

10. ENDOCRINE DISRUPTION TECHNICAL REPORT

(Conducted on behalf of the APVMA by the Office of Chemical Safety (OCS) within the Department of Health and Ageing)

10.1 INTRODUCTION

The APVMA interim report on the review of endosulfan (1998) assessed a comprehensive toxicity data package. The major hazard associated with endosulfan was the high acute toxicity through exposure by ingestion, skin contact or inhalation. It was found that endosulfan does not persist for long periods in the tissues or organs of animals, and it was concluded that endosulfan was unlikely to bioaccumulate in humans.

There was no increase noted in the incidence of cancer arising from high concentrations and long exposure periods to endosulfan in the diet. It was also concluded that endosulfan was not likely to have any harmful effects on reproduction or cause birth defects. Endosulfan was not found to cause damage to genetic material and there was no evidence of disruption to the endocrine hormonal system.

In examining the issue of whether endosulfan is a xenoestrogen, the interim report concluded that toxicology studies did not indicate that endosulfan induces any functional aberrations that might result from disruption of endocrine homeostasis. However, a US EPA RED (Reregistration Eligibility Decision), finalised in 2002, identified endosulfan as “a potential endocrine disruptor”.

Subsequent to the interim report, the APVMA decided to re-examine the issue of endocrine disruption for endosulfan. In doing so, the objective was to:

- 1) examine the US EPA RED report and attendant information regarding endosulfan, and identify and clarify variations from previous conclusions reported in the interim report;
- 2) specifically re-examine the issue of possible endocrine disruption caused by endosulfan.

In conducting this re-examination, the conclusions of the interim report relating to the chronic, developmental and reproductive studies have been reconsidered, together with the relevant findings of the US EPA RED report. Additionally all of the published literature relevant to the endocrine disrupting potential of endosulfan to the end of April 2003 has been evaluated.

Part 1 of this report considers the US EPA RED for endosulfan, which was finalised in November 2002, and compares it to the Australian ECRP review of endosulfan that was released in September 1999. The overall conclusions and regulatory recommendations of both documents are summarised and it can be seen that the overall conclusions and recommendations of both regulators are very similar.

Part II of this report examines the issue of whether endosulfan is a xenoestrogen. The ECRP review concluded that toxicology studies did not indicate that endosulfan induces any functional aberrations that might result from disruption of endocrine homeostasis. In contrast, the US EPA RED identifies endosulfan as “a potential endocrine disruptor”, a view strongly opposed by the Endosulfan Task Force (ETF), an industry grouping consisting of the technical registrants of endosulfan. This section summarises the current scientific understanding of endocrine disruption and the evidence that endosulfan is an EDC.

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the final US EPA RED report. Additionally all of the published literature relevant to the endocrine disrupting potential of endosulfan to the end of April 2003 has been evaluated.

10.2 US EPA AND APVMA REPORTS

10.2.1 APVMA review of endosulfan

Regulatory history of endosulfan in Australia

In 1968 the ADI for endosulfan was set at 0.007 mg/kg/day, it was included in schedule 6 of the SUSDP), and MRLs were established. In 1985 the clearance of endosulfan TGAC was reviewed. All available toxicology data were evaluated and the NOEL and ADI were confirmed. At this time, changes to product scheduling, particularly home garden uses were foreshadowed. During 1987-88 additional toxicology data supplied by the sponsors was evaluated and the TGAC clearance and the Poisons Scheduling were reviewed. Endosulfan products were withdrawn from the home market and the active was rescheduled from S6 to the more restrictive S7. In 1995 the NDPSC confirmed the S7 schedule and endosulfan was nominated onto the AVPMA ECRP Priority Review Candidate List. During 1997-98 the endosulfan review data call-in studies, public submissions and all available toxicology information were evaluated for the review. The AVPMA endosulfan review findings were released in August 1998; the toxicology, findings are summarised below.

Toxicology and Public Health Issues

The review of the mammalian toxicology and the metabolism/toxicokinetics of endosulfan concluded that the substance has high acute toxicity when administered via oral, dermal, and inhalational routes of exposure, with clinical signs of acute intoxication including piloerection, salivation, hyperactivity, respiratory distress, diarrhoea, tremors, hunching and convulsions. Long-term dietary studies in rodents indicated that endosulfan was not carcinogenic, it lacked genotoxicity in a range of tests, and it had no adverse effects on reproductive parameters. While evidence of delayed development was seen in rat fetuses, this was associated with maternotoxicity, and no treatment related teratogenicity was observed in any studies. In rats, the kidney appeared to be the main target in a number of studies. Renal effects seen included increases in kidney weights and granular pigment formation after shorter-term administration, and progressive chronic glomerulonephrosis or toxic nephropathy after longer-term exposure to endosulfan. The toxicology review noted that these renal findings are common in ageing laboratory rats and also occurred at a high incidence in non-exposed control animals.

The Acute Reference Dose (acute RfD) for endosulfan was set at 0.02 mg/kg bw derived from a NOEL of 2.0 mg/kg bw based on developmental effects, reduced food consumption and clinical signs (tonoclonic convulsions, hypersalivation) seen in a rat developmental study at the LOEL of 6.0 mg/kg bw/d.

The Acceptable Daily Intake (ADI) was set at 0.006 mg/kg bw/day derived by applying a 100-fold safety factor on a NOEL of ca. 0.6 mg/kg bw/day. This NOEL was common to a range of studies as detailed below.

No-observed-effect-level (NOEL) seen in a range of endosulfan studies
<ul style="list-style-type: none">• 0.58 mg/kg/day in female mice in a 78-week dietary study, the highest dose tested;• 0.64 mg/kg/day in rats in a 13-week dietary study, based on haematological changes and granular pigment formation in renal proximal tubules at 1.92 mg/kg/day;• 0.57 mg/kg/day (females) and 0.65 mg/kg/day (males) in dogs in a 1-year dietary study, based on clinical signs and reduced body weights at 2.3 mg/kg/day;• 0.66 mg/kg/day in female rats in a developmental study, based on decreased body weights at 2 mg/kg/day.• 0.6 mg/kg/day in a 2-year rat dietary study, based upon reduced body weights and kidney

pathology at 2.9 mg/kg/day.

10.2.2 The US EPA Reregistration Eligibility Decision

USA Regulatory history

In the USA, endosulfan is registered for use on a wide variety of vegetables, fruits, cereal grains, and cotton, as well as ornamental shrubs, trees, vines, and ornamentals for use in commercial agricultural settings. The use patterns and productspectrum in the USA are comparable to those seen in Australia.

The regulatory history of endosulfan in the USA is not dissimilar to that seen in Australia. The technical registrants amended product labels in 2000 to withdraw all home-garden or domestic uses.

The RED process was initiated in 1996 in accordance with the requirements of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). The Act calls for the development and submission of data to support the re-registration of an active ingredient, as well as a thorough review by the US EPA of the current scientific database underlying a pesticide's registration. The Food Quality Protection Act of 1996 (FQPA) requires a risk assessment of residue levels including an assessment of cumulative effects of chemicals with a common mechanism of toxicity. Endosulfan is broadly classed as a chlorinated cyclodiene, or more accurately as a dioxathiepin insecticide/acaricide. The US EPA has concluded that there are not any other chemical substances that share a common mechanism of toxicity with endosulfan and thus they did not perform a cumulative risk assessment as part of the RED.

The US EPA draft RED for endosulfan was released for comment in July, 2002, after consultation with the Endosulfan Task Force (ETF), an industry grouping made up of the technical registrants of endosulfan. The final review document was released in November 2002.

Summary conclusions of endosulfan Reregistration Eligibility Decision

Toxicology and Public health issues: The EPA assessed dietary risk by estimating exposure to endosulfan residues from consumption of food and drinking water that can occur over a single-day (acute) or longer (chronic). Based on the 99.9th percentile of exposure for the Population Adjusted Dose (PAD), the EPA concluded that residues of endosulfan in drinking water and food were both of concern for some population subgroups for the acute but not the chronic PAD. For the general population neither PAD was of regulatory concern. To mitigate the risks from acute food exposure, the EPA cancelled the use of endosulfan on succulent beans, succulent peas, grapes, and spinach. To mitigate the risks from drinking water, the EPA mandated buffer zones between treated areas and water bodies, reductions in maximum application rates, reductions in maximum seasonal application rates and reductions in the maximum number of applications allowed per use season.

The US Acute Reference Dose for endosulfan is 0.015 mg/kg bw, derived from a NOAEL of 1.5 mg/kg bw and applying a 100-fold safety factor; it is based on the increased incidence of convulsions seen in female rats within 8 hours after dosing at the LOAEL of 3 mg/kg bw in an acute neurotoxicity study.

The US Chronic Reference Dose is 0.006 mg/kg bw/day derived by applying a 100-fold safety factor to the NOAEL of 0.6 mg/kg bw/day; it is based on reduced body weight gain, enlarged kidneys, increased incidences of marked progressive glomerulonephrosis; and blood vessel aneurysms in male rats seen at the LOAEL of 2.9 mg/kg/day in a combined chronic toxicity/carcinogenicity study in rats.

FQPA safety factor: The FQPA Safety Factor of 10x for protection of children was retained for endosulfan. The RED comments that a weight-of-the-evidence approach indicated that there were no reliable data available to address 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 neuroendocrine effects in the young, and 4) the need for a developmental neurotoxicity study (DNT). Hence an extra 10-fold safety factor was applied to each of the acute and chronic RfDs to derive the respective acute and chronic PADs of 0.0015 mg/kg bw and 0.0006 mg/kg bw/d.

Occupational health and safety issues: The EPA review concluded that there are potential mixer, loader, applicator as well as post-application exposures to occupational handlers. Based on current use patterns, there are some short-term dermal and inhalation risks of concern for workers who mix, load and apply endosulfan to agricultural sites, as well as to those workers who re-enter a treated area following application of endosulfan. To mitigate these risks, the

US EPA mandated changes to packaging, deleted aerial application of WP products for some crops, and stipulated closed mixing/loading systems, closed cabs for air-blast equipment and restricted re-entry periods.

Environmental risks: For ecological effects, the EPA conducted a screening level assessment for terrestrial impacts and a refined exposure assessment for aquatic impacts of endosulfan use. These assessments indicated that endosulfan is likely to result in acute and chronic risk to both terrestrial and aquatic organisms. The report documents incidents where exposure to endosulfan has resulted in both reproductive and development effects in non-target animals, particularly birds, fish and mammals. The mitigation steps required are identical to those required for protecting drinking water, with the extra requirement of deletion of use on pecan nuts. The EPA also expressed concern regarding the persistence and long-range transport of endosulfan in the environment. Endosulfan is relatively volatile and moderately persistent and can migrate over a long distance through various environmental media such as air, water, and sediment.

Residue issues: In order to mitigate human and environmental risks, the EPA mandated that several MRLs be withdrawn as detailed above. Additional restrictions were placed on some allowable application rates and permitted geographical areas. Unlike the ECRP review, the RED did not identify restrictions on fodder and forage crops to minimise residues in meat products, but has requested studies to investigate this issue.

Data requirements listed in the US EPA RED: The EPA requires the following additional generic studies for endosulfan to confirm its regulatory assessments and conclusions:

- Avian acute oral toxicity of bobwhite quail and mallard ducks
- Avian subchronic oral toxicity of bobwhite quail and mallard ducks
- Avian reproduction study
- Freshwater fish acute toxicity study of bluegill sunfish
- Early life stage fish
- Life cycle invertebrate
- Freshwater fish full life cycle using rainbow trout
- Estuarine/marine fish acute toxicity study
- Estuarine/marine invertebrate acute toxicity study of mysid shrimp
- Whole sediment acute toxicity testing using a freshwater invertebrate
- Whole sediment acute toxicity testing using an estuarine/marine invertebrate
- Whole sediment chronic toxicity testing using a freshwater invertebrate
- Whole sediment chronic toxicity testing using an estuarine/marine invertebrate
- Vegetative buffer effectiveness study
- Groundwater monitoring study
- Surface drinking water monitoring study
- Subchronic Neurotoxicity - Rat
- Developmental Neurotoxicity Toxicity Study - Rat
- Storage stability (oils seed, non-oily grain and processed commodities)
- Crop field trials for the following raw agricultural commodities: barley hay, and pearled barley; oat forage, hay, and rolled oats; rye forage; wheat forage, and hay
- Crop field trials for tobacco and a pyrolysis
- Magnitude of residue in processed food/feed commodities
- Dermal outdoor exposure for applying dip treatments to trees and roots or whole plants
- Product use information for applying dip treatments to trees and roots or whole plants

10.3 IS ENDOSULFAN AN ENDOCRINE DISRUPTOR?

Definition and mechanisms

Several definitions for the term ‘endocrine disruptor’ have been proposed. According to the definition of the OECD, “an endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations. A **potential** endocrine disruptor is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny or (sub)populations” (OECD, 1998).

The working definition used in the final report of the US EPA Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) is as follows: an “endocrine disruptor is an exogenous chemical or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations or subpopulations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle” (EDSTAC, 1998). The National Research Council (NRC) of the USA has adopted the term “Hormonally Active Agents”, in place of the term “endocrine disruptor chemicals” (NRC, 1999).

The broad sweep of these current definitions is deliberate as they are framed to include all endocrine effects, not just those affecting sex hormones. EDCs can thus be expected, at a minimum, to disrupt at least one of the three major endocrine axes that affect reproductive development and function, these being the hypothalamic-pituitary-gonadal (HPG), the thymus-pituitary-thymus (HPT), and the adrenal-pituitary-adrenal (HPA) axes. It is clear that endocrine disruptors can affect other endocrine axes as well.

The mode of action of EDCs is potentially equally diverse. The IPCS review clearly states that: “The mechanism or mode of action of EDCs is not limited to those agents that interact directly with hormone receptors. Other mechanisms of interest include inhibition of hormone synthesis, transport, or metabolism and activation of receptor through processors such as receptor phosphorylation or the release of cellular complexes necessary for hormone action.”

Australian and US EPA policy relating to Endocrine Disruptor Effects

The Australian Government first produced a paper on EDCs in April 1998 in response to public concerns. This document was redrafted in 2002; it acknowledges that Australian policy on EDCs remains under ongoing review and lends support to the IPCS EDC framework and the development and/or extension of appropriate OECD Test Guidelines. Australian agencies consider that endocrine disruption is but one part of a spectrum of effects that chemicals can cause if animals and humans are exposed to levels that overwhelm normal inactivation processes such as metabolism and excretion. That is, endocrine disruption is not considered to be an adverse end-point per se, but rather is a mode or mechanism of action potentially leading to other toxicological or eco-toxicological outcomes eg. reproductive, developmental, carcinogenic or ecological effects; these effects are routinely considered in reaching regulatory decisions (at least for pesticides, food additive chemicals and high production volume industrial chemicals for which the required toxicology database is extensive). This position is quite similar to the US EPA position.

The US EPA view of endocrine disruption has resulted from changes in its underlying legislation. Under the FFDCAs as amended by FQPA, the EPA is required to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) “may have an effect in humans that is similar to an effect produced by a naturally occurring oestrogen, or other such endocrine effects as the Administrator may designate.” The EDSTAC made recommendations that the EPA should broaden its definition of endocrine disruption to include the androgen and thyroid hormone systems, in addition to the oestrogen hormone system. The US EPA adopted these recommendations as well the recommendation to include evaluations of potential effects in wildlife.

The Australian vs USA position on endosulfan as an endocrine disruptor

The ECRP review of endosulfan states that “Several recent studies have reported that endosulfan, alone or in combination with other pesticides, may have oestrogenic binding capability, and possibly potential for perturbation of the endocrine system. To date, the available studies show only very weak binding to hormone receptors *in vitro*, and the evidence for any relevance to adverse physiological effects *in vivo* is extremely limited.” And further, that “Long term bioassays, and reproductive and developmental toxicology studies in experimental animals, do not indicate that endosulfan induces any functional aberrations which might result from disruption of endocrine homeostasis.”

The RED states that “Exposure to endosulfan has resulted in both reproductive and developmental effects in non-target animals. Endosulfan exposure resulted in impaired development in amphibians, reduced cortisol secretion in fish, impaired development of the genital tract in birds and reduced hormone levels and sperm production and produced testicular atrophy in mammals. Additionally, endosulfan has been demonstrated to bind to the human oestrogen receptor and exhibit significant estrogenic activity. Whether the toxicity endpoints are a result of endocrine disruption is not known. However, it is clear that organisms treated with endosulfan did exhibit some toxic effects that have historically been associated with endocrine disrupting chemicals, e.g., developmental and reproductive.”

Both the ECRP report and the RED suggest that more information is needed.

The ECRP review: “Once such studies are available, it would be useful for the endocrine disruption potential of endosulfan to be tested under validated conditions, as the current evidence is not sufficient to make a regulatory decision on the endocrine disruption potential of endosulfan.”

The US EPA RED: “When the appropriate screening and/or testing protocols have been developed, endosulfan may be subjected to additional screening and/or testing to better characterise effects related to endocrine disruption.”

Hence the main difference between the Australian (as stated in the ECRP review) and US EPA positions on endosulfan as an endocrine disruptor is primarily a definitional one. The toxicology chapter in the ECRP report suggests that endosulfan does not appear to be an endocrine disruptor in mammals whereas the RED proposes that the weight of evidence from all studies supports the designation of endosulfan as a **potential** endocrine disruptor.

10.3.1 The toxicological database for endosulfan

A variety of chronic/carcinogenicity, reproductive and developmental studies on endosulfan, either published or submitted by the sponsors, have been evaluated for regulatory purposes. These studies are suitable for evaluating the endocrine disrupting ability of endosulfan because they encompass a broad dose range often including the MTD, they assess a range of endpoints including indicators of endocrine disruption and they generally demonstrate a NOEL for most treatment effects. Several generalities are evident from the individual studies evaluated below. The chronic studies in mice, rats and dogs indicate that oral doses of endosulfan above ca. 1 mg/kg/d lead to hepatotoxicity and renal toxicity as the most common findings.

A variety of special toxicology studies including many designed to assess endocrine related effects have also been conducted and evaluations of these are also presented below.

Chronic toxicity studies

Male and female B6C3FI mice were dosed with endosulfan at <1 mg/kg/d in the diet for 78 weeks (intakes were 3.5 - 6.9 ppm for the males, and 2 - 3.9 ppm for the females). While body weights and clinical scores in both males and females were unaffected by treatment there was an increase in the mortality rate of high dose males early in treatment. Pathological examination found no treatment related changes in the kidneys or sex organs of males or females (Powers et al, 1978).

Male and female NMRI mice were dosed with endosulfan in the diet for up to 24 months. The intake of endosulfan for males was calculated to be 0.28, 0.84, and 2.51 mg/kg/day, and in females were 0.32, 0.97, and 2.86 mg/kg/day, at dietary concentrations of 2, 6, and 18 ppm, respectively. At the high dose there were reductions in body weight in males and a statistically significant increase in mortality in females. No statistically significant changes were observed in haematology or clinical chemistry parameters and macroscopic examination did not reveal any findings that were related to treatment. At terminal sacrifice, no statistically significant changes in organ weights were seen in treated animals and histopathological examination did not reveal any effects that were related to the administration of endosulfan (Donaubauer, 1988, 1989).

Male and female Osborne-Mendel rats were dosed with endosulfan in the diet, with time-weighted average doses of 0, 223, and 445 ppm (0, 10, 20 mg/kg/d) for females, and 0, 408 and 952 ppm (0, 20, 40 mg/kg/d) for males for 78 weeks, with a return to control diets for a further 4 weeks. A dose related reduction in body weights was found at all doses in male rats as well as a highly significant morbidity rate such that by week 54, 52% of the high dose males had died. Histopathological examination revealed a high incidence of toxic nephropathy (>90%) in treated but not control males and females. Renal calcium deposits were also observed in treated males. The toxic nephropathy observed in animals was characterised as degenerative changes in the proximal convoluted tubules at the junction of the cortex and medulla, and associated cloudy swelling, fatty degeneration, and necrosis of the tubular epithelium. Parathyroid hyperplasia occurred in treated males, as did medial calcification of the aorta and medial calcification of the mesenteric artery, and calcium deposits in the stomach. A dose related increase in testicular atrophy occurred in treated

male rats, characterised by degeneration and necrosis of the germinal cells lining the seminiferous tubules, multinucleated cells (fusion bodies), and calcium deposition resulting in aspermatogenesis. No treatment related effects were noted on the reproductive organs in female rats (Powers et al, 1978).

Renal toxicity was seen in Sprague-Dawley rats dosed with endosulfan in the diet at up to 75 ppm (2.9-3.8 mg/kg/day) for two years. Reductions in body weights and body weight gains were observed in males and females at 75 ppm, but there were no clinical signs and no increase in mortality at this dose. Gross pathological examination revealed an increase in incidence of enlarged kidneys (females), blood vessel aneurysms and enlarged lumbar lymph nodes (males) at 75 ppm, while histopathological examination revealed an increased incidence of blood vessel aneurysms and marked progressive glomerulonephrosis (PGN) in males at 75 ppm (Ruckman et al, 1989).

Renal toxicity was also evident in Wistar rats treated with endosulfan in their diets at dose levels of 0, 10, 30 or 100 ppm (equivalent to 0, 0.5, 1.5, and 5 mg/kg/d/) for 2 years. There were no treatment related clinical signs, and body weights were unaffected. Histopathologic changes observed at a high incidence in kidneys of the high dose males at 104 weeks consisted of enlarged kidneys, mild to severe renal tubule dilatation, mild to moderate formation of irregular albuminous casts, pronounced focal nephritis, and mild to severe degeneration of the renal tubule epithelium. At 104 weeks, female rats at the high dose showed some minimal degeneration of renal tubules and some focal nephritis, but no extensive pathological renal tubule changes. The NOEL was 30 ppm (1.5 mg/kg/day), based on kidney effects at 100 ppm (5 mg/kg/day) (Hazelton Laboratories, 1959a).

Summary table of chronic/carcinogenicity, reproduction and developmental studies considered

Study type	Species	Duration	Clinical signs of Toxicity	LOEL mg/kg/d	Primary toxicity	Author
Chronic	Mouse – B6C3F1	78 weeks	NO*	<1.0	Nil	Powers et al, 1978
Chronic	Mouse - NMRI	104 weeks	Body weight ↓ Mortality ↑	2.86	Systemic	Donaubauer, 1988, 1989
Chronic	Rat – Osborne-M	78 weeks	Body weight ↓ Mortality ↑ Nephropathy Pituitary hyperplasia Testicular atrophy	10.0 F* 20.0 M*	Systemic Renal	Powers et al, 1978
Chronic	Rat – SD	104 weeks	Body weight ↓ Renal toxicity	2.9 F 3.8 M	Systemic Renal	Ruckman et al., 1989
Chronic	Rat – Wistar	104 weeks	Renal toxicity	5	Renal	Hazelton Laboratories, 1959a
Chronic	Dog – Beagle	52 weeks	Body weight ↓ Mortality ↑	2.3	Systemic	Brunk 1989, 1990
Chronic	Dog – mongrel	52 weeks	NA	0.75 2.5	Nil Systemic	Hazelton Laboratories, 1959b
Reproduction	Rats – SD	36 weeks	Renal Liver	5.72 M 6.92 F	Systemic Renal	Edwards et al., 1984; Offer, 1985
Developmental	Rats – albino	9 d	nil	10 F	Nil	Gupta et al., 1978
Developmental	Rats – Wistar	10 d	Body weight ↓ Mortality ↑	6 F	Maternotoxicity	Albrecht & Baeder, 1993
Developmental	Rat – SD	14 d	Body weight ↓	6 F	Maternotoxicity	MacKenzie, 1980
Developmental	Rabbit – NZW	23 d	Convulsions	1.8 F	Maternotoxicity	MacKenzie, 1981
Developmental	Rat – Druckrey	9 d	Spermatid count ↓ Sperm count ↓	1.0	foetotoxicity	Sinha et al, 2001
Developmental	Rat – Wistar	28 d	Sperm count ↓	3.0	Maternotoxicity	Dalsenter et al, 1999
Developmental	Rat – Wistar	63 d	NO	>1.5	Nil	Dalsenter et al, 2003

*NO - None observed; M – male; F - female

Technical endosulfan was administered in the diet to groups of Beagle dogs at dietary concentrations of 0, 3, 10, or 30 ppm (equivalent to 0, 0.23, 0.77, and 2.3 mg/kg/d) for one year. Another group dosed with endosulfan in increasing dietary concentrations of 30/45/60 ppm were killed in extremis due to poor condition before the study's scheduled completion, and displayed a number of signs of intoxication, including tonic contraction, and increased sensitivity to noise and optical stimuli. Treatment at the high dose induced lower body weights and body weight gains and abdominal cramping in some animals. No other effects related to treatment were observed (Brunk 1989, 1990).

In another dog study endosulfan was administered orally, via gelatine capsules, to adult mongrel dogs at dose levels of 0, 3, 10 and 30 ppm (equivalent to 0, 0.075, 0.25 and 0.75 mg/kg/day) on 6 days/week for one year. Attempts to dose at 2.5 mg/kg/d were abandoned due to frank toxicity. No clinical signs or treatment related effects on body weight gains were seen. Clinical chemistry and haematology were within normal limits and kidney function was unaffected by treatment. No gross or histopathologic changes associated with treatment were noted (Hazelton Laboratories, 1959b).

Reproductive Toxicity

Technical endosulfan was administered in the diet to Sprague Dawley rats at concentrations of 0, 3, 15, and 75 ppm (equivalent to 0.2-0.23, 1.0-1.18, and 4.99-5.72 mg/kg/day for males, and 0.24-0.26, 1.23-1.32, and 6.18-6.92 mg/kg/day for females) for two mating generations, with two mating phases in each. No clinical signs or mortality related to endosulfan administration were observed during the study. Mating performance and pregnancy rates were not affected by treatment during the study. There was no effect on the mean pup weights, litter sizes or on sex ratios at any dose tested. Statistically significant increases in relative kidney weights were seen at the high dose some males, and statistically significant increases in relative liver weights were observed in some males and females at the high dose. The NOEL for reproductive effects was 75 ppm (approximately 6 mg/kg/day), with no effects on reproductive parameters or treatment related abnormalities being seen at any dose level tested in this study (Edwards et al., 1984; Offer, 1985).

Developmental Toxicity

Female albino rats were orally dosed with endosulfan from days 6-14 of gestation at doses of 0, 5, and 10 mg/kg body weight/day. There were no clinical signs or bodyweight differences between control and treated animals. No abortions were observed in any group, but there was a significant increase in the percent of litters with resorptions (5.5% in controls, compared with 20% at 5 mg/kg/d, and 22.8% at 10 mg/kg/d). A variety of minor skeletal variations were increased in treated groups but these effects were not considered to be related to treatment, as the magnitude of the changes was small, and the effects were not dependent upon the endosulfan dose. No maternotoxicity was evident at any dose level. The level of reporting in this published paper is not adequate for the purposes of defining a NOEL for developmental toxicity (Gupta et al, 1978).

Female Wistar rats were orally dosed with endosulfan from days 7-16 of gestation, at of 0, 0.66, 2, and 6 mg/kg body weight/day. No clinical signs of toxicity were reported in females at 0.66 or 2 mg/kg/day but four dams died with typical convulsive symptoms at 6 mg/kg/day. Body weight and bodyweight gain were reduced at 6 mg/kg/day. No statistically significant changes in reproductive or pup parameters were observed at any dose level in this study, and the foetal sex ratio was relatively balanced. No statistically significant increase in the incidence of abnormalities was observed in foetuses during examination. Skeletal examination revealed a statistically significant increase in fragmented thoracic vertebral centra at 6 mg/kg, an effect considered to reflect the frank maternotoxicity of endosulfan seen at the high dose level (Albrecht & Baeder, 1993).

Female CD Sprague Dawley rats were dosed with endosulfan by gavage, on gestation days 6-19 at dose levels of 0, 0.66, 2 and 6 mg/kg/day. Maternotoxicity was evident in dams treated with 6 mg/kg/day with a dose-related decrease in maternal body weight gain seen at 2 and 6 mg/kg/day. The number of implantations, sex ratio and litter size were unaffected by endosulfan treatment. There was a slight reduction in foetal weight and length in the high dose group. No external variations, effects on soft tissue development or malformations were attributable to treatment, with the exception of the litter of one high dose dam. Evidence of delayed development and isolated low incidence of skeletal variations were seen in this litter at the maternotoxic dose of 6.0 mg/kg/day (MacKenzie, 1980).

New Zealand White rabbits were dosed with endosulfan by gavage on gestation days 6 to 28 at dose levels of 0, 0.3, 0.7 or 1.8 mg/kg/day. There were no changes in mean body weights with endosulfan treatment, no does aborted and no signs of toxicity or mortality were seen at the lower doses of 0.3 and 0.7 mg/kg/day. The high dose was associated with signs of maternotoxicity including noisy and rapid breathing, hyperactivity and convulsions. The number of implantations, litter size, sex ratio, mean foetal weight and length and the number of live and resorbed foetuses were unaffected by endosulfan treatment. Common skeletal variations and minor anomalies occurred with a similar incidence in control and treated foetuses. Endosulfan did not produce any teratogenic or developmental effects even at the maternotoxic dose of 1.8 mg/kg/day (MacKenzie, 1981).

In another study, pregnant Druckrey rats were orally dosed with endosulfan at 0, 1 or 2 mg/kg bw/d from day 12 of gestation through parturition. Male neonates were fostered to untreated dams. At 100 days of age, the male offspring were sacrificed. Statistically significant, dose related increases in testicular lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH) were observed. Treatment also induced a decrease in spermatid count in testis and sperm count in cauda epididymis, and a significant decrease in testis, epididymis and seminal vesicle weights (Sinha et al, 2001).

In a developmental study female Wistar rats were treated orally with 0, 1.5 or 3.0 mg endosulfan/kg from day 15 of pregnancy to postnatal day (PND) 21 of lactation. The male offspring rats were investigated at PND 65 or 140, corresponding to the pubertal and adulthood stage of development. Maternal body weight was decreased at 3.0 mg/kg/d but litter size and mean birth weight were not affected. Treatment had no effect on the weight of reproductive and accessory sex organ nor on the age of testis descent and preputial separation in male offspring. However, there was decreased daily sperm production at puberty at 1.5 and 3.0 mg/kg/d, and at 3.0 mg/kg/d in adults (Dalsenter et al, 1999).

Female Wistar rats were dosed with endosulfan orally at 0, 0.5 or 1.5 mg/kg bw/d for 21 d prior to mating, during the mating, pregnancy and lactation. Maternal and reproductive outcome data and male sexual development landmarks (testis descent and preputial separation) were assessed. Reproductive endpoints of the male offspring examined at adulthood included: sex organ weights, daily sperm production, spermatid number, sperm transit, sperm morphology and testosterone level. No signs of maternal toxicity were detected at the dose levels tested. Sexual development landmarks were also unaffected. There were no statistically significant adverse effects of treatment on the reproductive endpoints investigated at adulthood except for a significant increase in the relative epididymis weight, not dose-related as it was seen only in the 0.5 mg/kg group (Dalsenter et al, 2003).

Testicular toxicity

The effect of sub-chronic endosulfan treatments on plasma and testicular testosterone, and two of the four main enzymes of the testes involved in biosynthesis of testosterone from pregnenolone (3- β hydroxysteroid dehydrogenase (3- β HSD), and 17- β hydroxysteroid dehydrogenase (17- β HSD) was studied in Wistar rats. Testicular microsomes were assayed for cytosolic glutathione (GSH)-S-transferase to evaluate cellular toxicity of endosulfan treatment. Groups of male rats received endosulfan by gavage at 0, 2.5, 5.0, 7.5 and 10 mg/kg body weight for 7 and 15 days. Organ and body weights of the treated animals did not change significantly. Testicular protein content and serum testosterone increased significantly after 7 d (LOEL at 7.5 mg/kg/d) while testicular testosterone decreased, which suggests sex-hormone binding globulin (SHBG) may be affected. Results after the 15d exposure were highly variable and frequently not dose-related, making interpretation of the results difficult (Singh and Pandey, 1989).

In a later study by the same authors, the effect of sub-chronic endosulfan treatments on plasma and testicular testosterone, plasma gonadotrophins (follicle stimulating hormone (FSH), and luteinising hormone (LH)), and two of the four main enzymes of the testes involved in biosynthesis of testosterone from pregnenolone (3- β hydroxysteroid dehydrogenase (3- β HSD), and 17- β hydroxysteroid dehydrogenase (17- β HSD) was studied in Wistar rats. Testicular microsomes were assayed for several mixed-function oxidases involved in testicular steroidogenesis and cytosolic glutathione (GSH)-S-transferase in testes of treated animals was assayed to evaluate cellular toxicity of endosulfan treatment. Groups of male rats received endosulfan by gavage at 0, 7.5, and 10 mg/kg bw for 15 d, 30 d, or 30 d with 7d recovery before sacrifice.

Treatment with endosulfan did not affect body weight or testicular weights. The levels of plasma gonadotrophins (FSH and LH) along with plasma testosterone and testicular testosterone were significantly reduced at both doses at 30 days. 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. Plasma testosterone and testicular testosterone levels at the lower dose of 7.5 mg/kg were not significantly reduced after 15 days of treatment. Activities of the steroidogenic enzymes (3 beta- and 17 beta-hydroxysteroid dehydrogenases) were significantly lowered after 30 days of treatment. A significant decrease in the contents/activities of microsomal cytochrome P-450 and related mixed -unction oxidases in the testes of treated animals was observed, along with a marked inhibition in the activity of glutathione-S-transferase at both dose levels. All of the effects from 30 days of exposure were reversible during a 7-day recovery period, except for decreased testicular testosterone, which remained depressed. (Singh & Pandey 1990).

Technical grade endosulfan was administered via oral gavage to groups of male Druckrey rats at doses of 0, 2.5, 5, and 10 mg/kg/day, on 5 days/week for 70 days. No changes in body weights or testis weight were seen in treated animals compared with controls. Statistically significant, dose related increases in testicular lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), gamma glutamyl transpeptidase (GGT), and glucose-6-phosphate dehydrogenase (G6PDH) activity were seen at all endosulfan dose levels. Statistically significant decreases in cauda epididymis sperm counts were seen at all test doses, with reductions of 22%, 43%, and 47%, at 2.5, 5, and 10 mg/kg/day, respectively. In the absence of historical control data, it is unclear whether the decrease in sperm count at 2.5 mg/kg/day (22%) was

within the expected biological range for the test animals. Statistically significant reductions in spermatid count (about 16%) and sperm production rate (about 22%) were also reported at 5 and 10 mg/kg/day but the biological significance of these changes is unclear as there was no dose relationship. Thus, the administration of endosulfan at doses of 2.5 mg/kg/day and above for several months resulted in testicular toxicity as evidenced by increased testicular enzyme activity and marked reduction in sperm counts at 5 mg/kg/day and above (Sinha et al, 1995).

The genotoxicity potential of endosulfan in mouse germ cells was assessed *in vivo* in two tests: the dominant lethal and the sperm shape abnormality test. The intraperitoneal administration of endosulfan to Swiss mice at doses of 16.6 mg/kg/day for five days resulted in an increase in the incidence in sperm abnormalities, along with decreased sperm counts and decreased testis weights. The reporting in this paper is inadequate to determine when the sperm were obtained, and it appears that the males used for sperm morphology assessment were different to those used in the dominant lethal assay, given that different dose levels, group sizes, and positive control concentrations were used. The dominant lethal assay showed an increase in dominant lethal mutations, reductions in the number of live implants/pregnant females, total implants/pregnant females, and corpora lutea/pregnant females at a dose of 16.6 mg/kg/day but only in a single mating interval (36-42 days). No effects were seen on any of these parameters at any other mating intervals at 16.6 mg/kg/day, and no effects were seen at doses of 9.8 or 12.7 mg/kg/day. It appears likely that the increase in sperm abnormalities is causally related to the possibly artifactual adverse effects on fertility and other reproductive parameters seen at the single mating interval in this study, but the reporting in this report is not adequate to definitely discount the possibility. It is unlikely that a single isolated increase in dominant lethal mutations at the high dose is related to endosulfan administration. No adverse effects were seen in animals dosed with endosulfan at doses of 12.7 mg/kg/day or lower (Pandey et al, 1990).

Endosulfan (35% emulsifiable concentrate) was administered to groups of six male Swiss albino mice by oral gavage at 0 and 3 mg/kg/day (estimated to be the maximum tolerated dose) for 35 d. Treatment induced an increase in abnormal sperm from 5 to 14%. No historical control incidences for abnormal sperm from this testing laboratory were provided, and there is no indication of whether this incidence of 14% was biologically significant, and/or within normal biological variation for this strain of test animal. Significant reductions in sperm count (80%) were seen following the administration of endosulfan. The test material was a 35% emulsifiable concentrate and it is unclear whether these findings are related to endosulfan or the unknown non active constituents (Khan & Sinha, 1996).

A poorly reported study in rats found testicular toxicity possibly secondary to pituitary toxicity after endosulfan treatment at 10 mg/kg/d and above for 30 d (Choudhary & Joshi, 2003).

The effect of sub-chronic oral exposure to a mixture of contaminants including endosulfan was investigated in male SD rats. The dosing mixture contained organochlorines (2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD], polychlorinated biphenyls [PCBs], p,p'-dichlorodiphenoxydichloroethylene [p,p'-DDE], p,p'-dichlorodiphenoxytrichloroethane [p,p'-DDT], dieldrin, endosulfan, methoxychlor, hexachlorobenzene, and other chlorinated benzenes, hexachlorocyclohexane, mirex and heptachlor) as well as metals (lead and cadmium). Each chemical was included in the mixture at the tolerable daily intake or for TCDD, at the NOEL used to calculate the TDI (USA). Adult male rats were exposed to the mixture at 0, 1, 10, 100, and 1000 times the estimated safe levels daily for 70 days.

Signs of hepatotoxicity were dose related (liver enlargement, reduced serum LDH activity, increased serum cholesterol and protein levels) and elevated hepatic ethoxyresorufin-O-deethylase (EROD) activities indicated enzyme induction. Immunotoxicity was evident particularly at the high dose (decreased proliferation of splenic T cells, decreased natural killer cell lytic activity). Genotoxicity was not evident as no treatment-related effects were seen on bone marrow micronuclei. Reproductive and endocrine effects were not evident as there were no treatment-related effects on daily sperm production, serum LH, FSH, or prolactin levels or weights of most organs of the reproductive tract. The weights of the whole epididymis and of the caput epididymis were significantly decreased at 10x and higher doses, although no effect was seen on cauda epididymal weight. The sperm content of the cauda epididymis was increased at the 1x level but not significantly different from control at higher dose levels. A slight, but significant, increase in the relative numbers of spermatids was seen in the animals from the 1000x group with a trend towards reduced proportion of diploid cells at the same dose. The authors concluded that the mixture induced effects on the liver and kidney and on general metabolism at high doses. Additive or synergistic effects of exposure to these contaminants at non-toxic concentrations did not result in adverse effects on immune function or reproductive physiology in male rats. (Wade et al, 2002b)

The effects of 4-tert-octylphenol (OCP), endosulfan, bisphenol A (BPA), and 17 beta-estradiol on basal or hCG-stimulated testosterone formation was investigated in cultured Leydig cells from young adult male rats. Exposure of Leydig cells to increasing concentrations of OCP (1 to 2000 nM), 17 beta-estradiol (1 to 1000 nM), endosulfan (1 to 1000 nM) or BPA (1 to 1000 nM), alone or with 10 mIU/mL hCG, did not lower ambient testosterone levels or effect conversion of 22(R)hydroxycholesterol to testosterone (Muroño et al, 2001).

Thyroid toxicity

The effect of sub-chronic oral exposure to a mixture of contaminants including endosulfan was investigated in male SD rats. The dosing mixture contained organochlorines (2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD], polychlorinated biphenyls [PCBs], p,p'-dichlorodiphenoxydichloroethylene [p,p'-DDE], p,p'-dichlorodiphenoxytrichloroethane [p,p'-DDT], dieldrin, endosulfan, methoxychlor, hexachlorobenzene, and other chlorinated benzenes, hexachlorocyclohexane, mirex and heptachlor) as well as metals (lead and cadmium). Each chemical was included in the mixture at the tolerable daily intake or for TCDD, at the NOEL used to calculate the TDI (USA). Adult male rats were exposed to the mixture by gavage at 0, 1, 10, 100, and 1000 times the estimated safe levels daily for 70 days. Endpoints related to circulating thyroid hormone (serum thyroxine [T(4)], triiodothyronine [T(3)], thyroid stimulating hormone [TSH], and serum T(3) uptake [T(3)-up]), thyroid gland histomorphology (thyroid follicle cross sectional area, epithelial height, follicle roundness or aspect ratio, colloid/epithelial ratio) and hepatic metabolism of thyroid hormone (UDP-glucuronyl transferase [UGT] and outer-ring deiodinase [ORD]) were assessed.

There were treatment-related effects for most test parameters but the magnitude varied considerably between endpoints. While most endpoints did not show significant changes at mixture doses below 1000x, 2 endpoints, TSH and hepatic outer ring deiodinase activity, were significantly increased and decreased, respectively, by 1x dose and showed dose-related increases in severity with increasing dose. These two endpoints are directly responsive to thyroid hormone stimulation. Median thyroid follicle cross sectional area was also increased by the lowest dose of the mixture but decreased with subsequent increases in dose until, at the highest dose, this parameter was significantly reduced relative to control. The relative sensitivity of endpoints of thyroid function in detecting toxicity of the mixture was TSH = ORD = median follicle area >> T(3) > all other endpoints (Wade et al, 2002a).

Effects of endosulfan on thyroid physiology have been studied in the female freshwater catfish *Clarias batrachus* during the pre-spawning and spawning phases of its annual reproductive cycle. Effects of endosulfan varied with the length (96 h and 16 days) of exposure, and reproductive status of the fish and organ. The 96-h endosulfan exposure significantly increased the level of thyroxine (T4) in serum and pharyngeal thyroid follicles concurrent with induction of peroxidase activity. However, the triiodothyronine (T3) level and the T3/T4 ratio decreased in serum and pharyngeal thyroid gland. No change was noticed in any of these parameters in the anterior kidney but in the posterior kidney endosulfan reduced T3 and T3/T4 ratio without affecting T4 levels and peroxidase activity. Sixteen days of endosulfan treatment also had a similar impact, except that it did not influence the studied parameters in pharyngeal thyroid (abstract of Sinha et al, 1991).

Adrenal toxicity

An *in vitro* bioassay for detection and quantitative assessment of chemicals with the capacity to disrupt adrenal steroidogenesis was used to compare the cytotoxic and endocrine-disrupting potential of four pesticides. Enzymatically dispersed adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) were exposed *in vitro* to atrazine, diazinon, endosulfan, and mancozeb; cell viability and cortisol secretion in response to ACTH or dibutyryl-cAMP (dbcAMP) were then determined. The effective concentration, EC50 (concentration that inhibits cortisol secretion by 50%), the median lethal concentration, LC50 (concentration that kills 50% of the cells), and the LC50/EC50 ratio were established for the test pesticides. The pesticides were ranked as follows: EC50, endosulfan < diazinon < mancozeb < atrazine; LC50, diazinon < endosulfan < mancozeb < atrazine, with diazinon as the most cytotoxic. The authors state that endosulfan and mancozeb disrupted sites downstream of the cAMP-generating step of the cortisol synthetic pathway while atrazine seemed to act upstream from the cAMP step (Bisson & Hontela, 2002).

Pituitary toxicity

An *in vitro* study using a pituitary cell line (GH(3)) that responds to estrogens by increasing its secretion of prolactin (PRL) was conducted to assess the estrogenic activity endosulfan and chlordane. Prolactin is a hormone with diverse physiological functions, especially in foetal growth, development, and reproduction. The effect of treatment on the levels of PRL secretion and PRL mRNA transcription were measured using immunometric tests, Northern blots, and relative quantitative RT-PCR. The proliferation of GH(3) cells stimulated with 17-beta estradiol and endosulfan or chlordane was also quantified. Treatment with endosulfan and chlordane induced a significant increase of PRL expression but had no effect on cell growth. The results are interpretable as evidence for modulation of the inducible-inducible PRL by endosulfan and chlordane, possibly acting via second messenger-mediated cellular mechanisms instead of solely competing with estrogens for the nuclear oestrogen receptor sites (Rousseau et al, 2002).

Oestrogenic effects

A study primarily designed to examine the interaction between endosulfan and dieldrin in the activation of ER in or extracted from mammalian cells showed that endosulfan induced cell proliferation in the MCF-7 human breast cancer cell line between 2 and 4 times control levels at exposure levels of 10 and 50 µM, but had no proliferative effect at 2 µM. Endosulfan and dieldrin showed no synergism in displacing ³H-E2 from rat uterine ER or in inducing the

proliferation of MCF-7 breast cancer cells. Additionally endosulfan (0.1 mg per animal per d) or dieldrin (0.1 mg), alone or in combination, injected intraperitoneally daily for 3 d, did not stimulate any uterotrophic activity nor did it have any effect on pituitary prolactin or other endocrine-related endpoints in immature female rats (Wade et al, 1997).

Another study using the MCF7 cell line (human breast cancer, oestrogen-sensitive) assessed the oestrogenic effects of o,p-DDT, chlordecone, endosulfan, DDT, dieldrin and toxaphene. The concentration range for the weak oestrogenic activity seen for the pesticides was from 10-25 μ M, and at higher concentrations cytotoxicity was observed. There was no evidence of synergy when a mixture of the chemicals was administered to MCF7 cells at concentrations lower than that required to produce an oestrogenic effect when administered alone (Soto et al, 1994).

In another *in vitro* assay, both α - and β -endosulfan were weakly estrogenic in inducing foci in MCF-7 cultures at 10 μ M (but not at lower concentrations), and showed no estrogenic synergism when incubated in combination with dieldrin (Arcaro et al. 1998).

In addition to inducing cell proliferation, endosulfan induced proliferation of the progesterone receptor, another mimicking-mimicking effect (Soto et al, 1995).

In apparent contradiction of these positive findings, endosulfan (isomeric composition not reported) did not substantially affect the growth of either ER-positive (MCF-7) or ER-negative (SK-BR-3) cultured human breast cancer cell lines at concentrations of 35 μ M. Endosulfan did severely inhibit cell growth at higher concentrations, and this growth inhibition was synergistic when cultures were incubated with either dieldrin or chlordane (Hsu et al. 1998).

In a recent study which quantified the oestrogen receptor (ER) relative binding affinities of 188 compounds, endosulfan was found to have no detectable binding affinity for ER (Blair et al, 2000).

Another paper investigated the transcriptional activation of human oestrogen receptor (hER) in yeast in response to environmental chemicals (endosulfan, dieldrin, toxaphene, chlordane) alone and in combination. Three types of assay methods were used to test the chemicals: (1) a yeast oestrogen system (YES), genetically engineered to contain human oestrogen receptors; (2) competitive displacement of the binding of tritiated 17- β oestradiol to a recombinant human oestrogen receptor preparation *in vitro*; and (3) an endometrial cancer cell line transiently transfected with human oestrogen receptors and a coupled luciferase reporter system. Combinations of two compounds were reported to be 1000 times as potent in hER-mediated transactivation as any chemical alone (Arnold et al, 1996).

NB: This paper was subsequently withdrawn by the authors when the results appeared difficult to replicate in a number of laboratories, including the authors' own.

Other investigators reassessed the potential synergistic interactions of dieldrin and toxaphene using ten different oestrogen-responsive assays, and found that the combined activities of these compounds was essentially additive. In addition, the investigators reinvestigated all of the binary mixtures of organochlorine pesticides reported by Arnold et al (1996). in two yeast based assays, and found that the estrogenic activities of all of the binary mixtures of organochlorine pesticides were additive, not synergistic (Ramamoorthy et al, 1997).

Continuous exposure of adult male sheepshead minnow (*Cyprinodon variegates*) to p-nonylphenol, MXC, or endosulfan for up to 42 days was observed to induce a dose-dependent increase in hepatic vitellogenin mRNA and plasma protein within 5 days of exposure to all but endosulfan (Hemmer et al., 2001).

Neurobehavioural effects

Three rat studies conducted by the one laboratory were complicated by poor reporting and frank toxicity at the doses used. There was no unequivocal evidence of neurobehavioural effects in these studies (Paul et al, 1993, 1994 and 1995).

Immunotoxicity

In a study designed to investigate immune competence, male Wistar rats were treated with endosulfan in the diet for six weeks and immunised with tetanus toxoid after 25 days of pesticide exposure. There were no clinical signs or effects on body, spleen and thymus weights. A significant increase in liver weight was observed in rats exposed to 2.5 mg/kg/d endosulfan. Measures of immune response (serum antibody titre to tetanus toxoid, serum IgM and IgG levels) showed a significant dose-related decrease at 1.5 and especially 2.5 mg/kg/d (Banarjee & Hussain, 1987).

Another study by the same authors also investigated immune competence in male Wistar rats treated with endosulfan in the diet for 22 weeks with interim sacrifices. At 19 weeks of exposure the rats were immunised with tetanus toxoid. There were no clinical signs or effects on bodyweights but there was a decrease in thymus weight at the high dose of

1.0 mg/kg/d. Measures of immune response showed a significant time and dose-related decrease at 0.5 and 1.0 mg/kg/d (Banarjee & Hussain, 1986).

10.4 DISCUSSION

Chronic, developmental and reproductive toxicity

As stated above, the chronic studies in mice, rats and dogs indicate that oral doses of endosulfan above ca. 1 mg/kg/d lead to systemic toxicity with hepatotoxicity and renal toxicity the most common findings. It is not surprising then that signs of maternotoxicity were seen in the developmental studies where the doses were ca. 2-10 mg/kg/d. The detailed pathology examinations conducted during the chronic studies show no consistent evidence of endocrine related toxicity. The gross pathology and histopathology of sexual organs, reproductive organs, indicators of secondary sexual characteristics (eg muscle mass) do not generally indicate primary endocrine disturbance. The developmental studies show no unequivocal disturbances of sex ratios, sexual differentiation, gonad development (vaginal opening & testes descent), preputial separation, gross pathology or histopathology of reproductive tissues at low doses.

One criticism of the developmental studies is that the mandated observations do not address subtle endocrine-related changes that might only be evident in maturity. It is biologically plausible that the earliest life stages are the most sensitive to endocrine disruption, whether because the foetus is uniquely sensitivity or merely quantitatively more sensitive. The developmental effects of endocrine disruptors tend to be latent and traditional endpoints of toxicity (ie altered structure or function) may not be detectable until sexual maturity, which is 8-10 weeks after birth for common laboratory rodent species.

In one developmental study in Wistar rats (Dalsenter et al, 1999) where dams were dosed from day 15 of pregnancy to postnatal day (PND) 21 of lactation, the high dose of 3.0 mg endosulfan/kg induced maternotoxicity (decrease in body weight) and in male offspring, abnormal development of seminiferous tubules leading to a permanent decrease in sperm production. Litter size, mean birth weight, age at testis descent and preputial separation were not affected indicating that sperm production is the most sensitive endpoint. Another developmental study by the same laboratory found that oral doses of endosulfan at 0, 0.5 or 1.5 mg/kg bw/d administered to Wistar rats pre-mating and throughout mating, pregnancy and lactation, did not induce maternotoxicity and had no effect on sex organ weights, daily sperm production, spermatid number, sperm transit, sperm morphology and testosterone level in male offspring (Dalsenter et al, 2003). Another study dosed pregnant Druckrey rats with endosulfan at 0, 1 or 2 mg/kg bw/d from day 12 of gestation through parturition and reported dose related increases in testicular LDH and SDH as well as reduced spermatid and sperm counts and decreases in testis, epididymis and seminal vesicle weights (Sinha et al, 2001). These contrasting results indicate that there may be differences in susceptibility of the male reproductive system to endosulfan depending on the rat species and treatment period used.

The single reproduction study available provides an example of extended prenatal exposure to endosulfan, followed by assessment of sexual maturation and performance (including behaviour) through two generations at doses up to and including parental toxicity. The study provides no unequivocal evidence that endosulfan can induce endocrine disruption *in vivo*.

Testicular toxicity

Testicular toxicity is clearly demonstrated in a number of relatively high-dose studies in mice and rats but this disturbance of the HPG axis is regarded as being secondary to systemic toxicity. Testes have a relatively low ability to metabolise xenobiotics and are relatively lipid rich; these properties might be expected to render testes particularly sensitive to a lipophilic compound like endosulfan.

Thyroid toxicity

Endosulfan induced thyroid toxicity in a study in catfish but the relevance to humans is unclear. In a rat study where a mixture of contaminants including endosulfan was co-administered, thyroid toxicity was evident at doses causing systemic toxicity.

Adrenal toxicity

In an *in vitro* study using trout cells endosulfan was both cytotoxic and inhibited cortisol secretion.

Pituitary toxicity

Endosulfan was reported to modulate oestrogen-inducible gene expression in an *in vitro* study using pituitary cells.

Oestrogenicity

A number of *in vitro* and ex vivo studies report that endosulfan induces proliferation in human breast cancer cells and can displace oestrogen from the oestrogen receptor. Other studies found no uterotrophic activity, no proliferative effect and insignificant binding to the ER compared to oestrogen.

Immune toxicity

A study in rats using a complex mixture of contaminants including endosulfan showed dose-related decreases in immune response at doses equivalent to 1000–times the TDI. Another study in catfish found adverse effects of endosulfan on thyroid function that varied with length of exposure and reproductive status.

Synergy

A number of studies investigated the interaction of endosulfan with co-administration of one or more compounds. There was no unequivocal evidence of synergistic interactions, the most common interaction being less than additive. The one study demonstrating synergy (Arnold et al, 1996) was later withdrawn.

As shown, a number of studies investigated the effects of endosulfan in non-mammalian species. The relevance to humans of observations of endocrine disruption in non-mammalian species is not clear. Given the conserved nature of steroid hormone systems in mammals and perhaps vertebrates generally, it is reasonable to extrapolate effects across species and a variety of qualitative studies for a number of estrogenic chemicals support this approach. However, molecular evidence (differences in primary amino acid sequences) suggests that between species there will be quantitative differences in ligand-receptor binding interactions as well as species-specific ligands. This problem is likely to be magnified as observations cross animal kingdoms and hence the relevance of results obtained in amphibians, fish and avians is uncertain (Harris et al, 2002; Matthews et al, 2002).

Similar difficulties arise when extrapolating data obtained from the reported *in vitro* assays to effects observed *in vivo*, and for the extrapolation of evidence of endocrine activity in what are simple screening assays to the ability to induce adverse effects in more traditional testing protocols.

Exposure

Endosulfan is of particular interest to public health considerations because of its potential for long-range transport. Endosulfan is a semi-volatile cyclodiene pesticide that can migrate over a long distance through various environmental media such as air, water, and sediment. Once endosulfan is applied to crops, it can either persist in soil as a sorbed phase or be removed through several physical, chemical, and biological processes. Recent studies in the Northern Hemisphere suggest that secondary emissions of residual endosulfan continue to recycle in the global system while they slowly migrated and were redeposited via wet deposition. The occurrences of endosulfan in remote regions like the Great Lakes, the Arctic, and mountainous areas are well documented. Endosulfan can also enter the air in the adsorbed phase on suspended particulate matter, but this process does not appear to be a major contributor to long range transport like volatilisation. A validated global model has not been published because of uncertainties involved in the source inventories, chemical fate data, degradative pathways and exposure analyses.

Bioaccumulation

Australian Pesticides and Veterinary Medicines Authority (APVMA)

Endosulfan is a polychlorinated “cyclodiene-type” pesticide structurally related to chlordane, heptachlor, aldrin, endrin and dieldrin, chemicals that are no longer registered for use as pesticides in many countries. However, endosulfan is of higher water solubility and is significantly less persistent than each of the other polychlorinated cyclodiene insecticides; the physical data supporting this contention is shown in the table below.

Physical properties of selected cyclodiene insecticides (USDA-ARS database)

Compound	Solubility (ppm)	Log K _{OW}	Koc	Field dissipation half-life (d) and range
Endosulfan	0.33	4.77	11,000	60 (12-176)
Chlordane	0.056	6.0	60,000	365 (283-3500)
Dieldrin	0.14	4.55	12,000	1000 (225-1260)
Aldrin	0.027	5.52	17,500	365 (10-1237)
Heptachlor	0.056	4.4 – 5.5	24,500	250 (40-1277)

While the partition coefficient (log K_{OW}) may suggest similar bioaccumulation potential, endosulfan differs in its bioaccumulation behaviour in that it is rapidly excreted in the wide range of species studied.

10.5 CONCLUSION

The recent Australian APVMA and US EPA reviews of endosulfan evaluated comparable databases and adopted similar regulatory approaches on most issues. The specific issue of whether endosulfan should be categorised as an endocrine disruptor remains as one significant difference between the two agencies mainly arising from the US EPA inclusion of data from all endocrine systems as well as potential effects in wildlife. Both agencies state that further testing of endosulfan using validated assays would be valuable and might help to further characterise effects related to endocrine disruption.

The APVMA evaluation reported the endocrine-related effects seen in test animals, particularly testicular toxicity, but noted that these appear to arise from homeostatic disturbance resulting from systemic toxicity. The APVMA report concludes that endosulfan binding to the oestrogen receptor is insignificant and considers that the regulatory endpoint chosen (see section 10.2.1 for the NOEL table) is adequately sensitive and protective against potential endocrine disruption by endosulfan.

The US EPA evaluation noted the effects seen in test animals and argued additionally that effects seen in amphibians, fish, birds and hormone receptor studies are indicative of potential endocrine disruption.

This current report has evaluated recently published studies and considered the conclusions of the two agency reports. From the public health point of view there are no compelling reasons to change the conclusions of the APVMA ECRP review with respect to the endocrine disrupting potential of endosulfan. While the effects seen in wildlife indicate that endosulfan may have endocrine disrupting potential in some species, the overall weight of evidence is that endosulfan has limited endocrine disrupting potential in mammals. Furthermore, while endosulfan may be relatively persistent in the environment and is capable of long-range transfer, it does not appear to bioaccumulate. The endocrine disrupting potential of endosulfan is not a significant risk to public health under the risk management controls and health standards established by the recent review.

10.6 TOXICOLOGY REFERENCES

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