/kg/day established in the two-generation reproduction study based on the same endpoint (decreased body weight gain); and 3) the use of the dermal absorption factor (45%) in conjunction with the oral NOAEL (0.6 mg/kg/day) results in a dermal equivalent dose of 1.3 mg/kg/day (0.6 mg/kg/day÷0.45= 1.3 mg/kg/day) which supports the oral dose selected for this risk assessment.
Since an oral NOAEL was selected, a dermal absorption factor of 45% should be used for route-to-route extrapolation.

This risk assessment is required.

4. Long-Term Dermal (Several Months to Life-Time)

Study Selected: Combined Chronic/Carcinogenicity Study in Rats §83-5

MRID No.: 41099502

Executive Summary: See Chronic Dietary

Dose and Endpoint for Risk Assessment: NOAEL= 0.6 mg/kg/day based on reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats at 2.9 mg/kg/day (LOAEL).

Comments about Study/Endpoint: This dose, endpoint and study were used to establish the chronic RfD. In addition, this NOAEL is supported by the NOAEL of 0.65 mg/kg/day established in the chronic toxicity study in dogs and the parental/systemic toxicity NOAEL of 1.3 mg/kg/day established in the two-generation reproduction toxicity study in rats. Since an oral NOAEL was selected, a dermal absorption factor of

45% should be used for route-to-route extrapolation.

This risk assessment is required.

5. Inhalation Exposure

5a. Short-Term (1-7 Days)

Study Selected: 21 Day Inhalation in the Rat §82-4

MRID No.: 00147183

Executive Summary: . Groups of SPF Wistar rats (10/sex/concentration) were exposed "nose-only" to aerosol concentrations of endosulfan (97.2%) at 0, 0.0005, 0.0010, or 0.0020 mg a.i/L, 6 hours/day, 5 days/week for 21 days. Air and vehicle controls were used. An additional group of 5 animals each per concentration was held for a 4-week recovery period after receiving the test aerosol. No clinical signs of toxicity were noted in the study. Body weights in high dose males were lowered by 3-5% from day 20 through 29. Lowered body weight in this high dose recovery group of 5 males, was more pronounced (12-16%) from day 34-60. Also, although neither sex displayed any statistically significantly body weight changes during the exposure period and the number of recovery animals for each sex was only 5, the apparent effect suggests a possible delay in its manifestation. Erythrocyte counts in the low and mid dose males at the end of the exposure period (Day 29) were significantly elevated. No effects on erythrocyte counts were observed at the high dose, hence the changes do not demonstrate a pattern of toxicity that are clearly related to the test compound. In addition, the test report stated that the values were apparently within the norm for the species and strain studied. Some slight effects on clinical chemistry and in hematology counts were noted but these do not demonstrate significant toxicity of the test compound. The significant leucocyte counts depression (20.1%) in the high-dose males, and increased creatinine (21%) values in the high-dose females were judged to be related to treatment. The NOAEL was 0.0010 mg a.i./L and the LOAEL was 0.0020 mg a.i./L, based on decreased body-weight gain and decreased leukocyte counts in the males and increased creatinine values in the females.
This study is classified as **acceptable** and satisfies the guideline requirement for a subchronic inhalation toxicity study in rats (82-4).

Dose and Endpoint Selected for Risk Assessment: NOAEL = 0.0010 mg a.i./L based on decreased body-weight gain and decreased leukocyte counts in males and increased creatinine values in females at 0.0020 mg a.i./L (LOAEL).

Comments about Study/Endpoint: The HIARC determined that the exposure period (21 days) in this study is appropriate for assessing short-term inhalation exposure risks but

not adequate for evaluation of intermediate or long-term inhalation exposure risks.

This risk assessment is required.

5.b. Intermediate and Long-Term

The HIARC determined that for intermediate and long-term exposure risk assessments, the oral NOAEL (0.6 mg/kg/day) should be used because: 1) of the concern for the toxicity (marked a progressive glomerulonephrosis and blood vessel aneurysm) observed in male rats following long-term oral exposure; 2) lack of a subchronic or chronic inhalation toxicity study to evaluate these effects via this route; and 3) the available inhalation study is not appropriate to evaluate long-term effects due to the short treatment period (21-days). Since and oral NOELs was selected, route-to-route extrapolation should be as follows:

Step I	Convert the inhalation exposure (µg/lb ai) using 100% inhalation absorption rate, application rate and acres treated and to an **oral equivalent dose** (mg/kg/day).
Step II	Convert the dermal exposure (mg/kg/day) using 45% dermal absorption rate, application rate and acres treated and to an **oral equivalent dose** (mg/kg/day). This dose should be combined with the converted oral dose in Step I.
Step III	Compare this oral equivalent dose to: the Oral NOEL of 0.6 mg/kg/day to calculate the MOE for intermediate and long-term inhalation risks.

This risk assessment is required.

D. Margins of Exposure for Occupational/Residential Exposures

The HIARC determined that a Margins of Exposure (MOE) of 100 is adequate for occupational exposure risk assessments. The MOE for the residential exposure will be determined during the risk characterization by the FQPA Safety Factor Committee.

E. Recommendation for Aggregate Exposure Risk Assessments

For **acute** aggregate exposure risk assessment, combine the high end exposure values from food + water and compare it to the acute RfD.

A **short-term** aggregate exposure risk assessment is not feasible since dermal and inhalation NOAELs were selected for the respective routes without a common endpoint (i.e., the dermal NOAEL is based on hepatotoxicity while the inhalation NOAEL is based

hematopoietic toxicity). Therefore, the dermal and inhalation exposures cannot be combined with dietary (food + water)**exposure; separate MOEs should be calculated.**

For intermediate and long-term aggregate exposure risk assessments, the dermal and inhalation exposures should be converted to dermal and inhalation absorption values (oral equivalent dosages), and these should be added to the oral exposures from food, water and incidental oral exposure, and compared to the oral NOEL to calculate an aggregate risk MOE.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study in Rat §83-5

MRID No. 41099502

Executive Summary: See Chronic Dietary

Discussion of Tumor Data: There was no evidence of carcinogenicity.

Adequacy of the Dose Levels Tested: The Committee considered that the doses tested were adequate to test the carcinogenic potential of the test compound.

2. Carcinogenicity Study in Mice

MRID No. 40792401

Executive Summary: In a carcinogenicity study, groups of HOE:NMRKf (SPF71) 60 mice/sex/group were fed with technical endosulfan (97.9% purity) at 0, 2, 6 and 18 ppm (≈ 0, 0.3, 0.9 and 2.6 mg/kg/day) for 24 months. A satellite group of twenty mice/sex/group was dosed in a similar fashion and were used for hematology and clinical chemistry evaluations. No treatment-related effects were evident in clinical signs, food consumption, hematology, clinical chemistry, urinalysis, organ weights and gross and microscopic evaluations. At study termination, statistically significant increased mortality was noted in the high-dose females (only 28% survivors *versus* 45% of the controls). Increased mortality in the high-dose females is judged to be treatment-related. Mean body weights of the males and females were comparable among all groups. A slight reduction in body weight gain in high-dose males (between weeks 13 and 26) was noted. At 12 months, the lung and ovary weights of the 18 ppm females were significantly ($p<0.05$) decreased and at 18 months, the relative liver weights of males dosed at 18 ppm and relative ovary weights in females dosed at 18 ppm were slightly but significantly decreased. At 24 months, organ weights were comparable among all groups. Decreases in organ weights noted at various intervals during the study are not judged to be related to treatment, because they are within the normal historical ranges.

Histopathologically, slight increased incidences of epithelial thickening of the urinary bladder were noted in all treated males (0, 5, 8 and 12, in the control, low-, mid- and high-dose groups, respectively) and females (0, 6, 9 and 10, in the control, low-, mid- and high-dose groups, respectively). The original reviewer of this study concluded these increases were not toxicologically significant, because of the "absence of a progression to a clear proliferative change". Lymphosarcoma was found in practically all organs of male and female mice. In addition, since it was noted equally among all groups, these occurrences were judged to be spontaneous and strain-related changes. These occurrences were not considered to be treatment-related effects. The systemic NOAEL was 6 ppm (0.9 mg/kg/day) and the LOAEL was 18 ppm (2.65 mg/kg/day), based on increased incidences of mortality in females.

This study is classified as **acceptable/ guideline** and it satisfies the guideline requirements for an oncogenicity study in mice (§83.2).

Discussion of Tumor Data: There was no evidence of carcinogenicity. The lymphosarcoma observed in all organs of male and female mice of both treated and control mice at comparable incidences were judged to be spontaneous and strain-related changes, and were not considered to be treatment-related effects.

Adequacy of the Dose Levels Tested: The Committee considered that the doses used in this study were adequate to test the carcinogenic potential of endosulfan.

IV. MUTAGENICITY

Endosulfan technical was inactive in the primary rat hepatocyte unscheduled DNA synthesis (UDS) assay (MRID#00148265), and was non-mutagenic in the mouse lymphoma forward mutation assay (MRID#00148266).

V. FQPA CONSIDERATIONS

1.. Adequacy of the Database:

The toxicology data base is not complete. Datagap exists for a subchronic neurotoxicity study in rats (§82-5). Studies on an acute delayed neurotoxicity in hen and acute neurotoxicity screening in the rat were submitted to the Agency.

Acceptable prenatal toxicity studies in rats and rabbits, and a 2-generation reproduction toxicity study in rats using endosulfan were submitted to the Agency.

2. Neurotoxicity:

In a 42-day delayed neurotoxicity study (MRID# 00147181), endosulfan technical (97.2% a.i.) in corn oil was administered by oral gavage to 40 white leghorn hens at 96 mg/kg. The result of this neurotoxicity study is inconclusive; in the 9/40 animals examined at 42 days after initial and challenge (day 21) dosing, there was no evidence of progressive nerve damage in the brain, spinal cord, or peripheral nerve. No evidence of a delayed neurotoxicity or neuropathology was observed in an acute delayed neurotoxicity study using endosulfan in hens.

The acute neurotoxity study (MRID#.44403101) is described in Section II.1. Acute Reference dose. The NOAEL was 12.5 mg/kg for males and 1.5 mg/kg for females. The LOAEL was 25 mg/kg for males based increased incidences of stilted gait, squatting posture, and irregular respiration, as well as decreased spontaneous activity. The LOAEL was 3 mg/kg for females, based on an increased incidence of stilted gait, squatting posture, straddled hindlimbs, irregular respirations, panting and bristled coat; decreased spontaneous activity was also noted.

c. Other evidence of neurotoxic effects

In a subchronic feeding study, decreased serum ChE activity was noted in female rats dosed at 360 mg/kg/day (40% at week 13) (MRID#00145668).

In a subchronic dermal toxicity study, neurological signs (tremors, Straub-tail, trismus, saltatory spasms, extension spasms and tetanoid spasms) were noted right after dosing in males dosed at 81 mg/kg and in females dosed at 18 and 36 mg/kg, with isolated incidences noted in females dosed at 12 mg/kg. These signs occurred 1 hour after dosing and disappeared 30 minutes after the onset of neurological signs. Since the number of these neurological signs was not presented in the study report, the toxicological significance of these findings cannot be evaluated with certainty. Decreased serum ChE activity was seen in female rats 80 mg/kg/day. (MRID#41048505).

In another subchronic dermal toxicity study), decreased serum ChE activity was noted in female rats dosed at 192 mg/kg/day and tonoclonic convulsions were noted in females dosed at 48 mg/kg/day (ACC# 257682/257683).

In a chronic toxicity feeding study in dogs, neurological effects were only noted in dogs dosed at 30/45/60 ppm (1.75 mg/kg/day). Increased incidences of neurologic findings in males and females were characterized by loss or weakening of righting reactions, and tonic contractions of the abdominal and masticatory muscles (MRID#41099501).

In a developmental toxicity study in rats, dams dosed at 6 mg/kg exhibited tonoclonic seizures, increased salivation, and hyperactivity (MRID# 43129101).

In a developmental toxicity study in rabbits (MRID#00094837), does dosed at 1.8 mg/kg showed tonoclonic convulsions, rapid breathing, increased salivation, and hyperactivity.

There are no indicators for any special sensitivity to the fetuses that are evident in either the rat or the rabbit studies.

3. Developmental Toxicity

In a developmental toxicity study (MRID#43129101), endosulfan technical (97.3% a.i.) was administered by gavage to four groups of 20 pregnant female Wistar rats at dose levels of 0, 0.66, 2.00, and 6.00 mg/kg/day from days 6 through 18 of gestation. The following treatment-related, effects were noted in dams dosed at 6.00 mg/kg/day dose level: 1) the deaths of four dams and two non-pregnant females; 2) decreased body weight gain (23% of control) during the first week of dosing and concomitant food consumption decrease (68% of control); 3) tonoclonic convulsions in 16 dams, three of which showed increased salivation and one of the latter also exhibited hyperactivity. No statistically significant differences were noted in the number of corpora lutea/dam, implantations/dam, live fetuses/dam, resorptions/dam, dead fetuses/dam, pre- and post-implantation losses, litter weight, fetal body weight (combined and per sex), or fetal crown-rump length among the groups. Only one malformed fetus was found in the 6.00 mg/kg/day dose group. There was, however, an increased incidence in the number of "retarded" fetuses (fetal weights of less than 3 gms) at the 6.00 mg/kg/day dose group (8 *versus* 5 litters in controls). The original reviewer considered the increased incidences of thoracic vertebral centra fragmentation and an increased incidence of "retarded" fetuses (fetuses weighing less than 3 grams) in the 6.00 mg/kg/day dose group, to be treatment-related. No other significant fetal malformations were noted. For maternal toxicity, the NOAEL was 2.00 mg/kg/day and the LOAEL was 6.00 mg/kg/day, based on increased death, tonoclonic convulsions, increased salivation, and decreased bodyweight gains and food consumption. For developmental toxicity, the NOAEL was 2.00 mg/kg/day and the LOAEL was 6.0 mg/kg/day, based on a slight increase in the incidence of fragmented thoracic vertebral centra and a slight increase in the occurrence of "retarded" fetuses (fetuses weighing less than 3 grams). There are no indicators of any special sensitivity to the fetus in this study.

This study is classified as **acceptable-guideline,** and satisfies the guideline requirements for a developmental toxicity study in rats (83-3). This study is a repeat study for an unacceptable developmental toxicity study (ACC# 243707).

In a developmental toxicity study (MRID#: 00094837), endosulfan technical (97.3% a.i.) was administered by gavage to four groups of 20 pregnant female New Zealand White rabbits at dose levels of 0, 0.3, 0.7, and 1.8 mg/kg/day from days 6 through 28 of gestation. The does were sacrificed on gestation day 29. The following effects were noted in the 1.8 mg/kg/day dams: 1) Four does dosed at 1.8 mg/kg/day died on gestation days 7, 10, 21 and 29. Three of them were due to improper oral gavage as evidenced by the presence of oil in the trachea and the lungs and one doe dosed at 1.8 mg/kg/day that died showed evidence of hemorrhagic activity. 2) Increased incidences of convulsions, rapid breathing, salivation and hyperactivity were also noted in does dosed at 1.8

mg/kg/day.3) Body weight losses were noted in does dosed at 0.7 and 1.8 mg/kg/day (-16 and -47 g/rabbit *versus* 43 g/rabbit in the controls) during days 19-29 but these values were not statistically significant. The body weight (after corrected for uterine weight) was only negative in does dosed at 1.8 mg/kg/day as compared to the controls (-17 g *versus* 5 g/rabbit in the controls). No treatment-related effects on fetal deaths/resorptions, altered growth, developmental anomalies and malformations were noted. Developmental toxicity was not observed at any dose level. For maternal toxicity, the NOAEL was 0.7 mg/kg/day and the LOAEL was 1.8 mg/kg/day, based on decreased bodyweight, increased incidences of deaths, convulsions, rapid breathing, salivation and hyperactivity. For developmental toxicity, the NOAEL was greater than 1.8 mg/kg/day, the highest dose tested; a LOAEL was not established.

This study is classified as **acceptable-guideline** and it satisfies the guideline requirements for a developmental toxicity study in rabbits (83-3).

4. Reproductive Toxicity:

In a 2-generation reproduction study (MRID#: 00148264), exposure of Crl:COBS CD(SD)BR rats to endosulfan (97% purity) via the diet during premating and through gestation and lactation, at dose levels of 0, 3, 15, and 75 ppm (0, 0.20, 1.00, and 4.99 mg/kg/day in males and 0, 0.24, 1.23, and 6.18 mg/kg/day in females), produced minimal maternal toxicity at the high-dose level. There were 32 rats/sex/group in the FO generation and 26 rats/sex/group in the F1 generation. Mortality, food/water consumption, and body weight were not affected in either generation, but there was a decrease in body-weight gain in the F0 females at the high-dose level during the first week of study (67% of control). Pregnancy rate, gestation times, the ability to rear young to weaning, and pre-coital time were comparable among the groups at both matings in both generations. F0 males displayed increased heart weight at the mid- and high-dose levels and increased liver and kidney weights at the high-dose level. F0 females displayed increased brain and liver weights at the high-dose level. In the Flb adults, the high-dose males displayed increased kidney weights compared to the controls and the females displayed increased liver weights at the mid- and high-dose levels. These organ weight changes were not considered to be toxicologically significant (see notes below regarding RfD Committee memo dated October 13, 1992). The litter size throughout both matings in both generations was not affected by dosing. In the first mating of the F0 generation, there was an increase in the cumulative litter loss (8 litters) at the high-dose level.

Litter and pup weights were comparable at birth among the groups in both generations, but there was a decrease in litter weight observed during the lactation to weaning period in both matings in the F0 generation, which was significant at the high-dose level in the first mating and at the mid- and high-dose levels in the second mating (dose-related). Because there was no corroborative finding of a decrease in the number of pups per litter or in pup weight, the decrease in litter weight is not considered to be treatment-related. Increased pituitary weights (high-dose ♀ pups of 1st mating in FO generation) and

increased uterine weights (high-dose female pups of 1st mating of Flb generation) were observed in the offspring. There were no histopathological findings observed that could be attributed to treatment. Although there were no significant effects noted on the dams, the dose levels are considered adequate, based on the results of the range-finding study in which there was an increase in cumulative pup loss and a reduction in litter size at the 100 ppm dose level at days 24 and 28 days post weaning. For parental systemic toxicity, the NOAEL was 1.23 mg/kg/day and the LOAEL was 6.18 mg/kg/day, based on decreased body weight. For offspring toxicity, the NOAEL was 1.2 mg/kg/day and LOAEL was 6.18 mg/kg/day, based on increased pituitary and uterine weights. The offspring effects were not considered to be severe when compared to the maternal effects, since it was seen only in one generation (not consistent) and these were not the target organ for toxicity in other studies with endosulfan.

5. Open Literature Data

Lakshmana and Raju (1994) administered endosulfan via gastric intubation to Wistar rat pups of both sexes at 6 mg/kg body weight/day from post-natal days 2-25. Its effect on levels of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) was assayed in olfactory bulb (OB), hippocampus (HI), visual cortex (VC), brainstem (BS) and cerebellum (CB) on days 10 and 25 using high-performance liquid chromatography (HPLC). The activity of acetylcholinesterase (AChE) was also estimated in the same regions of the brain. Performance in operant conditioning for solid food reward was assessed in 25-day-old rats. NA levels were increased in OB (12%, P = 0.01) and BS (10%, P = 0.05) at 10 days of age and in HI (20%, P = 0.01) and CB (12%, P = 0.05) at 25 days of age. DA levels were decreased in HI at both 10 (42%, P = 0.001) and 25 (45%, P= 0.001) days. Serotonin levels were 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). The activity of AchE was not different from the control groups in any of the regions studied. The investigators suggested that monoaminergic systems in the developing rat brain respond to endosulfan by undergoing something like a 'reorganization'. However, such changes do not ameliorate certain functional losses following the exposure to endosulfan as operant conditioning revealed deficits in acquisition as well as retention of memory.

The HIARC recommended that this study be reviewed / evaluated and that a DER be prepared.

Reference: 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, 1994:139-50

5. Determination of Susceptibility

The data base is complete and there are no data gaps pertaining to developmental or reproductive toxicity. The data provided no indication of increased sensitivity of rats or rabbits to *in utero* and post-natal exposure to endosulfan. Two prenatal developmental toxicity studies, one in rats and one in rabbits, failed to show evidence of developmental

toxicity in the absence of maternal toxicity. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity.

6. Recommendation for a Developmental Neurotoxicity Study

As discussed earlier (Section V.2. Neurotoxicity), treatment-related clinical sign of neurotoxicity was seen following oral exposures in rats, rabbits and dogs and via dermal exposure in rats. Neither changes in brain weights nor histopathological lesions of the central or peripheral nervous system were seen in the hens in the acute delayed neurotoxicity study, in the acute neurotoxicity study in rats or in the other subchronic and chronic studies. The HIARC, however, placed the requirement for a developmental neurotoxicity study in rats in **reserve** status because of the datagap for a subchronic neurotoxicity study in rats.

7. Determination of the FQPA Safety Factor:

Based on the hazard assessment for endosulfan, the HIARC recommends to the FQPA Safety Committee, that 10X factor for the protection of infants and children be reduced to 3X because:

1) the developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits;

2) the two generation reproduction toxicity study in rats showed no increased susceptibility in pups when compared to adults; and

3) there was no evidence of abnormalities in the development of fetal nervous system in the pre/post natal studies. Neither brain weight nor histopathology (perfuse or nonperfused) of the nervous system was affected in the subchronic and chronic toxicity studies.

However, there is a datagap for a subchronic neurotoxicity study in rats. Data from this study will be used (in conjunction with other studies) in determining the need for a developmental neurotoxicity study (which is currently placed in reserve status). The developmental neurotoxicity study will provide additional data (e.g., functional parameter development, potential increased susceptibility, effects on the fetal nervous system etc.).

The final recommendation on the FQPA Safety Factor, however, will be made during risk characterization by the FQPA Safety Factor Committee.

VI. HAZARD CHARACTERIZATION

Except for a datagap for a subchronic neurotoxicity study, the toxicology database is complete to assess the chronic toxicity, carcinogenicity, mutagenicity as well as the developmental and reproductive toxicity potential of endosulfan.

No organophosphate induced delayed neurotoxicity (OPIDN) was seen following a 42 day exposure in hens. A single oral exposure to rats resulted in clinical signs and alterations several functional observation parameters but no histopathology. Following subchronic oral exposures in rats, plasma and RBC ChEI was seen at doses as a low as 0.45 mg/kg/day in rats. Following dermal exposure in rabbits, plasma and RBC ChEI were seen at 25 and 50 mg/kg/day, respectively. Clinical signs of neurotoxicity observed included salivation, convulsions, tremors, Straub-tail, trismus, saltatory spasms, extension spasms and tetanoid spasms, decreased activity etc. However, neither changes in brain weights nor neuropathological lesions were seen via the oral and dermal studies in any species.

Chronic dietary administration to dogs resulted in decreased weight gains in males and tonic contractions of the muscles of the abdomen and chaps a few hours post dosing in both sexes. No treatment-related effects were seen in clinical, gross or histopathology.

Following chronic dietary administration to mice and rats, no target organ for endosulfan-induced toxicity was identified in mice. Systemic toxicity was limited to increased mortality in females at the highest dose tested. In rats, the kidney was the target organ for endosulfan-induced toxicity; renal toxicity at the highest dose tested manifested as enlarged kidneys in females and progressive glomerulonephrosis and a renal aneurysm in males.

There was no evidence of carcinogenicity in mice and rats when tested at doses that were judged to be adequate for assessing carcinogenic potential

Endosulfan was shown to be non-mutagenic following *in vivo* and *in vitro*

There was no evidence of increased susceptibility in rat and rabbit fetuses following *in utero* exposures in prenatal toxicity studies in rats and rabbits or increased susceptibility in the offsprings as compared to parental animal following pre/post natal exposure in the two generation reproduction study.

It has been reported in the open literature that endosulfan is suspected to affect normal hormone metabolism and endocrine function. In studies submitted to the Agency, treatment-related effects were seen in the two-generation reproduction study in rats characterized as increases in the pituitary glands weights and as increased incidences of parathyroid hyperplasia in male rats in the carcinogenicity study.

VII. DATA GAPS

Subchronic Neurotoxicity - Rat	§82-5
Developmental Neurotoxicity Toxicity Study - Rat (reserve)	§83-6

VII. ACUTE TOXICITY

Guideline#	Study Type	MRID	Results	Tox Category
81-1	Acute Oral	00038307	LD_{50} = 40.38 mg/kg in ♂ LD_{50} = 9.58 mg/kg in ♀	I
81-2	Acute Dermal	41183503	LD_{50} = 2000 mg/kg	III
81-3	Acute Inhalation	41183504	LC_{50} = 0.16-0.5 mg/L	I
81-4	Primary Eye Irritation	255157	Eye irritant (Residual opacity at day 13)	I
81-5	Primary Skin Irritation	00038309 00128649	Non-irritant Slightly irritant	IV IV
81-6	Dermal Sensitization	00136994	Not a dermal sensitizer	

VIII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL=1.5	Increased incidences of convulsions seen within 8 hours after dosing in females	Acute neurotoxicity-Rat
	UF=100	**Acute RfD = 0.015 mg/kg**	
Chronic Dietary	NOAEL = 0.6	Reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats	2-year chronic toxicity/ carcinognicitiy-Rat
	UF=100	**Chronic RfD = 0.006 mg/kg/day**	
Short-Term (Dermal)	Dermal NOAEL= 3.0	Hepatotoxicity (enlargement of parenchymal cells ,loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes.	21-day dermal toxicity-Rat
Intermediate-Term (Dermal) [a]	Oral NOAEL= 0.6	Reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats	2-year chronic toxicity/ carcinognicitiy-Rat
Long-Term (Dermal) [a]	Oral NOAEL= 0.6	Reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats	2-year chronic toxicity/ carcinognicitiy-Rat
Short-Term (Inhalation)	Inhalation NOAEL= 0.0010 mg/L	Decreased body-weight gain and decreased leukocyte counts in males and increased creatinine values in females s	21-day inhalation-Rat
Intermediate-Term (Inhalation) [b]	Oral NOAEL= 0.6	Reduced body weight gains.	2-year chronic toxicity/ carcinognicitiy-Rat
Intermediate-Term (Inhalation) [b]	Oral NOAEL= 0.6	Reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats	2-year chronic toxicity/ carcinognicitiy-Rat

a = Since an Oral NOAEL was selected a dermal absorption factor of 45% should be used for route-to-route extrapolation.

b = Since and Oral NOAEL was selected an inhalation absorption (100%) factor should be used for route-to-route extrapolation.

APPENDIX B

HED DOC. NO. 012974

20-NOV-1998

MEMORANDUM

SUBJECT: ***ENDOSULFAN*** - Report of the FQPA Safety Factor Committee.

FROM: Brenda Tarplee, Executive Secretary
FQPA Safety Factor Committee
Health Effects Division (7509C)

THROUGH: Ed Zager, Chair
FQPA Safety Factor Committee
Health Effects Division (7509C)

TO: Steve DeVito, Risk Assessor
Reregistration Action Branch 2
Health Effects Division (7509C)

PC Code: 079401

The Health Effects Division (HED) FQPA Safety Factor Committee met on November 2, 1998 to evaluate the hazard and exposure data for endosulfan and recommended that the FQPA Safety Factor (as required by Food Quality Protection Act of August 3, 1996) be reduced (to 3x) in assessing the risk posed by this chemical.

I. HAZARD ASSESSMENT

1. Determination of Susceptibility

The Hazard Identification Assessment Review Committee (HIARC) determined that the available Agency Guideline studies indicated no increased susceptibility of rats or rabbits to *in utero* and/or postnatal exposure to Endosulfan. In the prenatal developmental toxicity studies in rats and rabbits, developmental toxicity was seen only in the presence of maternal toxicity. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity (*Memorandum:* D. Liem and J. Rowland to S. DeVito dated October 7, 1998).

2. Open Literature Data

Lakshmana and Raju (1994) administered endosulfan via gastric intubation to Wistar rat pups of both sexes at 6 mg/kg body weight/day from post-natal days 2-25. Its effect on levels of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) was assayed in olfactory bulb (OB), hippocampus (HI), visual cortex (VC), brainstem (BS) and cerebellum (CB) on days 10 and 25 using high-performance liquid chromatography (HPLC). The activity of acetylcholinesterase (AChE) was also estimated in the same regions of the brain. Performance in operant conditioning for solid food reward was assessed in 25-day-old rats. NA levels were increased in OB (12%, P = 0.01) and BS (10%, P = 0.05) at 10 days of age and in HI (20%, P = 0.01) and CB (12%, P = 0.05) at 25 days of age. DA levels were decreased in HI at both 10 (42%, P = 0.001) and 25 (45%, P= 0.001) days. Serotonin levels were 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). The activity of AchE was not different from the control groups in any of the regions studied. The investigators suggested that monoaminergic systems in the developing rat brain respond to endosulfan by undergoing something like a 'reorganization'. However, such changes do not ameliorate certain functional losses following the exposure to endosulfan as operant conditioning revealed deficits in acquisition as well as retention of memory (Reference: 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, 1994:139-50).

3. Adequacy of Toxicity Database

The HIARC determined that the requirement for a developmental neurotoxicity study in rats was reserved for endosulfan pending the receipt and review of a subchronic neurotoxicity studies in rats. However, **the FQPA Safety Factor Committee concluded that a developmental neurotoxicity study in rats is required for endosulfan** due to

concern by the Committee for: 1) the fetal effects reported in the open literature abstract (discussed above); 2) the severity of effects seen in the female offspring of the F_0

generation (increased pituitary) and F_1b generation (increased uterine weights) at the high-dose when compared to the toxicity observed in parental animals (decreased body weight) at this dose in the two-generation reproduction study in rats; and 3) the subchronic neurotoxicity study (requested by the HIARC) will only address the neuropathological concerns resulting from exposure to endosulfan - a developmental neurotoxicity study will provide the critical data demonstrating the toxic effects of endosulfan on the developing fetal nervous system.

NOTE: The Agency should be consulted with respect to the Developmental Neurotoxicity study design / protocol.

II. EXPOSURE ASSESSMENT AND RISK CHARACTERIZATION

1. Dietary (Food) Exposure Considerations

Endosulfan is widely used on many agricultural crops and also in residential settings as an insecticide and aracicide. The chemical is a mixture of isomers and the tolerance expression includes the alpha, beta isomers plus a sulfate metabolite.

Tolerances for residues of Endosulfan and its metabolites are established in/on many RACs including fruits, vegetables grains, milk and meat at levels ranging from 0.1 ppm to 2.0 ppm (40CFR180.182). Codex maximum residue limits (MRLs) for residues of Endosulfan are established in/on various plant and animal commodities.

There are numerous field trial data on various commodities, reflecting various application sites throughout the country. Additionally, PDP and FDA monitoring data are available for endosulfan. Residues of endosulfan have been reported by PDP and FDA in a variety of crops. For example, in 1995 endosulfan was detected in apples (7%), carrots (4%), grapes (4%), green beans (24%), peaches (8%), potatoes (20%), spinach (14%), corn (0.1%), peas (0.3%), and oranges (2%) - for which there is no tolerance. The Limit of Quantitation (LOQ) for these data is ~0.01 ppm.

The HED Dietary Exposure Evaluation Model (DEEM) is used to assess the risk from acute and chronic dietary exposure to residues of Endosulfan in food. These analyses are based on the consumption database used by DEEM using reassessed tolerance values.

2. Dietary (Drinking Water) Exposure Considerations

A drinking water exposure assessment for Endosulfan had not yet been performed at the time of this meeting. EFED is in the process of: 1) completing the environmental fate assessment (as quantitative an evaluation as possible); 2) modeling exposures for high

use crops and other potentially vulnerable sites (which will be determined after the Smart meeting with the registrant); and 3) evaluating the extent and quality of existing monitoring data, if necessary.

The environmental fate data base for Endosulfan is not complete. EFED is currently in the process of reviewing new studies submitted by the registrant to fulfill Agency data requirements and investigating other sources of data for endosulfan. Once data review is completed, a quantitative fate assessment sufficient for assessing drinking water exposure can be developed.

Supplemental studies listed in the EFGWB One-Liner Database suggest that endosulfan may be moderately persistent in soils but its high affinity to sorb to soil particles, reduces its susceptibility to leaching. Although endosulfan does not appear to be highly mobile, it may be persistent enough in some instances to move to ground water (detects have been reported in the EPA Pesticides in Ground Water Database). Movement to surface water sources of drinking water is likely to occur via spray drift and runoff adsorbed to soil particles. This is supported by several studies which have reported endosulfan detects in surface water.

Endosulfan consists of isomers which appear to have some differences in persistence. The extent to which these differences will affect the fate assessment, if any, is not yet known. Endosulfan sulfate is expected to be the degradate of concern. Further information on the fate of this degradate is under investigation.

Ground water and surface water EECs for Endosulfan will be based upon modeling and supported by any available monitoring data. The EFED models used for ground and surface source drinking water exposure assessments result in estimates that are considered to be upper-bound concentrations.

3. Residential Exposure Considerations

Residential uses of the insecticide, endosulfan, include applications made to ornamentals and small fruit trees and to home vegetable gardens. It is formulated as a liquid spray (maximum rate of one pound per acre) and as a dust (maximum rate of 3.5 pounds per acre). The frequency of application varies with each crop ranging from 1-5 applications per growing season. Exposure to infants and children could occur during and after application of Endosulfan. For example, toddlers could be exposed from incidental soil ingestion by hand-to-mouth activity in garden plots.

Chemical-specific product use information for endosulfan was provided via the LUIS report from BEAD. A large sample of labels are also being used to ascertain the use pattern, potential exposure scenarios, and exposed populations of concern.

There are currently no chemical- or site-specific data available to assess the exposure resulting from the residential use of endosulfan. The Pesticide Handler Exposure Database (PHED) and/or the Draft HED Standard Operating Procedures (SOP) for Residential Exposure Assessments will be used for all residential calculations with no

deviations made to the SOP assumptions. A dermal absorption factor of 45% will be used with the oral dose and endpoint selected for intermediate and chronic risk assessments. This value (45%) is based on data from two dermal absorption studies in rats (§85-2; MRID Nos. 40223601 and 41048504).

III. SAFETY FACTOR RECOMMENDATION AND RATIONALE

1. FQPA Safety Factor Recommendation

The Committee recommended that the **FQPA safety factor** for protection of infants and children (as required by FQPA) be **reduced to 3x.**

2. Rationale for Reducing the FQPA Safety Factor

The HIARC determined that there is: 1) no indication of increased susceptibility of rats or rabbit fetuses to *in utero* exposure in the developmental toxicity study for endosulfan; 2) quantitatively, no indication of increased susceptibility to rat offspring following pre- and/or post-natal exposure in reproductive study; and 3) no evidence of adverse effects on the developing fetal nervous system in any of these studies. Therefore, the HIARC, using a tiered approach, placed the requirement for a developmental neurotoxicity study in reserve pending the receipt of the subchronic neurotoxicity study.

However, the FQPA Safety Factor Committee concluded that it was appropriate to request the developmental neurotoxicity study in rats at this time because the subchronic neurotoxicity study will only address the neuropathological concerns in adults and not the concern for effects in developing fetuses. The developmental neurotoxicity study is requested at this time because of the concern for: 1) the fetal effects reported in the open literature abstract (Lakshmana et al., 1994); and 2) the severity of effects seen in the female offspring of the F_0 generation (increased pituitary) and F_1b generation (increased uterine weights) at the high-dose when compared to the toxicity observed in parental animals (decreased body weight) at this dose in the two-generation reproduction study in rats.

The FQPA Safety Factor Committee concluded that the **FQPA safety factor** is required, however can be **reduced to 3x** because: 1) there is no evidence of increased susceptibility in any study; 2) the severity of the fetal effects in the reproduction study were not consistent between generations and the target organ toxicity seen in this study was not seen in any other study; and 3) reliable data and conservative assumptions in screening level models were used to assess the potential dietary (food and water) and residential exposure to this chemical. Consequently the FQPA safety factor was reduced based on the uncertainty associated with the data gap for a developmental neurotoxicity study in rats.

3. Population Subgroups for Application of the Safety Factor

The Committee determined that the FQPA safety factor (3x) is applicable for the following subpopulations:

Acute Dietary Assessment: All populations which include Infants and Children. The FQPA factor is appropriate for these populations due to the uncertainty regarding the effects on the developing fetal nervous system (data gap). This uncertainty is being addressed by the requirement of a developmental neurotoxicity study in rats.

Chronic Dietary Assessment: All populations which include Infants and Children. The FQPA factor is appropriate for these populations due to the uncertainty regarding the effects on the developing fetal nervous system (data gap). This uncertainty is being addressed by the requirement of a developmental neurotoxicity study in rats.

Residential (Short-, Intermediate- and/or Long-Term) Assessment(s): All populations which include Infants and Children. The FQPA factor is appropriate for these populations since the potential for residential exposure to infants and children resulting from the use of endosulfan exists and there is uncertainty regarding the effects on the developing fetal nervous system after such exposure. This uncertainty is being addressed by the requirement of a developmental neurotoxicity study in rats.

APPENDIX C

HED DOC. NO. 012974

20-NOV-1998

MEMORANDUM

SUBJECT: ***ENDOSULFAN*** - Report of the FQPA Safety Factor Committee.

FROM: Brenda Tarplee, Executive Secretary
FQPA Safety Factor Committee
Health Effects Division (7509C)

THROUGH: Ed Zager, Chair
FQPA Safety Factor Committee
Health Effects Division (7509C)

TO: Steve DeVito, Risk Assessor
Reregistration Action Branch 2
Health Effects Division (7509C)

PC Code: 079401

The Health Effects Division (HED) FQPA Safety Factor Committee met on November 2, 1998 to evaluate the hazard and exposure data for endosulfan and recommended that the FQPA Safety Factor (as required by Food Quality Protection Act of August 3, 1996) be reduced (to 3x) in assessing the risk posed by this chemical.

I. HAZARD ASSESSMENT

1. Determination of Susceptibility

The Hazard Identification Assessment Review Committee (HIARC) determined that the available Agency Guideline studies indicated no increased susceptibility of rats or rabbits to *in utero* and/or postnatal exposure to Endosulfan. In the prenatal developmental toxicity studies in rats and rabbits, developmental toxicity was seen only in the presence of maternal toxicity. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity (*Memorandum:* D. Liem and J. Rowland to S. DeVito dated October 7, 1998).

2. Open Literature Data

Lakshmana and Raju (1994) administered endosulfan via gastric intubation to Wistar rat pups of both sexes at 6 mg/kg body weight/day from post-natal days 2-25. Its effect on levels of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) was assayed in olfactory bulb (OB), hippocampus (HI), visual cortex (VC), brainstem (BS) and cerebellum (CB) on days 10 and 25 using high-performance liquid chromatography (HPLC). The activity of acetylcholinesterase (AChE) was also estimated in the same regions of the brain. Performance in operant conditioning for solid food reward was assessed in 25-day-old rats. NA levels were increased in OB (12%, P = 0.01) and BS (10%, P = 0.05) at 10 days of age and in HI (20%, P = 0.01) and CB (12%, P = 0.05) at 25 days of age. DA levels were decreased in HI at both 10 (42%, P = 0.001) and 25 (45%, P= 0.001) days. Serotonin levels were 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). The activity of AchE was not different from the control groups in any of the regions studied. The investigators suggested that monoaminergic systems in the developing rat brain respond to endosulfan by undergoing something like a 'reorganization'. However, such changes do not ameliorate certain functional losses following the exposure to endosulfan as operant conditioning revealed deficits in acquisition as well as retention of memory (Reference: 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, 1994:139-50).

3. Adequacy of Toxicity Database

The HIARC determined that the requirement for a developmental neurotoxicity study in rats was reserved for endosulfan pending the receipt and review of a subchronic neurotoxicity studies in rats. However, **the FQPA Safety Factor Committee concluded that a developmental neurotoxicity study in rats is required for endosulfan** due to

concern by the Committee for: 1) the fetal effects reported in the open literature abstract (discussed above); 2) the severity of effects seen in the female offspring of the F_0

generation (increased pituitary) and F_1b generation (increased uterine weights) at the high-dose when compared to the toxicity observed in parental animals (decreased body weight) at this dose in the two-generation reproduction study in rats; and 3) the subchronic neurotoxicity study (requested by the HIARC) will only address the neuropathological concerns resulting from exposure to endosulfan - a developmental neurotoxicity study will provide the critical data demonstrating the toxic effects of endosulfan on the developing fetal nervous system.

NOTE: The Agency should be consulted with respect to the Developmental Neurotoxicity study design / protocol.

II. EXPOSURE ASSESSMENT AND RISK CHARACTERIZATION

1. Dietary (Food) Exposure Considerations

Endosulfan is widely used on many agricultural crops and also in residential settings as an insecticide and aracicide. The chemical is a mixture of isomers and the tolerance expression includes the alpha, beta isomers plus a sulfate metabolite.

Tolerances for residues of Endosulfan and its metabolites are established in/on many RACs including fruits, vegetables grains, milk and meat at levels ranging from 0.1 ppm to 2.0 ppm (40CFR180.182). Codex maximum residue limits (MRLs) for residues of Endosulfan are established in/on various plant and animal commodities.

There are numerous field trial data on various commodities, reflecting various application sites throughout the country. Additionally, PDP and FDA monitoring data are available for endosulfan. Residues of endosulfan have been reported by PDP and FDA in a variety of crops. For example, in 1995 endosulfan was detected in apples (7%), carrots (4%), grapes (4%), green beans (24%), peaches (8%), potatoes (20%), spinach (14%), corn (0.1%), peas (0.3%), and oranges (2%) - for which there is no tolerance. The Limit of Quantitation (LOQ) for these data is ~0.01 ppm.

The HED Dietary Exposure Evaluation Model (DEEM) is used to assess the risk from acute and chronic dietary exposure to residues of Endosulfan in food. These analyses are based on the consumption database used by DEEM using reassessed tolerance values.

2. Dietary (Drinking Water) Exposure Considerations

A drinking water exposure assessment for Endosulfan had not yet been performed at the time of this meeting. EFED is in the process of: 1) completing the environmental fate assessment (as quantitative an evaluation as possible); 2) modeling exposures for high

use crops and other potentially vulnerable sites (which will be determined after the Smart meeting with the registrant); and 3) evaluating the extent and quality of existing monitoring data, if necessary.

The environmental fate data base for Endosulfan is not complete. EFED is currently in the process of reviewing new studies submitted by the registrant to fulfill Agency data requirements and investigating other sources of data for endosulfan. Once data review is completed, a quantitative fate assessment sufficient for assessing drinking water exposure can be developed.

Supplemental studies listed in the EFGWB One-Liner Database suggest that endosulfan may be moderately persistent in soils but its high affinity to sorb to soil particles, reduces its susceptibility to leaching. Although endosulfan does not appear to be highly mobile, it may be persistent enough in some instances to move to ground water (detects have been reported in the EPA Pesticides in Ground Water Database). Movement to surface water sources of drinking water is likely to occur via spray drift and runoff adsorbed to soil particles. This is supported by several studies which have reported endosulfan detects in surface water.

Endosulfan consists of isomers which appear to have some differences in persistence. The extent to which these differences will affect the fate assessment, if any, is not yet known. Endosulfan sulfate is expected to be the degradate of concern. Further information on the fate of this degradate is under investigation.

Ground water and surface water EECs for Endosulfan will be based upon modeling and supported by any available monitoring data. The EFED models used for ground and surface source drinking water exposure assessments result in estimates that are considered to be upper-bound concentrations.

3. Residential Exposure Considerations

Residential uses of the insecticide, endosulfan, include applications made to ornamentals and small fruit trees and to home vegetable gardens. It is formulated as a liquid spray (maximum rate of one pound per acre) and as a dust (maximum rate of 3.5 pounds per acre). The frequency of application varies with each crop ranging from 1-5 applications per growing season. Exposure to infants and children could occur during and after application of Endosulfan. For example, toddlers could be exposed from incidental soil ingestion by hand-to-mouth activity in garden plots.

Chemical-specific product use information for endosulfan was provided via the LUIS report from BEAD. A large sample of labels are also being used to ascertain the use pattern, potential exposure scenarios, and exposed populations of concern.

There are currently no chemical- or site-specific data available to assess the exposure resulting from the residential use of endosulfan. The Pesticide Handler Exposure Database (PHED) and/or the Draft HED Standard Operating Procedures (SOP) for Residential Exposure Assessments will be used for all residential calculations with no

deviations made to the SOP assumptions. A dermal absorption factor of 45% will be used with the oral dose and endpoint selected for intermediate and chronic risk assessments. This value (45%) is based on data from two dermal absorption studies in rats (§85-2; MRID Nos. 40223601 and 41048504).

III. SAFETY FACTOR RECOMMENDATION AND RATIONALE

1. FQPA Safety Factor Recommendation

The Committee recommended that the **FQPA safety factor** for protection of infants and children (as required by FQPA) be **reduced to 3x.**

2. Rationale for Reducing the FQPA Safety Factor

The HIARC determined that there is: 1) no indication of increased susceptibility of rats or rabbit fetuses to *in utero* exposure in the developmental toxicity study for endosulfan; 2) quantitatively, no indication of increased susceptibility to rat offspring following pre- and/or post-natal exposure in reproductive study; and 3) no evidence of adverse effects on the developing fetal nervous system in any of these studies. Therefore, the HIARC, using a tiered approach, placed the requirement for a developmental neurotoxicity study in reserve pending the receipt of the subchronic neurotoxicity study.

However, the FQPA Safety Factor Committee concluded that it was appropriate to request the developmental neurotoxicity study in rats at this time because the subchronic neurotoxicity study will only address the neuropathological concerns in adults and not the concern for effects in developing fetuses. The developmental neurotoxicity study is requested at this time because of the concern for: 1) the fetal effects reported in the open literature abstract (Lakshmana et al., 1994); and 2) the severity of effects seen in the female offspring of the F_0 generation (increased pituitary) and F_1b generation (increased uterine weights) at the high-dose when compared to the toxicity observed in parental animals (decreased body weight) at this dose in the two-generation reproduction study in rats.

The FQPA Safety Factor Committee concluded that the **FQPA safety factor** is required, however can be **reduced to 3x** because: 1) there is no evidence of increased susceptibility in any study; 2) the severity of the fetal effects in the reproduction study were not consistent between generations and the target organ toxicity seen in this study was not seen in any other study; and 3) reliable data and conservative assumptions in screening level models were used to assess the potential dietary (food and water) and residential exposure to this chemical. Consequently the FQPA safety factor was reduced based on the uncertainty associated with the data gap for a developmental neurotoxicity study in rats.

3. Population Subgroups for Application of the Safety Factor

The Committee determined that the FQPA safety factor (3x) is applicable for the following subpopulations:

Acute Dietary Assessment: All populations which include Infants and Children. The FQPA factor is appropriate for these populations due to the uncertainty regarding the effects on the developing fetal nervous system (data gap). This uncertainty is being addressed by the requirement of a developmental neurotoxicity study in rats.

Chronic Dietary Assessment: All populations which include Infants and Children. The FQPA factor is appropriate for these populations due to the uncertainty regarding the effects on the developing fetal nervous system (data gap). This uncertainty is being addressed by the requirement of a developmental neurotoxicity study in rats.

Residential (Short-, Intermediate- and/or Long-Term) Assessment(s): All populations which include Infants and Children. The FQPA factor is appropriate for these populations since the potential for residential exposure to infants and children resulting from the use of endosulfan exists and there is uncertainty regarding the effects on the developing fetal nervous system after such exposure. This uncertainty is being addressed by the requirement of a developmental neurotoxicity study in rats.

Appendix A:
ENDOSULFAN
Evidences of Endocrine-related Effects
(David Liem 11/24/98)

The EDSTAC (1996) defines an "endocrine disruptor" as an exogenous substance that changes endocrine function and causes adverse effects at the level of the organism, its progeny, and/or (sub)populations of organisms.

Below is a summary of endocrine-related effects as it relates to endosulfan. Evidence of endocrine-related effects are based on data of submitted guideline studies and from the literature.

Conclusions

Based on the evidence and effects on the endocrine glands as well as the results of hormonal changes presented below, it could be concluded that endosulfan may affect endocrine systems as some other organochlorine pesticides which have estrogenic and enzyme-inducing properties.

In a standard chronic feeding study, testicular atrophy was noted in male rats dosed at 20.4 mg/kg/day, characterized by degeneration and necrosis of the germinal cells lining of the seminiferous tubules, multinucleated cells (fusions bodies), and calcium deposition resulting in aspermatogenesis (MRID#00004256). It was not clear whether the degeneration and necrosis of the germinal cells lining of the seminiferous tubules of the male rats were relate to endocrine related effects. Decreased sperm counts in the cauda epididymis and reduced intratesticular spermatid counts associated with elevation in the activities of specific testicular marker enzymes (sorbitol dehydrogenase, lactic dehydrogenase, gamma glutamyl transpeptidase, and glucose-6- phosphate dehydrogenase) were seen in male rats dosed with endosulfan at 2.5 mg/kg/day through oral intubation for 70 days (Sinha, et al, 1995). In another rat study, oral administration of endosulfan at 2.5 mg/kg for 60 days resulted in a slight decreased testes weight (Ansari et al, 1984).

Increased pituitary weight in female pups in the Fo generation and increased uterine weights in female pups of the F1b generation were noted in a 2-generation rat study at 6.18 mg/kg/day dose level (MRID#00148264). In addition, parathyroid hyperplasia was found in male rats dosed at 20.8 mg/kg/day in a chronic feeding study (MRID#00004256). In a special acute rat study, intraperitoneally injection of endosulfan at 4.1 mg/kg resulted in thyroid follicle damage (Cerkezkayabekir et al, 1997).

Adverse effects on the ovaries and adrenal glands were not reported in experimental animals.

It should be noted that the Acute RfD for endosulfan was based on a NOAEL of 1.5 mg/kg, based on increased incidences of convulsions in female rats at 3 mg/kg in an Acute Neurotoxicity Study in Rats (MRID No.:44403101). The Chronic RfD was based on a NOAEL of 0.6 mg/kg/day, based on reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats dosed at 2.9 mg/kg/day in a Combined Chronic/Carcinogenicity Study in Rats (MRID#41099502).

Evidences of endosulfan affecting the hormonal system based on changes of estrogenic, androgenic, and progesteronic activities are as follows:

1. Evidence of Estrogenic Effects

In an *in vitro* bioassay (E-screen test) using human breast estrogen-sensitive MCF7 cells, endosulfan was shown to have estrogenic properties comparable to those of DDT and chlordecone, which are known to be estrogenic in rodent models. Thus, endosulfan has estrogenic effects on human estrogen-sensitive cells (Soto, et al, 1994). For endosulfan the Relative Proliferative Effect was reported between 77 - 81% that of estradiol (Soto, et al., 1995).

The recombinant yeast bioassay was used for the screening and determining the direct interaction between ER and estrogenic compounds. This system was used in parallel with a more elaborate biological system, trout

hepatocyte aggregate cultures, to examine the estrogenic potency of a wide spectrum of chemicals. In hepatocyte cultures, the vitellogenin gene whose expression is principally dependent upon estradiol was used as a biomarker. The competitive binding assays were performed to determine the direct interaction between rtER (estrogen receptor) and xenobiotics. In this study, endosulfan exhibited estrogenic activity in the two bioassays (Petit et al, 1977).

In a competition binding assays with a synthetic progestin [3H]R5020, endosulfan inhibited the binding capacity of [3H]R5020 to aPR (progesterone receptor). These results provided evidence supporting the hypothesis that the reported reproductive abnormalities may be related to the modulation of endocrine-related responses (Vonier et al, 1996).

Although there are questions regarding the validity or reliability of the *in vitro* testing methods, it should be noted that the results of these assays on known "*endocrine disruptor"* chemicals revealed consistent results among the three assays used (the receptor binding, the transcriptional activation, and the *in vivo* effect in an estrogen-responsive tissue) in respect to what is known about the estrogenic activities of the chemicals tested and their requirements for metabolic activation (Shelby et al, 1996).

2. Evidence of Androgenic Effects

Male adult rats fed endosulfan (po) at 7.5 and 10 mg/kg body weight dose levels for 15 and 30 days, significantly inhibited testicular androgen biosynthesis. No appreciable alterations were apparent in body weights, testicular wet weights, and cytosolic and microsomal protein contents of testes in treated rats (Singh et al, 1990).

Endosulfan lactone caused the greatest reduction in binding of androgen to the androgen receptor, alpha endosulfan and endosulfan sulfate caused slight reduction in binding at high concentrations (1 mM) when tested in a cell-free in vitro binding assay using cytosolic prostate tissue extracted from mature rats and ^{3}H-methyltrienolone, a synthetic androgen (Brieske, 1997).

Chronic endosulfan exposure in rats led to a considerable increase in the activities of drug metabolizing enzymes, whereas it had inhibitory effect on the activities of enzymes involved in the androgen biotransformation (Singh, 1989).

3. Evidence of Progesteronic Effects

The mammalian sperm acrosome reaction (AR) is essential to fertilization. The acrosome reaction (AR) can be initiated *in vitro* by progesterone, a putative physiological initiator that helps to activate sperm GABA receptor/chloride channels, and by glycine. Glycine is a substitute for the egg zona pellucida, which activates sperm glycine receptor/chloride channels. At 1 nM (0.41 ng/ml or 0.41 ppb), chlordane and endosulfan, chlorinated cyclodiene blockers of insect neuronal GABA receptor/chloride channels, strongly inhibited the AR (acrosome reaction) initiated by progesterone or glycine. Inhibitory concentrations of these cyclodienes are well within the range detected in human and wildlife tissue and fluids as a result of environmental contamination (Turner, 1997).

The ability of chemicals to bind the estrogen receptor (aER) and progesterone receptor (aPR) in a protein extract prepared from the oviduct of alligators was evaluated. A competition binding assay with the synthetic progestin (3H)R5020 was conducted to assess the ability of chemicals to interact with aPR. This assay showed that endosulfan inhibited (3H)R5020 binding to aPR (progesterone receptor). There is evidence that environmental chemicals bind with the aER and aPR of the American alligator, supporting the hypothesis that the reported reproductive abnormalities in American alligators may be related to modulation of endocrine-related responses (Vonier, 1996).

Table 2. Summary Table of the Effects of Endosulfan on Endocrine Glands

Organ/Hormone Affected	Study/Species	Dose mg/kg/d	Notes
Testes Weight↓	* Rat - 60-day study	2.5	* Slightly increased
	* Rat - 70-day study	2.5	* Accompanied by decreased sperm counts in the cauda epididymis and reduced intratesticular spermatid counts associated with elevation in the activities of specific testicular marker enzymes (sorbitol dehydrogenase, lactic dehydrogenase, gamma glutamyl transpeptidase, and glucose-6-phosphatedehydrogenase)
	* Rat - Chronic study	20.8	* Testicular atrophy characterized by degeneration and necrosis of the germinal cells lining of the seminiferous tubules, multi-nucleated cells (fusions bodies), and calcium deposition resulting in aspermatogenesis.
Uterine Weight↑	*Rat - Two-generation study	6.18	*Female pups of the F1b generation
Pituitary Gland Weight ↑	*Rat - Two-generation study	6.18	* Female pups in the Fo generation
Parathyroid Hyperplasia ↑	* Rat - Chronic study	20.8	* In males
Thyroid follicle damage ↑	* Rat - Acute intraperitoneal Injection	4.16	* Follicle damage

I. Evidence Affecting Endocrine Glands and Functions

A. Testes:

1. Two groups of 50 Osborne-Mendel rats/sex/group, were given endosulfan (98.8%) in the diet at the time weighted average concentration of 408 and 952 ppm to males (20.4 mg/kg/day and 40.8 mg/kg/day) and 223 and 445 ppm (11.1 mg/kg/day and 22.0 mg/kg/day) to females, respectively. After a 78-week period of chemical administration, observation of female rats continued for 33 additional weeks.

Dose-related depression in the rates of growth and survival were shown in the male rats. At week 54, 52% of the high-dose males died (the Tarone test for a positive dose-related trend in mortality was highly significant). The low- and high-dose male rats were terminated during week 74 and week 82, respectively. No appreciable difference in mean body weight among the females was noted. At termination (week 102), 70% of the controls, 62% of the low-dose and 50% of the high-dose groups survived.

At the doses administered to rats in this study endosulfan was toxic, inducing a high incidence of toxic nephropathy in both sexes. In the males, 47/50 and 43/47 were observed in the low- and high-dose (20.4 and 40.8 mg/kg/day), respectively. In the females, 27/50 and 29/50 toxic nephropathy was also observed low- and high-dose (11.1 and 22.0 mg/kg/day), respectively.

A parathyroid hyperplasia was reported to be associated with renal lesions and occurred in 21/48 low-dose (20.4 mg/kg/day) and in 18/47 high-dose (40.8 mg/kg/day) males. Only 1/49 parathyroid lesion was noted in the low dose female.

Testicular atrophy was noted in 3/19 controls, 18/47 low-dose (20.4 mg/kg/day) , and 24/47 high-dose (40.8 mg/kg/day) male rats; this testicular atrophy was characterized by degeneration and necrosis of the germinal cells lining of the seminiferous tubules, multinucleated cells (fusions bodies), and calcium deposition resulting in aspermatogenesis.

In the high dose male rats early mortality, associated with toxic nephropathy, was noted. Probably as a result of a high mortality rate, the incidence of tumors in the males was higher in the controls as compared to the low- or high-dose groups. Early deaths of the male rats preclude the usefulness of any analysis of late developing tumors.

This study was classified as unacceptable guideline study for a carcinogenicity study in rats (NCI, 1978. Bioassay of Endosulfan for Possible Carcinogenicity. (CAS# 115-29-7) and NCI-CG-TR-62. Report. Hazelton Lab. Inc., Vienna, Virginia. DHEW Publ# (NIH) 78-1312. NCI Technical Report No.62, 1978. MRID#:00004256).

2. Adult male rats were exposed to 0, 2.5, 5.0 or 10.0 mg endosulfan/kg body weight through oral intubation for 70 days. Decreased sperm counts in the cauda epididymis and reduced intratesticular spermatid counts associated with elevation in the activities of specific testicular marker enzymes (sorbitol dehydrogenase, lactic dehydrogenase, gamma glutamyl transpeptidase, and glucose-6-phosphatedehydrogenase) were seen in all the endosulfan-dosed groups. Endosulfan caused impairment in testicular functions by altering activities of the enzymes responsible for spermatogenesis, thereby influencing intratesticular spermatid count and causing low sperm production and sperm deformity (Sinha N, Narayan R, Shanker R, Saxena DK. Endosulfan-induced biochemical changes in the testis of rats. Vet Hum Toxicol 1995 Dec; 37(6):547-549).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

3. Endosulfan was administered orally (2.5 and 7.5 mg/kg) daily to male rats for a period of 60 days. The distribution pattern of alpha- and beta-isomers was studied using a gas-liquid chromatograph equipped with an electron capture detector. There was a significant increase in liver and lung weights. The testes weight was slightly decreased. At both dose levels, the concentration of alpha-isomer was highest in kidney (574 and 1655 ng/g, respectively), followed by lung, ventral prostate, spleen, testes and brain. In the seminal vesicle, epididymis, heart and liver, the concentration of beta-isomer was higher than the alpha-isomer. The results of the study indicated a differential ability to accumulate the two isomers of endosulfan which may help to explain the difference in the toxic potential of the alpha- and beta-isomers [Ansari R.A.; Siddiqui M.K.J.; Gupta P.K. Toxicol. Lett., (1984) 21/1 (29-33)].

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

B. Ovaries

No evidence of any treatment-related effects was found in animals studies.

C. Pituitary Glands:

1. In a 2-generation reproduction study (MRID#: 00148264), exposure of Crl:COBS CD(SD)BR rats to Endosulfan (97% purity) via the diet during premating and through gestation and lactation, at dose levels of 0, 3, 15, and 75 ppm (0, 0.20, 1.00, and 4.99 mg/kg/day in males and 0, 0.24, 1.23, and 6.18 mg/kg/day in females), produced minimal maternal toxicity at the high-dose level. There were 32 rats/sex/group in the F_O generation and 26 rats/sex/group in the F1 generation.

Increased pituitary weights (high-dose female pups of 1st mating in F_O generation) and increased uterine weights (high-dose female pups of 1st mating of F_{lb} generation) were observed in the offspring. There were no histopathological findings observed that could be attributed to treatment.

The NOEL for parental toxicity is 15 ppm (1.23 mg/kg/day), and the parental LOEL is 75 ppm (≈ 6.18 mg/kg/day), based on decreased body weight.The NOEL for reproductive effects is 75 ppm (6.18 mg/kg/day), the highest dose tested. The reproductive LOEL is greater than 75 ppm (6.18 mg/kg/day).The developmental toxicity NOEL is 15 ppm (1.23 mg/kg/day), and the developmental toxicity LOEL is 75 ppm (6.18 mg/kg/day), based on increased pituitary and uterine weights.

This study was classified as acceptable-guideline, and it satisfied the guideline requirements (83-4) for a 2-generation reproduction study in rats. (Edwards, J.A., et al, 1984. Effect of Endosulfan-Technical (Code 02671 0 I AT209) on Reproductive Function in the Rat. Hoechst Aktien-gesellschaft. Huntingdon Research Centre, Study#: HST204/83768. July 19, 1984. HED Doc# 004881, 008868, 009552; Tox. Chem.#: 420; ACC#: 256127; 41799301; TRID#: 460002-031; MRID#: 00148264).

D. Thyroid/Parathyroid Glands:

1. Two groups of 50 Osborne-Mendel rats/sex/group, were given endosulfan (98.8%) in the diet at the time weighted average concentration of 408 and 952 ppm to males and 223 and 445 ppm to females, respectively. After a 78-week period of chemical administration, observation of female rats continued for 33 additional weeks.

Dose-related depression in the rates of growth and survival were shown in the male rats. At week 54, 52% of the high-dose males died (the Tarone test for a positive dose-related trend in mortality was highly significant). The low- and high-dose male rats were terminated during week 74 and week 82, respectively. No appreciable difference in mean body weight among the females was noted. At termination (week 102), 70% of the controls, 62% of the low-dose and 50% of the high-dose groups survived. At the doses administered to rats in this study endosulfan was toxic, inducing a high incidence of toxic nephropathy in both sexes. In the males, 47/50 and 43/47 were observed in the low- and high-dose, respectively. In the females, 27/50 and 29/50 toxic nephropathy was also observed low- and high-dose, respectively. A parathyroid hyperplasia was reported to be associated with renal lesions and occurred in 21/48 low-dose and in 18/47 high-dose males. Only 1/49 parathyroid lesion was noted in the low dose female.

Testicular atrophy was noted in 3/19 controls, 18/47 low-dose, and 24/47 high-dose male rats; testicular atrophy is characterized by degeneration and necrosis of the germinal cells lining of the seminiferous tubules, multinucleated cells (fusions bodies), and calcium deposition resulting in aspermatogenesis. In the high dose male rats early mortality, associated with toxic nephropathy, was noted. Probably as a result of a high mortality rate, the incidence of tumors in the males was higher in the controls as compared to the low- or high-dose groups. Early deaths of the male rats preclude the usefulness of any analysis of late developing tumors.

This study was classified as unacceptable guideline study for a carcinogenicity study in rats. (NCI, 1978. Bioassay of Endosulfan for Possible Carcinogenicity. (CAS# 115-29-7) and NCI-CG-TR-62. DHEW Publ# (NIH) 78-1312. NCI Tech. Rept No.62, 1978. MRID#: 00004256).

2. In a non-guideline study, the acute toxic effects of endosulfan on thyroid glands were investigated by light and electron microscopy. Endosulfan (LD-30 4.16 mg/kg) was injected intraperitoneally into adult male mice. In the light microscopic studies, it was observed that some of the follicles had been damaged and joined together. Furthermore, colloidal fluids had diffused from follicles and dispersed into connective tissues. Swelling in endoplasmic reticulum sacs, expansion in perinuclear area, pycnotic nuclei and accumulation of heterochromatin were determined by electron microscopy (Cerkezkayabekir A; Aktac T. Turkish Journal of Biology 21 (4). 1997. 439-444.

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

E. Adrenal Glands

No evidence of any treatment-related effects were found in animals studies.

II. Evidence Affecting Hormonal Activities

There are several hormones that affect sexual and reproductive functions of an animal include estrogen, progesterone, and androgen including testosterone.

A. Evidence Affecting Estrogen Activities:

Estrogens are hormones produced by humans and other animals and are secreted primarily by the ovaries (female). Estrogens are defined by their ability to induce the proliferation of cells of the female genital tract. Estrogens stimulate sexual maturation of females, and play an important role in reproductive function. Hormones act through specific receptors. A complex feedback mechanism provides balance. Mammals have both "male" and "female" hormones.

Studies that provide evidence that endosulfan affecting the normal estrogen functions are:

1. The E-SCREEN assay was developed to assess the estrogenicity of environmental chemicals using the proliferative effect of estrogens on their target cells as an end point. This quantitative assay compares the cell number achieved by similar inocula of MCF-7 cells in the absence of estrogens (negative control) and in the presence of 17 beta-estradiol (positive control) and a range of concentrations of chemicals suspected to be estrogenic. Concentration describes the dose at which an estrogenic effect is detected; maximal cell yield is obtained at concentrations between 10 and 100 pM estradiol. Most xenobiotics are active at 10M. The RPE (Relative Proliferative Effect) measures the ratio between the maximal cell yield achieved by the xenobiotic and that of estradiol. The RPP (Relative Proliferative Potency) is the ratio between the minimal concentration of estradiol and the minimal dose of the xenoestrogen test compound needed to produce maximal cell yields x 100. For endosulfan the concentration detected was at 0.0001 and maximum cell yield obtained at 10 M concentration and the Relative Proliferative Effect was between 77.17-81.25% that of estradiol (Soto, A.M, Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N. , and Serrano, F.O. Environ Health Perspect 1995 Oct; 103 Suppl 7:113-122).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

2. An *in vitro* bioassay was used to assess the estrogenicity of several pesticides. The E-screen test uses human breast estrogen-sensitive MCF7 cells and compares the cell yield achieved after 6 days of culture in medium supplemented with 5% charcoal-dextran stripped human serum in the presence (positive control) or absence (negative control) of estradiol and with diverse concentrations of xenobiotics suspected of being estrogenic. Among the organochlorine pesticides tested, toxaphene, dieldrin, and endosulfan had estrogenic properties comparable to those of DDT and chlordecone; the latter are known to be estrogenic in rodent models. The E-screen test also revealed that estrogenic chemicals may act cumulatively; when mixed together they induce estrogenic responses at concentrations lower than those required when each compound is administered alone. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells (Soto, A.M., Chung, K.L., and Sonnenschein, C., 1994. Environ-Health-Perspect; VOL 102, ISS 4, 1994, P380-3).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

3. Reports of reproductive abnormalities in the American alligator from Lake Apopka, Florida, have been linked to a spill of DDT and other pesticides suspected of having hormonelike activity. To determine whether

environmental chemicals had the potential to function as exogenous hormones in the American alligator, the ability of chemicals to bind the estrogen receptor (aER) and progesterone receptor (aPR) in a protein extract prepared from the oviduct of the alligator. In competition binding assays with [3H]17beta-estradiol, some DDT metabolites showed inhibition of [3H]17beta-estradiol binding to aER. A combination of DDTs demonstrated an additive decrease in [3H]17beta-estradiol binding to aER. Modern-use chemicals such as alachlor, trans-nonachlor, endosulfan, and atrazine also competed with [3H]17beta-estradiol for binding to the aER. To test the effect of chemicals identified in alligator eggs from Lake Apopka on [3H]17beta-estradiol binding, we mixed these chemicals at concentrations measured in eggs in the competition binding assay. P,p'-DDD and trans-nonachlor, both found in Lake Apopka, interacted with aER, whereas others such as chlordane and toxaphene did not. Surprisingly, combinations of these chemicals decreased [3H]17beta-estradiol binding in a greater than an additive manner. To assess the ability of chemicals to interact with aPR, we performed competition binding assays with the synthetic progestin [3H]R5020. Most of the chemicals tested did not reduce [3H]R5020 binding to aPR, whereas endosulfan, alachlor, and Kepone inhibited binding. These results provide the first evidence that environmental chemicals bind the aER and aPR from the American alligator, supporting the hypothesis that the reported reproductive abnormalities may be related to the modulation of endocrine-related responses. The findings that combinations of chemicals demonstrated a greater than additive interaction with the aER and some chemicals bind to the aPR in the competition binding assay are novel. This suggests that interactions of these chemicals with the endocrine system are complex (Vonier PM; Crain DA; Mclachlan JA; Guillette LJ Jr; Arnold SF Environ. Health Perspect, (1996). Vol. 104, No. 12, pp. 1318-1322.).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

4. A yeast system highly and stably expressing a rainbow trout estrogen receptor (rtER) is used to analyze the biological activity of the receptor. The recombinant yeast system appears to be a reliable, rapid and sensitive bioassay for the screening and determination of the direct interaction between ER and estrogenic compounds. This system was used in parallel with a more elaborate biological system, trout hepatocyte aggregate cultures, to examine the estrogenic potency of a wide spectrum of chemicals commonly found in the environment. In hepatocyte cultures, the vitellogenin gene whose expression is principally dependent upon estradiol was used as a biomarker. Moreover, competitive binding assays were performed to determine direct interaction between rtER and xenobiotics. In this study, 50% of the 49 chemical compounds tested exhibited estrogenic activity in the two bioassays: the herbicide diclofop-methyl; the fungicides biphenyl, dodemorph, and triadimefon; the insecticides lindane, methyl parathion, chlordecone, dieldrin, and endosulfan; polychlorinated biphenyl mixtures; the plasticizers or detergents alkylphenols and phthalates; and phytoestrogens. To investigate further biphenyl estrogenic activity, Its principal metabolites were also tested in both bioassays. Among these estrogenic compounds, 70% were able to activate rtER in yeast and hepatocytes with variable induction levels according to the system. Nevertheless, 30% of these estrogenic compounds exhibited estrogenic activity in only one of the bioassays, suggesting the implication of metabolites or different pathways in the activation of gene transcription. This paper shows that it is important to combine in vivo bioassays with *in vitro* approaches to elucidate the mechanism of xenoestrogen actions (Petit F; Le Goff P; Cravedi J-P; Valotaire Y; Pakdel F, 1977).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

5. Reliable methods for detecting and characterizing estrogenic chemicals are needed. A general agreement should be reached on which tests to use and that these tests should then be applied to the testing of both man-made and naturally occurring chemicals. As a step toward developing a comprehensive approach to screening chemicals for estrogenic activity, three assays for detecting estrogenicity were conducted on 10 chemicals with known or suspected estrogenic activity. The assays were:

1) competitive binding with the mouse uterine estrogen receptor,
2) transcriptional activation in HeLa transfected with plasmids containing an estrogen receptor and a response element, and
3) the uterotropic assay in mice.

The chemicals studied were 7-beta-estradiol, diethylstilbestrol, tamoxifen, 4-hydroxytamoxifen, methoxychlor, the methoxychlor metabolite 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), endosulfan , nonylphenol, o,p'-DDT, and kepone. These studies were conducted to assess the utility of this three-assay combination in the routine screening of chemicals, or combinations of chemicals, for estrogenic activity. Results were consistent among the three assays with respect to what is known about the estrogenic activities of the chemicals tested and their requirements for metabolic activation. By providing information on three levels of hormonal activity (receptor binding, transcriptional activation, and an in vivo effect in an estrogen- responsive tissue), an informative profile of estrogenic activity is obtained with a reasonable investment of resources (Shelby M D; Newbold R R; Tully D B; Chae K; Davis V L. Environmental Health Perspectives 104 (12). 1996. 1296-1300).

B. Evidence Affecting the Androgen Activities:

The testes, ovary and adrenal cortex are responsible for the normal synthesis of androgens. In males, this hormone regulates the spermatogenesis and the maturation of sperm in the testes. Testoterone is the dominant steroid testicular hormone. Studies on endosulfan showing evidence on affecting the androgen function are as follows:

1. Endosulfan significantly inhibited testicular androgen biosythesis in adult rats, when fed (po) at 7.5 and 10 mg/kg body weight dose levels, consecutively for 15 and 30 days. No appreciable alterations were apparent in body weights, testicular wet weights, and cytosolic and microsomal protein contents of testes in treated rats. Profound decrease in the levels of plasma gonadotrophins (FSH and LH) along with plasma testosterone and testicular testosterone were observed at both the doses of endosulfan, particularly after the longer exposure of 30 days. Activities of steroidogenic enzymes studied (3beta- and 17beta-hydroxysteroid dehydrogenases) were considerably lowered on longer exposure of endosulfan. A significant decrease in the contents/activities of microsomal cytochrome P-450 and related mixed function oxidases (MFOs) in testes of treated rats was also observed, along with a marked inhibition in the activity of cytosolic conjugation enzyme, glutathione-S-transferase both doses studied. These biochemical changes were reversed when the endosulfan treatment was withdrawn (Singh S K; Pandey R S Indian J Exp Biol, (1990). Vol. 28, No. 10, pp. 953-956).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

2. Effects of endosulfan and chlorpyrifos were examined on the growth of neonatal male and female rats reproductive organs and serum testosterone and estradiol concentrations. The rats received subcutaneous sub-lethal injections daily after seven days of birth for 15 days with one of the following 4.5 mg/kg and 9.0 mg/kg weak and strong doses of endosulfan respectively and 7.0 mg/kg and 14.0 mg/kg weak and strong doses of chlorpyrifos or 1 ml corl oil. Endosulfan and chlorpyrifos did not affect the body weights or mortality. Endosulfan and chlorpyrifos decreased the weights of the male and female reproductive organs and suppressed the testosterone and estradiol concentrations. The insecticides probably suppress the Leydig cell activity of testis and interstitial cell activity of an ovary (Ahmad M M; Ahmad M M; Sarvat S. Pakistan Journal of Zoology, (1993). Vol. 25, No. 1, pp. 11-14).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

3. The potential of endosulfan and its microbial transformation products to cause reproductive disturbances through androgen-receptor mediated activity was determined in a cell-free in vitro binding assay using cytosolic prostate tissue extracted from mature rats and 3H-methyltrienolone, a synthetic androgen. Among the compounds tested, endosulfan lactone caused the greatest reduction in binding of androgen to the androgen-receptor, endosulfan alpha and endosulfan sulfate caused slight reduction in binding at high concentrations (1 mM). Preliminary experimental testing binary combinations of these compounds suggest additive effects (Brieske, J. A.; Mousa, M.; Madhukar, B. V.; Boyd, S. A.; Chou, K. Organohalogen Compound (1997), 34(Dioxin '97), 357-359).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

4. Chronic endosulfan exposure in rats led to a considerable increase in the activities of drug metabolizing enzymes, whereas it had inhibitory effect on the activities of enzymes involved in the androgen biotransformation. Endosulfan also produced a dose- and duration-dependent increase in microsomal lipid peroxidation. The alterations produced after shorter duration showed much variation with respect to the dose levels and exposure period of endosulfan studied. The above biochemical changes were reversed after endosulfan withdrawal (Singh S K; Pandey R S . Indian J Biochem Biophys, (1989). Vol. 26, No. 4, pp. 262-267).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

C. Evidence Affecting the Progesterone Activities:

1. The mammalian sperm acrosome reaction (AR) is essential to fertilization. It can be initiated *in vitro* by progesterone, a putative physiological initiator that helps to activate sperm GABA receptor/chloride channels and by glycine, a substitute for the egg zona pellucida, which activates sperm glycine receptor/chloride channels. Even at 1 nM (0.41 ng/ml or 0.41 ppb), chlordane and endosulfan, chlorinated cyclodiene blockers of insect neuronal GABA, receptor/ chloride channels, strongly inhibited the AR initiated by progesterone or glycine. Inhibition of the latter was also seen at 0.1 nM chlordane and endosulfan, but neither cyclodiene inhibited either AR initiator at 0.01 nM. Inhibitory concentrations of these cyclodienes are well within the range detected in human and wildlife tissue and fluids as a result of environmental contamination (Turner, K.O. Sylvanen, M; Meizel S, 1997. Journal of Andrology, (1997). Vol. 18, No. 6, pp. 571-575).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

2. Reports of reproductive abnormalities in the American alligator from Lake Apopka, Florida, have been linked to a spill of DDT and other pesticides suspected of having hormonelike activity. To determine whether environmental chemicals had the potential to function as exogenous hormones in the American alligator, the ability of chemicals to bind the estrogen receptor (aER) and progesterone receptor (aPR) in a protein extract prepared from the oviduct of the alligator were evaluated. In competition binding assays with (3H)17-beta-estradiol, some DDT metabolites showed inhibition of (3H)17-beta-estradiol binding to aER. A combination of DDTs demonstrated an additive decrease in (3H)17-beta-estradiol binding to aER. Modern-use chemicals such as alachlor, trans-nonachlor, endosulfan , and atrazine also competed with (3H)17-beta-estradiol for binding to the aER. To test the effect of chemicals identified in alligator eggs from Lake Apopka on (3H)17-beta-estradiol binding, these chemicals were mixed at concentrations measured in eggs in the competition binding assay. 2,2-bis(4-chlorophenyl)-N-(methoxymethyl)acetamide (p,p'-DDD) and trans-nonachlor, both found in Lake Apopka, interacted with aER, whereas others such as chlordane and toxaphene did not. Surprisingly, combinations of these chemicals decreased (3H)17-beta-estradiol binding in a greater than an additive manner. To assess the ability of chemicals to interact with aPR, competition binding assays with the synthetic progestin (3H)R5020 were conducted. Most of the chemicals tested did not reduce (3H)R5020 binding to aPR, whereas endosulfan, alachlor, and kepone inhibited binding. These results provide the first evidence that environmental chemicals bind the aER and aPR from the American alligator, supporting the hypothesis that the reported reproductive abnormalities may be related to the modulation of endocrine-related responses. The findings that combinations of chemicals demonstrated a greater than additive interaction with the aER and some chemicals bind to the aPR in the competition binding assay are novel. This suggests that interactions of these chemicals with the endocrine system are complex (Vonier P M; Crain D A; McLachlan J A; Guillette L J Jr; Arnold S F Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. Environmental Health Perspectives 104 (12). 1996. 1318-1322).

D. Evidence in Decrease of Urinary Ketosteroid

1. The dietary exposure to endosulfan at two different dose levels (12.5 and 37.5 mg/250g) daily to female goats for a period of 90 days caused a significant decrease in urinary 17-ketosteroid level in the treatment groups when compared to that of the control group (C = 3.27± 0.22; T1 = 2.73 ± 0.14 and T2 = 2.49 ±0.19), but was not significant between the two treatment groups. Decreased level was more perceptible on day 90 than day 45 when compared with day 0. The decrease in level may be associated with decreased hormone synthesis and hypofunction of the adrenal as well as ovarian glands. Thus, endosulfan might have adverse effect on the normal formation and metabolism of the sex hormones (Bose K K; Mukherjee S K; Prasad R L Indian J Anim Health, (1991). Vol. 30, No. 1, pp. 63-66).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

In-Vitro Testing

Estrogen Receptor Testing

Estrogens are hormones produced by humans and other animals; secreted primarily by the ovaries (female); Estrogens are defined by their ability to induce the proliferation of cells of the female genital tract. Estrogens stimulate sexual maturation of females, and play an important role in reproductive function. Hormones act through specific receptors.

1. The E-SCREEN assay was developed to assess the estrogenicity of environmental chemicals using the proliferative effect of estrogens on their target cells as an end point. This quantitative assay compares the cell number achieved by similar inocula of MCF-7 cells in the absence of estrogens (negative control) and in the presence of 17 beta-estradiol (positive control) and a range of concentrations of chemicals suspected to be estrogenic. Concentration describes the dose at which an estrogenic effect is detected; maximal cell yield is obtained at concentrations between 10 and 100 pM estradiol. Most xenobiotics are active at 10M. The RPE (Relative Proliferative Effect) measures the ratio between the maximal cell yield achieved by the xenobiotic and that of estradiol. The RPP (Relative Proliferative Potency) is the ratio between the minimal concentration of estradiol and the minimal dose of the xenoestrogen test compound needed to produce maximal cell yields x 100. For endosulfan the concentration detected was at 0.0001 and maximum cell yield obtained at 10 M concentration. The Relative Proliferative Effect was between 77.17-81.25% that of estradiol (Soto, A.M, Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N. , and Serrano, F.O.., 1995. Environ Health Perspect 1995 Oct; 103 Suppl 7:113-122).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

2. An "in culture" bioassay was used to assess the estrogenicity of several pesticides. The E-screen test uses human breast estrogen-sensitive MCF7 cells and compares the cell yield achieved after 6 days of culture in medium supplemented with 5% charcoal-dextran stripped human serum in the presence (positive control) or absence (negative control) of estradiol and with diverse concentrations of xenobiotics suspected of being estrogenic. Among the organochlorine pesticides tested, toxaphene, dieldrin, and endosulfan had estrogenic properties comparable to those of DDT and chlordecone; the latter are known to be estrogenic in rodent models. The E-screen test also revealed that estrogenic chemicals may act cumulatively; when mixed together they induce estrogenic responses at concentrations lower than those required when each compound is administered alone (Soto, A.M., Chung, K.L., and Sonnenschein, C., 1994. The pesticides endosulfan , toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environ-Health-Perspect; VOL 102, ISS 4, 1994, P380-3).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

3. A yeast system highly and stably expressing a rainbow trout estrogen receptor (rtER) is used to analyze the biological activity of the receptor. The recombinant yeast system appears to be a reliable, rapid and sensitive

bioassay for the screening and determination of the direct interaction between ER and estrogenic compounds. This system was used in parallel with a more elaborate biological system, trout hepatocyte aggregate cultures, to examine the estrogenic potency of a wide spectrum of chemicals commonly found in the environment. In hepatocyte cultures, the vitellogenin gene whose expression is principally dependent upon estradiol was used as a biomarker. Moreover, competitive binding assays were performed to determine direct interaction between rtER and xenobiotics. In this study, 50% of the 49 chemical compounds tested exhibited estrogenic activity in the two bioassays: the herbicide diclofop-methyl; the fungicides biphenyl, dodemorph, and triadimefon; the insecticides lindane, methyl parathion, chlordecone, dieldrin, and endosulfan; polychlorinated biphenyl mixtures; the plasticizers or detergents alkylphenols and phthalates; and phytoestrogens. To investigate further biphenyl estrogenic activity, Its principal metabolites were also tested in both bioassays. Among these estrogenic compounds, 70% were able to activate rtER in yeast and hepatocytes with variable induction levels according to the system. Nevertheless, 30% of these estrogenic compounds exhibited estrogenic activity in only one of the bioassays, suggesting the implication of metabolites or different pathways in the activation of gene transcription. This paper shows that it is important to combine in vivo bioassays with in vitro approaches to elucidate the mechanism of xenoestrogen actions (Petit F; Le Goff P; Cravedi J-P; Valotaire Y; Pakdel F, 1977).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

4. A number of chemicals released into the environment have the potential to interfere with physiological and developmental processes by disrupting endocrine pathways. Among the best known of these endocrine disruptors are compounds that mimic the action of the steroid hormone 17 beta -estradiol. These xenobiotic estrogens are believed to pose health risks to both humans and wildlife. Our laboratories are designing in vivo bioassays for xenobiotic estrogens based on induction of the egg-yolk precursor protein vitellogenin. Vitellogenin is normally produced by the liver of adult female non-mammalian vertebrates under estrogen stimulation. In immature or male animals, which have low levels of endogenous estrogens, vitellogenin can serve as a reliable biomarker for exposure to xenobiotic estrogens. Our model system used the African clawed frog, Xenopus laevis, an ideal species for laboratory screening of endocrine disruptors. Xenopus laevis vitellogenin was purified by diethylaminoethyl (DEAE) chromatography and used to generate polyclonal antibodies in rabbits. The resulting antiserum was used to develop an enzyme-linked immunosorbent assay (ELISA) for measurement of serum vitellogenin. Frogs were exposed to compounds by immersion in order to mimic environmental exposure to aquatic contaminants. Initially, frogs were immersed in the potent estrogenic agent diethylstilbestrol (DES) at a concentration of 1 ppm for 11 days to test the efficacy of the immersion protocol. Diethylstilbestrol exposed animals showed substantial induction of serum vitellogenin, indicating that the frogs are capable of responding to estrogenic agents present in their aquatic environment. Vitellogenin induction was then investigated for chlordane, dieldrin,endosulfan, and toxaphene, compounds that have been shown through in vitro assays to be weakly estrogenic when administered individually but more strongly estrogenic in combination. Adult male frogs were immersed in water containing the compounds (1 ppm, 11 days), both singly and in paired combinations. Endosulfan proved toxic at this concentration. Toxaphene- and dieldrin-treated frogs showed significant levels of vitellogenin induction, while chlordane-treated animals did not differ from controls. There was no evidence of a synergistic response between any of the combinations. This research demonstrates the utility of vitellogenin as a biomarker for exposure to estrogenic agents. The assays developed could be used to screen chemicals for estrogenic properties, to test waters for the presence of estrogenic agents, or to assess wildlife exposure to environmental estrogens (Palmer, B.D.; Huth, L.K.; Pieto, D.L.; Selcer, K.w. Environ. Toxicol. Chem., (19980100) vol. 17, no. 1, pp. 30-36.).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Date: December 11, 2000

MEMORANDUM

SUBJECT: **ENDOSULFAN:** Evaluation of Registrant Submission *Endosulfan: Evaluation of Possible Endocrine Effects in Mammalian Species.*

TO: Diana Locke, Ph.D.
Risk Assessor
Reregistration Branch 2/HED (7509C)

FROM: Elizabeth Méndez, Ph.D.
Toxicologist
Reregistration Branch 1/HED (7509C)

THROUGH: Whang Phang, Ph.D.
Branch Senior Scientist
Reregistration Branch 1/HED (7509C)

PC Code: 079401 **DP Barcode: D270808**
Submission Code: S588693 **MRID No.: 44939102**

On June 16, 2000 the Health Effects Division (HED) in the Office of Pesticide Programs (OPP) issued a Risk Assessment for the Endosulfan Reregistration Eligibility Decision (RED) Document.
As part of the hazard characterization required for a risk assessment, the toxicological database for endosulfan was reviewed and evaluated. In the process of this evaluation, endosulfan was identified as a potential endocrine disruptor.

The registrant, AgrEvo, has submitted a literature review in response to the Agency's characterization of the endosulfan database as providing "suggestive evidence that endosulfan may be an endocrine disruptor." After reviewing several published articles, the registrant concludes that "endosulfan does not meet the criteria of an endocrine disruptor." The registrant states that *in vitro* studies show that endosulfan has a low binding potency to the human estrogen

receptors and that "no effects were found on endocrine, reproductive or sexually regulated systems *in vivo* at doses causing clear toxicity."

The Agency identifies an environmental endocrine disruptor as an exogenous agent that interferes with the synthesis, secretion, transport, binding action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior.[3] Based on these criteria, the Agency disagrees with the conclusion by the registrant that endosulfan does not meet the definition of an endocrine disruptor. Binding to the estrogen receptor is only one potential mode of action for endocrine disruptors, namely direct interaction with a receptor in the target cells. Substances that act as endocrine disruptors may perturb the endocrine system in a variety of ways including but not limited to interfering with the synthesis, secretion, or transport of hormones in the organism. Consequently, the absence of high binding affinity to the estrogen receptor should not be interpreted as lack of endocrine disruption potential. The Agency notes that other organochlorines (i.e. DDT, DDE, dieldrin, and methoxychlor) have been demonstrated to interact with the endocrine system in spite of differing binding affinities to the estrogen receptor . Finally, the registrant states that no effects were reported after administration of endosulfan on the endocrine, reproductive or sexually regulated systems at doses causing clear toxicity. However, it is noteworthy that testicular atrophy was reported during a Chronic Oral Toxicity Study in Rats (MRID 00004256) submitted to the Agency. Additionally, increased pituitary and uterine weights were also observed during a Multi-Generation Reproduction Study (MRID 00148264). Furthermore, an increase in the incidence of parathyroid hyperplasia was also reported during the Chronic Oral Toxicity study in Rats. The Agency emphasizes the fact that the endocrine system integrates a variety of CNS-pituitary-target organ pathways that not only affect reproductive or sexually regulated parameters but also regulates a wide array of bodily functions and homeostasis.[4] Though this is not the case for endosulfan, it is important to note that a lack of overt toxicity to the reproductive system should not be interpreted as conclusive evidence of a lack of endocrine disruption. Given the effects noted in the Chronic Oral Toxicity Study in Rats and the Multi-Generation Reproduction Study submitted to the Agency, the potential of endosulfan to act as an endocrine disruptor can not be discounted. The Agency has requested that a Developmental Neurotoxicity Study be conducted; the Agency believes that this study will provide additional data that may help elucidate this matter.

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[3] Crisp, T.M. *et al. Environmental Endocrine Disruption: An Effects Assessment and Analysis.* **Environmental Health Perpectives 106 pp. 11-56.**

[4] R.L. Cooper and R.J. Kavlock. *Endocrine Disruptors and Reproductive Development: a Weight-of Evidence Overview.* **J. Endocrinology 152 pp. 159.-166**

PUBLISHED LITERATURE DATA ON ENDOCRINE DISRUPTOR PROPERTIES OF ENDOSULFAN RESULTING IN RESIDUAL UNCERTAINTY

Reference	Species	Dosing Paradigm	Comments
Agency for Toxic Substances and Disease Registry (2000)			
Singh & Pandey (1989)	Rats	7.5 - 10 mg/kg/day for 7 days	Adult male rats used. Decreased testicular testosterone in conjunction with increased serum testosterone; effects not seen at ≤ 5 mg/kg/day. Suggests sex-hormone binding globulin (SHBG) may be affected.
Singh & Pandey (1990)	Wistar rats	7.5 or 10 mg/kg/day for either 15 or 30 days	Decreased testicular testosterone, plasma testosterone, LH, and FSH as well as decreased steroidogenic enzyme and cytochrome P-450-dependent monooxygenase. Decreases in LH may lead to decreases in the activity of Steroidogenic Acute Regulatory Protein (responsible for translocation of cholesterol to the inner mitochondria).
Vonier et al. (1996)		Competitive Binding Assay using alligator oviduct tissue	Endosulfan exposure significantly inhibited ^{3}H-17β-estradiol binding to the estrogen receptor and progestin ^{3}H-R5020 binding to the progesterone receptor.
Ramamoorthy *et al.*	Yeast reporter system	100 μM added to the reporter gene assay.	Endosulfan induced human-ER-mediated β-gal activation. Endosulfan induced to about 32% of the induction seen after estradiol treatment at 0.01 μM.

Reference	Species	Dosing Paradigm	Comments
David Liem Evaluation (11/24/98)			
MRID # 00004256 Chronic Oral Tox Study	Rats	20.4 mg/kg/day	Testicular atrophy characterized by degeneration and necrosis of germinal cells lining the seminiferous tubules, multinucleated cells, aspermatogenesis.
Sinha *et al.* (1995)	Rats	2.5 mg/kg/day for 70 days	Decreased sperm counts in the cauda epididymis and decreased intratesticular spermatid counts.
Ansari *et al.* (1984)	Rats	2.5 mg/kg/day for 60 days	Decreased testes weight
MRID # 00148264 Multi-gen repro	Rats	6.18 mg/kg/day	Increased pituitary weight in female pups in the F_0 generation and increased uterine weights in female pups in the F_{1B} generation
Children's Susceptibility			
Zaidi *et al.* (1985)	Rats	1 mg/kg/day for 25 days to neonates (starting dosing on PND1) intraperitoneally	Serotonin binding to the frontal cortical membranes of the brain increased. Correlates with increase in aggressive behavior. Adults exposed in a similar manner for 30 days did not exhibit the same effects.
Kiran & Varma (1988)	Rats	12.5 mg.kg.day for 4 days to different age groups (15, 30, 70, and 365 day old rats)	Older animals exhibited tremors and muscular contractions, hyperglycemia, and reduced liver glycogen. None of these effects seen in the 15 day old rats

Reference	Species	Dosing Paradigm	Comments
Sinha *et al.* (1995 & 1997)	Rats	Oral treatment of 3-week or 3-month old rats for 90 days	Decreased intratesticular spermatid count and increased % of abnormal sperm seen in 3-week old rats at doses lower than those eliciting similar effects in 3-month old rats

APPENDIX D

TXR NO. 0050473

February 14, 2002

MEMORANDUM

SUBJECT: ***ENDOSULFAN*** - Report of the FQPA Safety Factor Committee.

NOTE: THIS REPORT REPLACES THE PREVIOUS REPORT OF THE FQPA SAFETY FACTOR COMMITTEE DATED NOVEMBER 20, 1998 (HED DOC. NO. 012974).

FROM: Carol Christensen, Acting Executive Secretary
FQPA Safety Factor Committee
Health Effects Division (7509C)

THROUGH: Ed Zager, Chair
FQPA Safety Factor Committee
Health Effects Division (7509C)

TO: Diana Locke, Risk Assessor
Reregistration Action Branch 2
Health Effects Division (7509C)

PC Code: 079401

The Health Effects Division (HED) FQPA Safety Factor Committee met on February 11, 2002 to re-evaluate the hazard and exposure data for endosulfan and recommended that the FQPA Safety Factor (as required by Food Quality Protection Act of August 3, 1996) be retained **(10x)** in assessing the risks posed by this chemical.

I. HAZARD ASSESSMENT

(Correspondence: D. Locke and E. Mendez to C. Christensen February 7, 2002)

1. Adequacy of the Toxicity Database

The endosulfan database contains acceptable guideline studies to evaluate the effects of endosulfan exposure both *in utero* and in young animals. The HIARC determined that the requirement for a developmental neurotoxicity study in rats was reserved for endosulfan pending the receipt and review of a subchronic neurotoxicity studies in rats. However, **the FQPA Safety Factor Committee concluded that a developmental neurotoxicity study in rats is required for endosulfan** due to concern by the Committee for: 1) the fetal effects reported in the open literature abstract; 2) the severity of effects seen in the female offspring of the F_0 generation (increased pituitary weight) and F_1b generation (increased uterine weights) at the high-dose when compared to the toxicity observed in parental animals (decreased body weight) at this dose in the two-generation reproduction study in rats; and 3) the subchronic neurotoxicity study (requested by the HIARC) will only address the neuropathological concerns resulting from exposure to endosulfan - a developmental neurotoxicity study will provide the critical data demonstrating the toxic effects of endosulfan on the developing fetal nervous system.

2. Determination of Susceptibility

A. The Hazard Identification Assessment Review Committee (HIARC) determined that under the conditions of the available Agency Guideline studies, there is no evidence of enhanced susceptibility of the offspring to exposure to endosulfan. In the prenatal developmental toxicity studies in rats and rabbits, developmental toxicity was seen only in the presence of maternal toxicity. Furthermore, the severity of developmental effects seen in these studies is comparable to the severity of effects seen in adult animals. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity (*Memorandum:* D. Liem and J. Rowland to S. DeVito dated October 7, 1998).

B. A recent review by the Agency for Toxic Substances and Disease Registry (ATSDR) reported the results of non-guideline studies which demonstrated that young rats may be more susceptible than older rats upon exposure to endosulfan. Studies conducted by Zaidi *et al.* (1985) and Sinha *et al.* (1995 & 1997) illustrate effects to the offspring at doses lower than those showing effects in adults. In the first, neonatal rat pups were dosed for 25 days intraperitoneally and displayed increased serotonin binding to the frontal cortical membranes of the brain and increased aggressive behavior. Adults exposed in a similar manner did not display these effects. In a study by Sinha *et al*., both three week and three months old rats were treated orally; decreased intratesticular spermatid count and increased percentage of abnormal sperm were seen in three week old rats at doses lower than those eliciting similar effects in three month old rats.

4. Evidence for Endocrine Disruption

A. There is evidence for endocrine disruption both in studies submitted to the Agency and those published in the open literature. In the chronic toxicity/carcinogenicity study in rats, endosulfan induced testicular atrophy and parathyroid hyperplasia. In the multi-generation reproduction study, increased pituitary and uterine weights were seen. Endosulfan is considered to be an endocrine disruptor. Substances that act as endocrine disruptors may perturb the endocrine system in a variety of ways including but not limited to interfering with the synthesis, secretion, or transport of hormones in the organism. The Agency emphasizes the fact that the endocrine system integrates a variety of CNS-pituitary-target organ pathways that not only affect reproductive or sexually regulated parameters but also regulates a wide array of bodily functions and homeostasis.

B. The ATSDR, 2000 reported a number of studies that assessed endosulfan's effects on the endocrine system. Singh and Pandey (1989) dosed adult rats orally for 7 days and observed decreased testicular testosterone in conjunction with increased serum testosterone which suggests sec-hormone binding globulin (SHBG) may be affected. In a subsequent study, these researchers dosed rats orally for 15-30 days. Under the conditions of this study, decreases in testicular testosterone, plasma testosterone, LH, and FSH as well as decreased steroidogenic enzyme and cytochrome P-450-dependent monooxygenase were reported. These decreases in LH may lead to decreases in the activity of Steroidogenic Acute Regulatory Protein (responsible for translocation of cholesterol to the inner mitochondria) and may therefore affect the conversion of cholesterol to testosterone. Vonier *et al.* (1996) conducted a competitive binding assay using alligator oviduct tissue and found endosulfan exposure significantly inhibited ^{3}H-17-estradiol binding to the estrogen receptor and progestin ^{3}H-R5020 binding to the progesterone receptor. Ramamoorthy *et al.* used the yeast reporter system to discover endosulfan induced human-ER-mediated-gal activation. Endosulfan induced galactosidase transcription/expression to about 32% of the induction seen after estradiol treatment at 0.01 μM. In a study conducted by Sinha *et al.* (1995) rats dosed orally with endosulfan for 70 days exhibited decreases in sperm counts in the cauda epididymis as well as decreased intratesticular spermatid counts. Finally, Lakshmana *et al.* (1994) showed 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 suggesting possible effects on the neuroendocrine system.

II. EXPOSURE ASSESSMENT AND RISK CHARACTERIZATION

(*Note: The only change to the exposure assessment and risk characterization since the 20 November 1998 FQPA SFC Meeting is the cancellation of the residential uses for endosulfan.)*

1. Dietary (Food) Exposure Considerations

Endosulfan is widely used on many agricultural crops and also in residential settings as an insecticide and acaricide. The chemical is a mixture of isomers and the tolerance expression includes the alpha, beta isomers plus a sulfate metabolite.

Tolerances for residues of endosulfan and its metabolites are established in/on many RACs including fruits, vegetables grains, milk and meat at levels ranging from 0.1 ppm to 2.0 ppm (40CFR180.182). Codex maximum residue limits (MRLs) for residues of endosulfan are established in/on various plant and animal commodities.

There are numerous field trial data on various commodities, reflecting various application sites throughout the country. Additionally, PDP and FDA monitoring data are available for endosulfan. Residues of endosulfan have been reported by PDP and FDA in a variety of crops. For example, in 1995 endosulfan was detected in apples (7%), carrots (4%), grapes (4%), green beans (24%), peaches (8%), potatoes (20%), spinach (14%), corn (0.1%), peas (0.3%), and oranges (2%) - for which there is no tolerance. The Limit of Quantitation (LOQ) for these data is ~0.01 ppm.

The HED Dietary Exposure Evaluation Model (DEEM) is used to assess the risk from acute and chronic dietary exposure to residues of endosulfan in food. These analyses are based on the consumption database used by DEEM and residue information from monitoring studies.

2. Dietary (Drinking Water) Exposure Considerations

The environmental fate data base for endosulfan is adequate for risk assessment. Endosulfan may be moderately persistent in soils but its high affinity to sorb to soil particles, reduces its susceptibility to leaching. Although endosulfan does not appear to be highly mobile, it may be persistent enough in some instances to move to ground water (detects have been reported in the EPA Pesticides in Ground Water Database). Movement to surface water sources of drinking water is likely to occur via spray drift and runoff adsorbed to soil particles. This is supported by several studies which have reported endosulfan detects in surface water.

Endosulfan consists of isomers which appear to have some differences in persistence. Endosulfan sulfate and its isomers are the degradates of concern. Ground water and surface water EECs for endosulfan will be based upon modeling and supported by any available monitoring data. The EFED models are used for ground and surface source drinking water exposure assessments.

3. Residential Exposure Considerations

Uses of endosulfan in the residential environment have been canceled.

III. SAFETY FACTOR RECOMMENDATION AND RATIONALE

1. FQPA Safety Factor Recommendation

The Committee recommended that the **FQPA safety factor** for protection of infants and children (as required by FQPA) be **retained (10x).**

2. Rationale for Retaining the FQPA Safety Factor

The Committee concluded that the **10x** FQPA Safety Factor should be retained. Previously (November 20, 1998), the Committee recommended a 3x FQPA Safety Factor due to the lack of a DNT. At the current meeting, however, the Committee recommended that the 10x FQPA Safety Factor should be retained because there was not reliable data available to address the following concerns or uncertainties raised by the following matters: 1) evidence for increased susceptibility of young rats, 2) additional evidence for endocrine disruption, 3) uncertainty regarding the neuroendocrine effects in the young, and 4) the need for a DNT.

3. Population Subgroups for Application of the Safety Factor

The Committee determined that the FQPA safety factor (10x) is applicable for all populations when assessing acute and chronic dietary exposure. There are no longer any residential uses for this chemical, so the FQPA Safety factor does not apply to the short-term or intermediate-term exposure scenarios.