# ENDOSULFAN AND ENDOCRINE DISRUPTION: HUMAN RISK CHARACTERIZATION

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## I. Overview of Endocrine Disruption as an Endpoint of Concern in Pesticide Risk Assessment

The endocrine system is one of the basic systems in mammalian physiology that is involved in variety of body functions including such functions as growth, development, reproduction, and behavior. The activity of the endocrine system involves endocrine glands which are collections of specialized cells in various tissues of the body that synthesize, store and release substances into the bloodstream. These substances are known as hormones. Hormones act as chemical messengers, traveling through the bloodstream to distant organs and tissues where they can bind to receptors located on those tissues and organs and as a result of receptor binding trigger a physiological response. The major endocrine glands include the pituitary gland, the thyroid gland, the pancreas, the adrenal gland, the testes, and the ovary.

"Endocrine disruption"[1] is a term that has arisen in human health risk assessment and is defined as the action of an external agent, such as a chemical, to interfere with normal activity of circulating hormones. This "interference" would include altering the synthesis, storage, release, or degradation of hormones, as well as actions at hormone receptors such as stimulating receptor activity (receptor agonist activity) or inhibiting receptor activity (receptor antagonist activity).

The potential effects of chemicals, including pesticides, as endocrine disruptors have been a focus of recent regulatory actions. The U.S. Environmental Protection Agency (EPA) has developed a program and is currently validating methods for testing for potential endocrine disrupting effects of pesticides (see 63 FR 42852; 63 FR 71542). There are three components to the program: priority setting, Tier 1 screening, and Tier 2 testing. Priority setting involves generating a list of chemicals to be tested for potential endocrine disrupting effects based on exposure potential.

[1] In 1997 the term "endocrine disruptor" was defined by the Organization of Economic Cooperation and Development (OECD) as follows: "An endocrine disruptor is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function."

A list of chemicals has already been published for public comment (see 72 FR 33486-33503) and includes 73 chemicals that are active ingredients or inert ingredients in pesticides; many of the 73 chemicals also have uses apart from pesticide products. Tier 1 screening, which is still undergoing peer review and validation, focuses on testing for effects of chemicals on the activity and function of estrogen, androgens, and thyroid hormone. The purpose of Tier 1 screening is to identify substances that have the potential to interact with the estrogen, androgen or thyroid systems through use of a battery of short-term *in vitro* and *in vivo* screens. Tier 2 testing, which is also still undergoing validation, is designed to identify and establish dose-response relationships for any significant findings from the Tier 1 screening. In the following discussion of endosulfan studies, the similarity between endosulfan studies and tests that are recommended as part of the EPA endocrine disruptor screening and testing program will be discussed.

Since there is a great deal of diversity in functions controlled by endocrine systems active in mammalian organisms, including humans, the endpoints of concern in pesticide risk assessment and the EPA screening program are varied. Many of the endpoints of concern in the EPA endocrine disruptor screening and testing program are already being addressed in current EPA guideline toxicology testing for evaluating pesticide safety. Therefore, the risks of endocrine disruption for currently registered pesticides are being assessed already based on data collected in a variety of toxicology tests including subchronic and chronic toxicity tests, carcinogenicity testing, reproductive toxicity tests, developmental toxicity tests, and neurotoxicity tests. For example, standard EPA guideline test protocols for subchronic and chronic toxicity testing include gross and histopathologic evaluations of endocrine tissues such as the thyroid, pancreas, pituitary, testes and ovary. Standard EPA guideline test protocols for multi-generation reproductive and developmental toxicity testing evaluate these same endocrine tissues in animals exposed at various lifestages (*in utero*, postnatally and adulthood). Therefore, even without data collected from validated endocrine disruptor screening tests (Tier 1 screens and Tier 2 tests), there will be a good deal of relevant toxicological data collected from animal testing that has been already

performed as part of the registration process for pesticides. It is important to note as well that a key study in the proposed Tier II testing for endocrine disruption potential is a multi-generation reproductive and developmental toxicity study in rodents, a study that is already required as part of standard food-use pesticide product development.

The goal of this assessment is to examine and analyze the available toxicological data on endosulfan and to determine whether endosulfan exhibits endocrine toxicity, as well as to assess whether endosulfan presents a risk to human health based on any identified effects on endocrine function.

## II. Endosulfan and Endocrine Toxicity: Overview of the Available Data

There are several sources of data that can be reviewed and analyzed that relate to the potential endocrine effects of endosulfan. These sources include the published, peer-reviewed literature, as well as unpublished studies submitted to EPA as part of the endosulfan pesticide registration process. The following is a review of the studies from each source, focusing on the relevancy of the study findings to assessing the risk to human health that may be related to any endosulfan-induced endocrine effects or toxicity.

### A. Published, Peer-Reviewed Studies

#### 1. Studies Reporting *In Vitro* Data

Over the last 15 years, studies have been published that report effects of endosulfan *in vitro* on some aspect of endocrine function. Table 1 below categorizes and describes the available studies based on the type of testing, endpoints examined, and findings related to endosulfan. It should be noted that several of the papers describe results from studies similar to proposed Tier 1 endocrine assays. These include the estrogen receptor binding assays and the uterotrophic assay. In all cases, these *in vitro* screening studies employ a method of exposure, direct cell contact

without consideration of metabolism, an exposure method that is not directly relevant to human pharmacodynamics.

**Table 1**
***In Vitro* Studies Examining Potential Endocrine Effects of Endosulfan**

| STUDY TYPE AND CITATION | ENDPOINT EXAMINED | REPORTED EFFECTS |
|---|---|---|
| MCF-7 cell proliferation assay (Soto et al. 1994. *Environ. Health Perspect.* 102:380-383) | Cell proliferating potency of pesticides as compared to estradiol. | Endosulfan was $10^6$ times less potent than the standard, 17β-estradiol. Effects only at doses of 10 μM endosulfan or greater.<br><br>LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| Progesterone receptor binding assay in human cells (Soto et al. 1995. *Environ. Health Perspect.* 103:113-122) | Relative binding affinity of pesticides to human progesterone receptor (hPR) as compared to estradiol. | Endosulfan hPR receptor binding affinity was much less ($10^5$ less) than 17β-estradiol. A weak effect.<br><br>LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| Estrogen receptor binding assay in human cells (Soto et al. 1995. *Environ. Health Perspect.* 103:113-122) | Relative binding affinity of pesticides to human estrogen receptor (hER) as compared to estradiol. | Endosulfan hER receptor binding affinity was much less ($2.4x10^6$ less) than 17β-estradiol. A weak effect.<br><br>LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| Estrogen and progesterone receptor binding assays in alligator cells (Vonier et al. 1996. *Environ. Health Perspect.* 104:1318-1322) | Relative binding affinity to alligator oviduct cell progesterone and estrogen receptors as compared to estradiol. | Endosulfan was much less potent than estradiol with an $IC_{50}$ value of greater than 50 μM (estradiol $IC_{50}$ = 0.0078 μM).<br><br>LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| MCF-7 cell proliferation assay (Wade et al. 1997. *Reproduct. Toxicol.* 11:791-798) | Cell proliferating potency of pesticides as compared to estradiol. | Endosulfan was much less potent ($10^5$ less) as compared to 17β-estradiol. The only effect reported was at the highest soluble dose.<br><br>LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| Yeast-based estrogen receptor assay, both mouse and human receptors (Ramamoorthy et al. 1997. *Endocrinology* 138:1520- | Estrogen receptor binding affinity of pesticides as compared to diethylstilbestrol (DES). | Endosulfan was much less potent as compared to DES in competing at both mouse ($10^5$ less) and human ($10^5$ less) estrogen receptors. |

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| 1527) | | LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| MCF-7 cell proliferation assay (Arcaro et al. 1998. *Environ. Health Perspect.* 106:1041-1046) | Cell proliferating potential of pesticides as compared to estradiol. | Endosulfan was $10^6$ times less potent than 17β-estradiol. Effects were only seen at the highest dose tested.<br><br>LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| Induction of luciferase activity in HepG2 cells transiently cotransfected with human estrogen receptor, or rat estrogen receptor subtypes (Gaido et al. 1998. *Environ. Health Perspect.* 106:1347-1351) | Luciferase activity induction in presence of pesticides as compared to activity in presence of estradiol. | Endosulfan was much less potent at inducing activity as compared to estradiol.<br><br>LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| Rat testicular cells in culture (Sertoli-germ cell co-culture) (Sinha et al. 1999. *Reproduct. Toxicol.* 13:291-294) | Testicular cell cytotoxicity in the presence of endosulfan as measured by cell viability and lactate dehydrogenase leakage. | Endosulfan produced cytotoxic changes after 24 and 48 hours of treatment, including a loss of cell viability. Effects reported were associated with exposure to levels of endosulfan at or above 20 μM.<br><br>LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| Rat testicular cells in culture (Sertoli-germ cell co-culture) (Sinha et al. 2001. *Bull. Environ. Contam. Toxicol.* 67:821-827) | Effects of endosulfan on activity of polyol pathway enzymes (sorbitol dehydrogenase and aldose reductase). | Endosulfan treatment increased aldose reductase activity and decreased sorbitol dehydrogenase activity, but only at high doses (at or above 20 μM).<br><br>LIMITATIONS: Effects only at doses consistent with human blood levels seen in poisoning episodes. |
| Human estrogen receptor mRNA expression assay (Grunfeld and Bonefeld-Jorgensen. 2004. *Toxicol. Lett.* 151:467-480) | Levels of human ERα and ERβ mRNA expression in MCF-7BUS cells using RT-PCR. Nine pesticides examined (including endosulfan). | Endosulfan decreased human ERα mRNA levels "weakly" but had no effect on human EBβ expression.<br><br>LIMITATIONS: These gene expression results were not directly correlated to functional changes. |

Review of the studies in Table 1 indicates that endosulfan exhibited weak estrogenic activity in the various *in vitro* assays. In almost all cases, the estrogenic effects elicited by endosulfan were compared with those elicited by a positive control, estradiol. In all cases where such comparisons are presented, estrogenic

effects are only seen at doses much higher than the doses of estradiol that were needed to elicit similar responses (in the range of $10^5$ to $10^6$ times higher concentrations required for endosulfan). In the two studies that examined the effects of endosulfan *in vitro* on androgen-responsive systems, endosulfan exhibited cytotoxic effects on testicular cells and their affected function, but only at high doses (equal to or greater than 20 μM)[2]. Unlike the studies on estrogenic effects of endosulfan that examined the ability of the pesticide to mimic the activity of a naturally-occurring hormone, the studies related to androgen function focused on potential anti-androgenic activity of endosulfan, or its ability to produce toxic effects on tissues sensitive to androgen hormones. Regardless of the system studied, however, the reported *in vitro* effects of endosulfan were only seen at doses that were much higher than the levels of endosulfan that might be encountered in the environment or levels that are biologically relevant for human risk assessment.

### 2. Studies Reporting *In Vivo* Animal Toxicology Data

Over the last two decades, studies have been published that report effects of endosulfan *in vivo* in various laboratory animal species, including the effects of endosulfan on some aspect of endocrine function. Table 2 below categorizes and describes the available studies based on the study type, the endpoints examined, and the findings related to endosulfan. It should be noted that none of the studies in Table 2 are of the same or similar design to proposed Tier 1 or Tier 2 assays, although some of the endpoints examined are similar.

[2] It is well understood that use of *in vitro* doses in the micromolar (μM) range is a "high" dose as compared to levels of chemicals that are usually detected in blood or tissues *in vivo* following dosing of animals and humans. For example, a 20 μM solution of endosulfan if converted to a level in blood or tissue would equate to about 8 mg/L, a high level for a chemical in biological tissues *in vivo*, and in fact is a level associated with high dose intentional poisonings in humans.

**Table 2**
***In Vivo* Administration of Endosulfan in Laboratory Animals and Effects on Endocrine Function**

| STUDY TYPE | DOSES AND ENDPOINTS EXAMINED | REPORTED EFFECTS |
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| *Studies Focusing on Potential Androgenic Activity* | | |
| Repeat dose effects of endosulfan in Wistar rats (oral gavage study) (Singh and Pandey. 1989a. *Ind. J. Exp. Biol.* 27:341-346) | Endosulfan doses of 0, 2.5, 5, 7.5 and 10 mg/kg for 7 or 15 days.<br><br>Endpoints examined included testicular weight, testicular protein content, testicular steroidogenic enzyme activity, and testosterone levels in serum and testes. | No effects on testes organ weight.<br>Increased testicular protein content.<br>The authors reported a "variable" effect on testosterone production, an effect that was not dose-related.<br><br>LIMITATIONS: ATSDR concluded that these data were too variable for use in risk assessment. |
| Repeat dose effects of endosulfan in Wistar rats (oral gavage study) (Singh and Pandey 1989b. *Ind. J. Biochem. Biophys.* 26:262-267) | Endosulfan doses of 0, 7.5 and 10 mg/kg for 15 or 30 days (one group with a recovery period of 7 days).<br><br>Endpoints examined included body and liver weights, hepatic protein content, and activity of androgen biotransformation enzymes, as well as levels of serum and liver testosterone. | No effects on organ or body weight.<br>Increased hepatic protein content was reported after 30 days of treatment only.<br>Cytochrome P450 enzyme activity was increased while steroid metabolic activity decreased. However, the changes reported were only seen after 30 days of endosulfan exposure and were reversible.<br><br>LIMITATIONS: ATSDR concluded that these data were too variable for use in risk assessment. |
| Repeat dose effects of endosulfan in Wistar rats (oral gavage study) (Singh and Pandey. 1990. *Ind. J. Exp. Biol.* 28:953-956) | Endosulfan doses of 0, 7.5 or 10 mg/kg for 15 or 30 days (one group with a recovery period of 7 days).<br><br>Endpoints examined included body and testicular weights, changes in testicular protein content, levels of plasma FSH, LH, and testosterone, testicular testosterone levels, and activity of testicular steroidogenic enzymes. | No effects on body or testes weight.<br>No changes in protein content.<br>Decreased plasma levels of FSH, LH and testosterone. Decreased levels of testicular testosterone. These effects were most pronounced after 30 days treatment and appeared reversible.<br>Effects on steroidogenic enzymes were small and reversible.<br><br>LIMITATIONS: Oral gavage dosing methods produce pharmacokinetics that are not directly relevant to human exposures. |
| Repeat dose effects of endosulfan in adult Druckrey rats (oral gavage study) (Sinha et al. 1995. *Vet. Human* | Endosulfan doses of 0, 2.5, 5 or 10 mg/kg, 5 days per week for 70 days. | Increased testicular enzyme activities at all doses tested.<br>Sperm count decreased in a dose-dependent manner. |

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| *Toxicol.* 37:547-549) | Endpoints examined included testicular enzyme activities, sperm count, sperm morphology, and intra-testicular spermatid count. | Decreased spermatid counts and reduced daily sperm production at the two highest test doses was reported.<br><br>LIMITATIONS: Relevancy of data to human risk assessment has been questioned by EPA due to strain of rat used (non-standard, purity of test chemical employed, and duration of dosing). Results not consistent with data collected in guideline studies with endosulfan. |
| Repeat dose effects of endosulfan in weanling Druckrey rats (oral gavage study)<br>(Sinha et al. 1997. *Bull. Environ. Contam. Toxicol.* 58:79-86) | Endosulfan doses of 0, 2.5, 5 or 10 mg/kg, 5 days per week for 90 days.<br><br>Endpoints examined included testicular enzyme activity, testes weights, sperm count, sperm abnormality, spermatid count, and daily sperm production. | No effects on testes weights.<br>Increased levels of testicular enzyme activity.<br>Sperm count decreased in a dose-dependent manner.<br>Decreased spermatid count and sperm production rate.<br><br>LIMITATIONS: Relevancy of data to human risk assessment has been questioned by EPA due to strain of rat used (non-standard, purity of test chemical employed, and duration of dosing). Results not consistent with data collected in guideline studies with endosulfan. |
| Repeat dose effects of endosulfan in CD-1 mice (administered in the diet)<br>(Wilson and LeBlanc. 1998. *Toxicol. Appl. Pharmacol*. 148:158-168) | Endosulfan doses of 0, 3.8, 7.5 or 15 mg/kg/day for 7 days.<br><br>Endpoints examined included body and liver weights, hepatic testosterone metabolizing enzyme activities, rate of production of testosterone metabolites, and rate of elimination of fecal and urinary androgens (high dose female mice only). | No changes in liver weights.<br>Decreased body weights in high dose animals.<br>Female mice showed dose-dependent increases in the rate of testosterone metabolite formation, which was associated with increases in elimination of the steroid.<br>Authors reported that "homeostatic processes apparently compensate for the effect and minimize any consequences on serum hormone levels."<br><br>LIMITATIONS: Changes in serum testosterone in female mice is not a standard measure. Data in guideline toxicity studies with endosulfan showed no effects on female reproductive capacity. |
| Repeat dose effects of endosulfan in offspring of Wistar rats (oral gavage study)<br>(Dalsenter et al. 1999. *Hum. Exp. Toxicol.* 18:583-589) | Endosulfan doses of 0. 1.5 or 3 mg/kg from GD15 to PND21.<br><br>Endpoints examined included body weight, sexual development, reproductive | At high dose, a decrease in maternal body weight.<br>Decreased daily sperm production at the high test dose, with decrease seen at low dose only at puberty.<br>Decreased spermatogenesis at both dose |

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| | organ weights, sperm count, sperm morphology, histology of testes, testosterone levels, and reproductive performance. | levels.<br>Authors reported that "low" doses of endosulfan did not affect developmental landmarks.<br><br>LIMITATIONS: Small number of animals included in test groups limits data utility. Oral gavage dosing methods produce pharmacokinetics that are not directly relevant to human exposures. |
| Repeat dose effects of a mixture of chemicals in sexually mature male SD rats (oral gavage study)<br>(Wade et al. 2002. *Toxicol. Sci*. 67:131-143) | Complex chemical mixture administered at a dose of 1 µl/g for 70 days. The mixture included compounds at their minimal risk level (endosulfan was included).<br><br>Endpoints examined included proliferation of splenic cells, natural killer cell activity levels, daily sperm production, serum LH, serum FSH, serum prolactin, epididymal weights, sperm content of the cauda epididymus. | Authors reported only "minor" effects on immune function, reproductive hormone levels, or general indices of reproductive function measures.<br>Authors concluded that additive or synergistic effects of the chemicals in the complex mixture were unlikely to result in adverse effects on immune function or reproductive physiology.<br><br>LIMITATIONS: Results with a mixture not useful for endosulfan human health risk assessment. Oral gavage dosing methods produce pharmacokinetics that are not directly relevant to human exposures. |
| Repeat dose effects of endosulfan in offspring of male Wistar rats exposed pre- and post-natally (oral gavage study)<br>(Dalsenter et al. 2003. *Hum Exp. Toxicol.* 22:171-175) | Endosulfan doses of 0, 0.5 and 1.5 mg/kg/day from 21 days prior to mating (dams), during mating, pregnancy and lactation.<br><br>Endpoints examined included body weight, sexual development, reproductive organ weights, sperm production and morphology, testosterone level. | No maternal toxicity at either dose.<br>No adverse effects on sperm production or morphology.<br>No effects on sexual development.<br><br>LIMITATIONS: Small number of animals included in test groups limits data utility. Oral gavage dosing methods produce pharmacokinetics that are not directly relevant to human exposures. |
| Repeat dose effects of endosulfan in male pubertal Wistar rats (oral gavage study)<br>(Chitra et al. 1999. *Asian J. Androl*. 1:203-206) | Endosulfan administered at a dose of 1 mg/kg for 30 days.<br><br>Endpoints examined included body and organ weights, testicular biochemical parameters, and testicular steroidogenic enzyme levels. | Reported decreased body weights and reproductive organ weights.<br>Increased testicular protein content reported.<br>Decreased steroidogenic enzyme levels reported.<br><br>LIMITATIONS: Small number of animals included in test groups limits data utility. Oral gavage dosing methods produce pharmacokinetics that are not directly relevant to human exposures.<br>Results not consistent with data collected |

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| | | in guideline studies with endosulfan. |
| Repeat dose effects of endosulfan in immature Wistar rats (oral gavage study) (Rao et al. 2005. *Ind. J. Phyisol. Pharmacol.* 49:331-336) | Endosulfan administered from postnatal day 7 to postnatal day 60 at doses of 0, 3, 6, 9, and 12 mg/kg/day.<br><br>Endpoints examined included body and testes weights, sperm count, sperm motility, and sperm morphology. | Decreased testes weights reported as well as decreased sperm counts and motility.<br>Sperm abnormalities seen in rats dosed with 6 mg/kg and greater doses of endosulfan.<br>LIMITATIONS: Small number of animals included in test groups limits data utility. Oral gavage dosing methods produce pharmacokinetics that are not directly relevant to human exposures. Results not consistent with data collected in guideline studies with endosulfan. |
| *Studies Focusing on Potential Estrogenic Activity* | | |
| Repeat dose effects of endosulfan in ovarectomized Wistar rats (oral gavage study) (Raizada et al. 1991. *Nat. Acad. Sci. Lett.* 14:103-107) | Rats administered endosulfan, 1.5 mg/kg daily for 30 days and injected daily with estradiol (1 µg/rat i.p.).<br><br>Endpoints examined included organs weights, glycogen content of organs, and histopathological changes in organs. | No effects on organ weights (uterus, cervix, vagina, pituitary).<br>No effects on glycogen content of the organs examined.<br>No histopathological changes were reported.<br>Authors did report transient clinical signs.<br><br>LIMITATIONS: Oral gavage dosing methods produce pharmacokinetics that are not directly relevant to human exposures. |
| Repeat dose effects of endosulfan in immature CD-1 mice determined *ex vivo* (subcutaneous injection) (Shelby et al. 1996. *Environ. Health Perspect.* 104:1296-1300) | Endosulfan administered on postnatal days 17-19 at a dose of 10 mg/kg.<br><br>Endpoints examined included competitive binding of endosulfan to mouse uterine estrogen receptor *ex vivo* and uterine growth. | No competitive inhibition of estrogen receptor binding reported at a dose $10^{3}$-fold in excess.<br>No increase in uterine wet mass.<br>Authors noted that other compounds studied (DES, estradiol, tamoxifen, DDT, methoxychlor) significantly affected uterine growth.<br><br>LIMITATIONS: Dosing methods produce pharmacokinetics that are not directly relevant to human exposures. |

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| Repeat dose effects of endosulfan in immature rats determined *ex vivo* (Wade et al. 1997. *Reproduct. Toxicol.* 11:791-798) | Rats injected daily for 3 days starting at day 18 after birth with 3 mg/kg/day endosulfan.<br><br>Endpoint was uterine estrogen receptor binding. | Endosulfan inhibited estradiol binding only at high doses ($10^5$ M).<br>Authors reported no change in uterine weights, uterine peroxidase activity, or numbers of uterine estrogen and progesterone receptors.<br><br>LIMITATIONS: Dosing methods produce pharmacokinetics that are not directly relevant to human exposures. |

First focusing on the results of the studies related to estrogenic effects, review of the studies in Table 2 reveals that endosulfan administration *in vivo* to laboratory animals did not produce significant estrogenic effects. Although the *in vitro* studies had indicated endosulfan was a weak estrogenic compound, the *in vivo* studies did not reveal any significant toxicity or organ changes that would be linked to such effects. As was seen in the *in vitro* studies, the binding potency of endosulfan to estrogen receptors as determined *ex vivo* was also many orders of magnitude lower than the binding affinity of the natural hormone, estradiol.

When the *in vivo* effects of endosulfan on androgen function are examined, results were variable although in some studies endosulfan was shown to be associated with alterations in testicular function. Like the effects seen *in vitro*, however, the testicular effects in animals were associated with high doses of endosulfan. As will be discussed in the following section of this paper related to unpublished toxicology data, the doses of endosulfan that produced significant toxicity on non-endocrine endpoints in a multi-generation reproductive toxicity study and a chronic toxicity/carcinogenicity study were lower than the doses administered in the published studies. Moreover, EPA in assessing some of these studies as a group (*e.g.,* the Sinha studies

and the studies by Dalsenter et al.) concluded that the variability in the study results in some cases could be attributed to the strain of rats tested, the duration of exposure, and the purity of the compound tested (see April 2, 2007 Reaves memorandum; TXR#0054554). As a result, although high dose exposure to endosulfan may be a risk for toxicity to male reproductive organs, not all of these data are relevant to assessing the risk and potential endocrine effects of the levels of endosulfan exposure that are encountered in the human environment.

### 3. Studies Reporting Effects in Humans

With the recognition that humans can be exposed to chemicals such as pesticides both through occupational activities and through contact with environmental media such as food, soil, and water, researchers have become interested in examining the potential for such chemicals to affect the health of human populations. Since it is generally considered unethical to intentionally dose humans with pesticides in order to specifically investigate toxicity, the published literature relating to potential human toxicity of pesticide exposures is limited to observational studies. These are studies where populations are defined not by a specific exposure dose but generally by the likelihood that exposure has or has not occurred. Therefore the data on actual exposures in any individual within a population being studied is usually inferred, not directly measured. The exception to this would be occupational exposure studies where workers may wear biomonitoring devices that are able to measure either exposure on the skin or exposure in the breathing zone. In some occupational studies, individual measures of internal levels of chemical exposure may be available (*e.g.*, blood, urine). Unfortunately, such human studies where exposure of individuals is reliably

quantified are rare. This is a major limitation of human studies in the area of pesticide toxicology and needs to be considered as studies are interpreted. Without adequate exposure information, any reported biological effects are difficult if not impossible to interpret as being related to a particular chemical.

In the case of endosulfan, several studies have been identified in the published literature addressing the issue of potential endocrine effects of the pesticide. These studies are described below in Table 3.

**Table 3**
**Studies Reporting Potential Endocrine Effects of Endosulfan in Humans**

| STUDY TYPE AND CITATION | POPULATION DESCRIPTION AND ENDPOINTS EXAMINED | REPORTED EFFECTS |
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| Cohort study in a population of lactating women in Spain (Campoy et al. 2001a. *Early Hum. Develop.* 65:173-182; Campoy et al. 2001b. *Early Hum. Develop*. 65:183-190) | Comparison of breast milk residues of pesticides in a population from an area of intensive agricultural activity (Almeria) with the level found in a population living in an urban area (Granada).<br><br>Endpoints examined included levels of a variety of pesticides, including endosulfan. | Authors reported that women living in both areas had detectable levels of many pesticides in breast milk, including endosulfan.<br><br>LIMITATIONS: There was no information collected on sources of exposure to any one particular chemical. No data linking exposures to any effects. |
| Cohort study in a population of male schoolchildren (10-19 years old) in India (Saiyed et al. 2003. *Environ. Health Perspect.* 111:1958-1962) | Exposed children living in a village at the foothills of cashew plantations where endosulfan had been aerially sprayed for more than 20 years. Control children from a village 20 km away.<br><br>Endpoints examined included growth-related parameters, sexual maturity rating assessment, serum testosterone, LH, and FSH, as well as endosulfan residues in blood. | The authors reported that sexual maturity was delayed in the exposed population as compared to the control population.<br><br>Serum endosulfan levels were higher in the exposed population as compared to controls, although even the control population had detectable levels of endosulfan in serum. 78% of the exposed group had detectable endosulfan in serum as compared to 29% of the control population.<br><br>Only serum LH levels appeared to be different between the populations when age was considered. |

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| | | LIMITATIONS: No information on other exposures that could also be related to potential endocrine toxicity were collected. |
| Case-control study in a population of women in Spain diagnosed with breast cancer (Ibarluzea et al. 2004. *Cancer Causes Cont*. 15:591-600) | Cases were women diagnosed with breast cancer at 3 hospitals in Almeria and Granada provinces from April 1996-June 1998. Controls were cancer-free and matched for age and hospital.<br><br>Endpoint examined was the relationship between cancer diagnosis and the levels of pesticides in adipose tissue, as well as body mass index. | Endosulfan residues were not associated with an increased risk of breast cancer. Leaner women had an increased risk of breast cancer; in the leaner women, the estrogenicity of adipose tissue, accumulated xenoestrogens, was associated with a higher risk of cancer as well.<br><br>LIMITATIONS: No information on sources of exposure to any of the chemicals studied. No mention of other potential chemicals that could be related to risks. |
| Survey study of women in Spain (Cerillo et al. 2005. *Environ. Res.* 98:233-239) | Adipose tissue, placental sample, and blood from the umbilical cord were collected from women entering hospitals for surgery or delivery of newborns. | Authors reported that endosulfan was detected in adipose, tissue, placental samples, and blood.<br><br>LIMITATIONS: Again, no information on exposure sources. Survey study only with no controls. |
| Review paper on the issue of human puberty and endocrine disruptors (Den Hond and Schoeters. 2006. *Int. J. Androl*. 29:264-271) | Reviews the published literature in the areas of female and male puberty onset and exposure to chemicals linked to endocrine disruption. | Since this is a review paper, no data specific to endosulfan or its risks to human health are presented.<br><br>The authors state that the literature does not allow for conclusions to be drawn regarding cause and effect.<br><br>LIMITATIONS: No actual data described so not useful for risk assessment. |
| Prospective cohort study of the sons of pregnant women employed in greenhouses in Denmark (Andersen et al. 2008. *Environ. Health Perspect. Online January 22*) | "Exposed" were mother-son pairs who had been exposed to pesticides in their working area more than once a month, applied pesticides, or handled treated plants. "Unexposed" mother-son pairs did not meet those criteria.<br><br>Endpoints examined included pregnancy outcomes, testicular volume and position, penile length, | No specific pesticide was the focus of the study. Dozens of products were in use.<br><br>Decreased penile length was reported as statistically significant.<br><br>LIMITATIONS: Since this study did not collect exposure information, the study cannot be used to establish any relationship between pesticide exposure and risk to human health. |

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| | position of urethral opening, and levels of serum reproductive hormones. | |

Review of the studies in Table 3 reveals that because of a lack of information on actual exposures within study groups, the available human data cannot be used in human health risk assessment in any scientifically defensible method. All of the studies suffer from a lack of reliable information on actual exposures of the populations to endosulfan. Only one of the human studies, Saiyed et al. (2003), had a design that was amenable to examining the potential for endosulfan to affect endocrine function. However, even the study by Saiyed et al. (2003), which attempted to focus on the issue of endosulfan exposure, failed to provide evidence that endosulfan is the only chemical that could be linked to the reported associations in the study. The study of Saiyed et al. (2003) is at best a hypothesis-generation study that could be used to design another more specific analysis of endosulfan exposure and potential health effects. Considered together the human data do not provide evidence that endosulfan is an endocrine disrupting chemical nor that endosulfan has endocrine effects that pose a risk to human health.

**B. Unpublished Studies**

In addition to studies in the published literature, any assessment of the potential endocrine toxicity of endosulfan must include consideration of toxicology studies performed as part of the pesticide registration process, studies that are usually unpublished. During the registration process for endosulfan, hazard evaluation and risk assessments performed by regulatory bodies around the world included assessment of reproductive effects, using a study design like the one proposed as an EPA Tier 2 test. It

is a general principle of reproductive toxicity testing that potential hormonal activity of the test chemical is assessed through evaluation of a variety of endpoints (Stevens et al. 1998. *J. Toxicol. Environ. Health* 102:380-383). Table 4 below lists the array of toxicity tests that were conducted with endosulfan and included as part of the endosulfan "Re-registration Eligibility Decision" or RED (EPA. 2002. EPA 738-R-02-013, November). The table lists the tests, the endpoints examined related to potential endocrine toxicity, as well as the findings in the studies. In the table, a finding listed as "negative" means that that there was no statistically significant association between exposure to endosulfan and that endpoint. A discussion of the studies follows Table 4.

**Table 4**
**Endocrine Effects Examined in Guideline Pesticide Toxicity Studies for Endosulfan**

| ENDPOINT | SUBCHRONIC TOXICITY TESTS | | | | DEVELOPMENTAL TOXICITY TESTS | | MULTI-GENERATION REPRODUCTIVE TOXICITY TEST | CHRONIC TOXICITY TESTS (CANCER) | |
|---|---|---|---|---|---|---|---|---|---|
| Species | Rat | Mouse | Rat | Dog | Rat | Rabbit | Rat | Rat | Mouse |
| *EFFECTS REPORTED IN ADULT ANIMALS* | | | | | | | | | |
| Reproduction | | | | | | | neg.[1] | | |
| Fertility | | | | | | | neg. | | |
| Fecundity | | | | | | | neg. | | |
| Gestation length | | | | | neg. | neg. | neg. | | |
| Abortion | | | | | neg. | neg. | neg. | | |
| Premature Delivery | | | | | neg. | neg. | neg. | | |
| Difficult labor | | | | | | | neg. | | |
| Time to mating | | | | | | | neg. | | |
| Mating and sexual behavior | | | | | | | neg. | | |
| Estrus cycle | | | | | | | neg. | | |
| Ovulation | neg. | neg. | neg. | neg. | | | neg. | neg. | neg. |
| Spermatogenesis | neg. | neg. | neg. | neg. | | | neg. | neg. | neg. |
| Sperm count | | | | | | | | | |
| Gonad development | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Secondary sexual characteristics (muscle mass, etc.) | neg. | neg. | neg. | neg. | | | neg. | neg. | neg. |
| Gross pathology of reproductive organs | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. | neg. |
| Histology of reproductive organs | neg. | neg. | neg. | neg. | | | neg. | neg. | neg. |
| Hormone levels | | | | | | | | | |
| Major sex differences | neg. | neg. | neg. | neg. | | | neg. | neg. | neg. |
| Endocrine tumor incidence | neg. | neg. | neg. | neg. | | | neg. | neg. | neg. |
| *EFFECTS REPORTED IN OFFSPRING* | | | | | | | | | |
| Sexual differentiation | | | | | neg. | neg. | neg. | | |
| Offspring sex ratio | | | | | neg. | neg. | neg. | | |
| Gonad development (size, morphology, weight) | | | | | neg. | neg. | neg. | | |
| Accessory organ development | | | | | neg. | neg. | neg. | | |
| Accessory sex organ function | | | | | | | neg. | | |
| Sexual development (vaginal opening, testes descent, etc.) | | | | | | | neg. | | |
| Malformations of genital tract | | | | | neg. | neg. | neg. | | |
| Gross pathology of reproductive organs | | | | | neg. | neg. | neg. | | |
| Histology of reproductive organs | | | | | | | neg. | | |
| Viability of the conceptus | | | | | | | neg. | | |
| Viability of offspring (neonatal) | | | | | neg. | neg. | neg. | | |
| Growth of conceptus (weight) | | | | | neg. | neg. | neg. | | |
| Growth of | | | | | | | neg. | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| offspring | | | | | | | | | |
| Major sex differences | | | | | neg. | neg. | neg. | | |

Two of the study types described above in Table 4 provided data permitting assessment of direct toxicity of endosulfan to endocrine organs as evidenced by changes in organ size and organ morphology and histology. In the subchronic toxicity studies in multiple species (MRID 00145668, 00147182, 41099501), no effects were reported on endocrine or reproductive organs. Although hormone levels were not measured in these studies, consequences of hormonal changes were monitored, such as organ weight changes of the pituitary gland, thyroid gland, uterus, ovaries, testes, adrenal gland, mammary gland, epididymides, seminal vesicles, and vagina. In chronic studies (MRID 41009502, 40792401), which involve exposure over the lifetime of the animal, minor hormone-related effects of a test substance should become evident.

In the chronic studies with endosulfan, there were no changes evident in any of the endocrine-related organs, nor was there an increased tumor incidence in such organs. In the rat carcinogenicity study (NCI 1978. DHEW Publication No. NIH-78-1312) endosulfan doses were associated with testicular atrophy and parathyroid hyperplasia, effects that can be related to direct or indirect endocrine toxicity. However, review of the study report reveals that these effects on two selected endocrine organs were accompanied by significant renal and liver toxicity as well as mortality rates of 38% and 50% (two endosulfan dose groups). It has been established that severe intoxication that involves organs such as the liver and kidney results in significant disruption of physiological homeostasis and indirect effects on organs in the major endocrine axis. This fact, combined with the fact that no such effects were seen in the other repeat dose toxicity studies, at higher dose levels, indicates that the effects reported in the

chronic oral rat study were not indicative of direct endocrine toxicity related to endosulfan exposure itself but instead indirect effects related to severe systemic toxicity.

The other study types described in Table 4 (MRID 43129101, 00094837), reproductive and developmental toxicity studies, provided data not only on direct toxicity of endosulfan to endocrine organs but also data on the functional consequences of altering endocrine organ physiology and hormone levels. In the developmental toxicity study, endosulfan treatment during the period of organogenesis of the developing fetus did not affect the development of the endocrine system.

In the 2-generation reproductive study in rats, possible disturbances of reproductive performance, development and maturation, including development of sex organs (vaginal opening, testis descent, cryptorchidism, *etc.*) were examined at doses up to and including doses that caused parental toxicity. Endosulfan did not produce any such endocrine-related toxicity in the study through two successive generations. There was an increase in pituitary weights, without any accompanying histological changes, in the high dose $F_0$ pups of the first mating as well as an increase in uterine weights, also without histological changes, in the $F_{1b}$ pups from the first mating. These organ weight changes, however, must be viewed in relation to the other available data. Neither the uterus nor the pituitary gland was a target organ in any other study, and the effects were not consistent across generations. The increase in pituitary weight was attributable to a result in a single high dose female. Moreover, results of *in vitro* uterotrophic assays failed to demonstrate that endosulfan has specific effects on the uterus at doses up to 100 mg/kg/day.

Considered together, the combined data indicated that endosulfan did not disrupt the endocrine system in parental animals or their offspring at exposure levels up to 3-6 mg/kg/day (dietary levels of

75 ppm), a level that was toxic in adult animals. This is an important point to consider, since the studies in the published literature that described endocrine-related toxicity in animals were either performed at higher doses or involved use of oral gavage methods, a dosing method that results in a spike of endosulfan in the blood with initial dosing.[3] It is generally accepted that human pesticide exposure conditions are better approximated with dosing methods other than gavage dosing (*e.g.,* dietary exposure, drinking water exposure, dermal exposure).

There is one additional unpublished study to consider, a developmental neurotoxicity study in rats (MRID 46968301). In the study female rats were administered endosulfan in their diet at doses of 0, 3.74, 10.8, or 29.8 mg/kg/day from gestation day 6 through postnatal day 21. Offspring were dosed through passage of drug across the placenta *in utero* and through nursing. The endpoints examined were the standard endpoints for a guideline study and included assessment of dams and offspring. The only finding noted that could be related to endocrine effects was a small delay in preputial separation in males but only at the mid test dose level (10.8 mg/kg/day). The lack of a dose-response in the finding noted argues against endosulfan being an endocrine toxicant in this study. There were no accompanying changes in sperm parameters measured at termination in this group. The results of the developmental neurotoxicity study provide further support for the finding that endosulfan is not an endocrine toxicant.

Therefore, the unpublished endosulfan laboratory animal data when considered together indicate that endosulfan does not pose a risk to human health when endocrine effects are considered.

[3] Gavage dosing pharmacokinetics (bolus, rapid exposure) can result in very different internal exposure than the exposure likely seen in humans, where exposure is through environmental media at smaller doses, at repeated intervals.

## III. Does the Weight-of-the-Evidence Indicate that Endosulfan is a Potential Endocrine Disrupting Chemical in Humans?

Considering all of the available data, published *in vitro* data, published *in vivo* data, published human data, and the toxicological data submitted as part of the pesticide registration process for endosulfan, a weight-of-the-evidence evaluation can be performed. Such evaluations are routinely performed by regulatory bodies as well as independent scientists when attempting to answer questions concerning the relevance of toxicological data or observations collected in cells or laboratory animals to predicting the toxicity potential of a chemical for humans. Such evaluations are based on patterns in the data that may be present, on the reliability of studies used in the assessment, on the consideration of the limitations of the individual studies used in the evaluation, and on consideration of the relevance of the data to human physiology and human exposure situations. Such evaluations can also rely on data collected for similar chemicals, if such data exists, as a way to fill data gaps in the assessment.

In the case of endosulfan, the *in vitro* studies indicated that endosulfan had some effects on estrogen and androgen functions and/or receptors. *In vitro* studies, however, are only relevant as indicators of the potential binding of a chemical to hormone receptors or indicators of the potential to affect hormone metabolism. This is because the conditions of *in vitro* assays do not allow for the known relationship of pharmacokinetics and metabolism to the pharmacodynamics of chemical responses and chemical toxicity. Further, in the case of endocrine system function, such isolated assays have limited predictive value for the conditions of living organisms where endocrine regulation is complex and interrelated. Therefore, the *in vitro* studies with endosulfan are useful for identifying the need for collection of *in vivo* data that should be evaluated for potential effects on estrogen-related and androgen-related functions (*e.g.,* reproductive function). It must also be remembered that the potency of endosulfan in these *in vitro* studies was very low, with potencies in the range of $10^5$ to $10^6$ times less than the naturally occurring hormones and even natural phytoestrogens that are present in the human diet.

When evaluating the *in vivo* toxicological data, the types of studies and the dose levels administered must both be considered. The studies performed to support the registration of endosulfan (unpublished studies) were consistent in showing that endosulfan was not toxic to endocrine organs, even following lifetime exposures. These studies, like all such guideline *in vivo* toxicology studies, were designed purposely to be valid and realistic assessments of possible effects in humans. Therefore, regulatory bodies rely on such studies to make decisions regarding safe levels for human exposure. The published *in vivo* animal studies that did report effects of endosulfan on endocrine systems, however, were not always designed in order to provide such realistic assessments of possible effects in humans. For example, many of the studies employed gavage dosing methods, a method of dosing which results in a pattern of exposure that is not realistic, except possibly for consideration of intentional high dose ingestion scenarios (suicide). Other studies reported effects only at high doses, doses beyond the levels shown to produce frank non-endocrine related toxicity in the guideline animal studies. In the case of studies reporting effects on steroidogenesis and hormone levels, the effects were also shown to be reversible. When considered together, the weight-of-the-evidence of the *in vivo* animal studies indicates that endosulfan is not directly toxic to endocrine organs, and that effects observed were limited to situations where exposure conditions were unrealistic as compared to human exposures.

In a typical assessment of potential to produce harm in humans, the process of evaluation would be complete after consideration of the *in vitro* and *in vivo* laboratory data. However, in some cases, human data are also available and should be part of the evaluation process. In the case of endosulfan, the available human data are not amenable to human risk assessment because the studies available generally lack the exposure data necessary in order to draw conclusions regarding exposure to any chemical at all, including endosulfan. Therefore, consideration of the human studies does not change the conclusions drawn from the laboratory data that endosulfan is not toxic to endocrine systems.

There is one other source of information that could be factored into the endosulfan assessment, studies that have described potential effects of pesticide

mixtures on endocrine responses. In 1996, a paper was published that reported dramatic synergism by a factor of as much as 1600-fold when weakly estrogenic chemicals were tested together in an *in vitro* genetically engineered yeast cell culture system (Arnold et al. 1996. *Science* 272:1489-1492). This finding led to studies my other groups on the issue of synergism and the potential hazard of mixtures of chemicals that might be present at low levels in the environment. However, the 1996 paper was retracted by the authors the following year because they and others could not reproduce the results (McLachlan 1997. *Science* 277:459-463). In fact, a review of the published literature on the issue of endosulfan and synergistic endocrine effects reveals that synergy is not an issue for concern (*e.g.,* Ashby et al. 1997. *Nature* 385:494; Wade et al. 1997. *Toxicology* 11:791-798; Ramamoorthy et al. 1997. *Endocrinology* 138:1520-1527).

In conclusion, after consideration of all of the available data, the weight-of-the-evidence indicates that endosulfan is not an endocrine-disrupting compound at environmentally relevant concentrations.

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