Australian Government

Australian Pesticides and Veterinary Medicines Authority

ENDOSULFAN

REVIEW OF NEW INFORMATION SINCE THE 1998 AND 2005 REVIEWS

Volume 2 –Toxicology and occupational health and safety assessments

Prepared for the APVMA by The Office of Chemical Safety and Environmental Health (OCSEH), Office of Health Protection, Department of Health and Ageing, Canberra

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TOXICOLOGY ASSESSMENT

FOREWARD

The APVMA has requested that the OCSEH addresses three issues:

(1) examine the United States Environmental Protection Agency (US EPA) Re-registration Eligibility Decision (RED) on endosulfan and attendant information regarding endosulfan, and identify and clarify variations from the OCSEH's earlier conclusions on toxicology matters published in the initial "Evaluation of the Mammalian Toxicology and Metabolism/Toxicokinetics" Review Report on endosulfan (1998/99);

(2) re-examine the issue of possible endocrine disruption caused by endosulfan; and

(3) examine new neurotoxicity data that has become available since the completion of the initial review of endosulfan.

Part I of this report considers the US EPA RED for endosulfan which was finalised in November 2002, and compares it to the Australian Existing Chemical Review Program (ECRP) review of endosulfan which 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 which 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 endocrine disrupting chemical (EDC).

Part III of this report examines a new developmental neurotoxicity study (September 2006). This study, as well as other studies from the published literature that had not previously been assessed by the OCSEH, were evaluated to address concerns regarding adult and foetal neuropathological and developmental endocrine effects.

In conducting this review the conclusions of the ECRP report with respect to the chronic, developmental and reproductive studies have been reconsidered along with the relevant findings of the final US EPA RED report. Additionally, all of the published literature relevant to the endocrine disrupting potential of endosulfan to the present date has been evaluated. A recent air monitoring study (PANNA 2007, 2008) was also evaluated.

GLOSSARY OF TERMS AND ABBREVIATIONS

a.i.	Active Ingredient
AAAA	Australian Aerial Agricultural Association
ACAHS	Australian Centre for Agricultural Health & Safety
ADI	Acceptable Daily Intake
aPAD	Acute Population Adjusted Dose
APVMA	Australian Pesticides & Veterinary Medicines Authority
ARfD	Acute Reference Dose
ATV	All Terrain Vehicles
BCF	Bioconcentration Factor
bw	body weight
Cal DPR	California Department of Pesticide Regulation
CAS	Chemical Abstracts Service
CNS	Central Nervous System
CP	Pressure control nozzles
cPAD	Chronic Population Adjusted Dose
C-PAS	Centre for Pesticide Application Safety
CRDC	Cotton Research & Development Corporation
CRP	(Existing) Chemical Review Program
d	day
DFR	Dislodgeable Foliar Residues
EC	Emulsifiable concentrate
ECRP	Existing Chemical Review Program
ER	Oestrogen Receptor
ERMA	Environmental Risk Management Authority New Zealand
FFDCA	Food Quality Protection Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FOB	Functional Observation Battery
g	gram
h	hour
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadal
HPT	Hypothalamic-pituitary-thyroid
HRDC	Horticulture Research & Development Corporation
IPM	Integrated Pest Management
JMPR	Joint Meeting on Pesticide Residues
kg	kilogram
L	litre
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
M/L/A/C	Mixing/loading/application/cleaning
mg	milligram
Mg/kg bw/day	milligrams/kilogram body weight/day

mL	millilitre
MOE	Margin of Exposure
NOAEL	No Observed Adverse Effect Level
NOEC	No Observable Effect Concentration
NOEL	No Observable Effect Level
NOHSC	National Occupational Health & Safety Commission
OCSEH	Office of Chemical Safety and Environmental Health
OHS	Occupational Health and Safety
OP	Organophosphorus compound
OPP	EPA Office of Pesticide Programs
OPPTS	EPA Office of Prevention, Pesticides and Toxic Substances
PAD	Population Adjusted Dose
PADI	Provisional Acceptable Daily Intake
PHED	Pesticide Handler Exposure Database
PMRA	Pest Management Regulatory Agency
ppb	Parts Per Billion
PPE	Personal Protective Equipment
ppm	Parts Per Million
PVC	Polyvinyl chloride
RBC	Red blood cell
RED	Reregistration Eligibility Decision
REI	Restricted Entry Interval
RfD	Reference Dose
SHBG	Sex hormone–binding globulin
SUSDP	Standards for the Uniform Scheduling of Drugs and Poisons
TC	Transfer Coefficient
ULV	Ultra Low Volume
US EPA	United States Environmental Protection Agency

EXECUTIVE SUMMARY

Endosulfan is a broad spectrum insecticide/acaricide which is registered in Australia for the control of a large variety of insects and mites in ranges of horticultural and agricultural crops. Among the pest/crop combinations for which this insecticide is registered are aphids, thrips, beetles, foliar feeding larvae, mites, cutworms, Helicoverpa spp, bugs, whiteflies and leafhoppers on citrus, pome and small fruits, fibre and forage crops, grains, nuts, oilseeds, pulses, ornamentals, tobacco and vegetables. It is not used in animal production. This use profile is similar to that elsewhere such as the USA and southern European countries. Current labels include instructions for application by ground and by air, with endosulfan being applied aerially in significant quantities since the major crop is cotton. Ground applications are either by boomspray, airblast, airshear or knapsack with hand wand/nozzle. Technical grade endosulfan is composed of two stereochemical isomers: α-endosulfan and β-endosulfan, in concentrations of approximately 70% and 30%, respectively.

Like other organochlorine pesticides, the toxicity of endosulfan to both insects and humans arises from over-stimulation of the nervous system. Specifically, endosulfan acts as a non-competitive gamma-aminobutyric acid (GABA) receptor antagonist and interferes with the transmission of nerve impulses. Binding of GABA to it receptor induces the uptake of chloride ions by neurones, resulting in hyperpolarisation of the membrane. The blockage of this activity results in only partial repolarisation of the neuron and a state of uncontrolled excitation.

The current Australian acceptable daily intake (ADI) for endosulfan is 0.006 mg/kg/day based on a NOEL of 0.6 mg/kg bw/day. This NOEL was based on a number of effects including decreased body weights and kidney pathology and was common to a range of studies, including a 13-week dietary study in rats, a 28-week dietary study in mice, a 1-year dietary study in dogs, a 2-year dietary study in rats, and a developmental study in rats.

The acute reference dose (ARfD) for endosulfan of 0.02 mg/kg bw was established in 2000 and is derived from a NOEL of 2 mg/kg bw from a developmental study in rats. This NOEL is based on developmental effects, reduced food consumption and clinical signs (tonoclonic convulsions and hypersalivation.

The OCSEH has conducted an assessment of existing commercial data holdings and currently available published information on endosulfan to address human health concerns and ensure that the continued use of endosulfan would not present an unacceptable human health risk to those using the chemical in an occupational environment or to members of the general public who may be exposed to the chemical.

This current report has evaluated recently published studies and considered the conclusions of the US EPA and ERMA New Zealand. From the public health point of view, there are no compelling reasons to change the conclusions of the 1998 and 2005 APVMA reviews 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. 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.

PART I: US EPA AND APVMA REPORTS

1 BACKGROUND

1.1 Current public health standards in Australia

Endosulfan is listed in Schedule 7 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) and Schedule 6 for aqueous preparations containing 33% or less of microencapsulated endosulfan. The acceptable daily intake (ADI) for endosulfan established in 1997 is 0.006 mg/kg bw/day based on a NOEL of 0.6 mg/kg bw/day and a safety factor of 100. This NOEL was common to a range of studies, including a 28-week dietary study in mice, a 13-week dietary study in rats, a 1-year dietary study in dogs, a 2-year dietary study in rats, and a developmental study in rats. The NOEL was based on a number of effects including decreased body weights and kidney pathology. The acute reference dose (ARfD) for endosulfan established in 2000, is 0.02 mg/kg bw derived from a NOEL of 2 mg/kg bw and a safety factor of 100. The NOEL is based on developmental effects, reduced food consumption and clinical signs (tonoclonic convulsions and hypersalivation) seen in a developmental study in rats.

1.2 Regulatory history of endosulfan in Australia

Several major assessments of the toxicology of endosulfan have been conducted in Australia.

In 1968, the ADI for endosulfan was set at 0.007 mg/kg bw/day, it was included in Schedule 6 of the SUSDP. In 1985, the clearance of endosulfan Technical Grade Active Constituent (TGAC) was reviewed. All available toxicology data were evaluated and the NOEL and ADI were confirmed. In 1987 and 1988, additional toxicology data supplied by the sponsors were 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 APVMA Existing Chemical Review Program (ECRP).

In 1995 endosulfan was nominated onto the APVMA ECRP Priority Review Candidate List. The APVMA then began a review of endosulfan due to concerns about possible risks to the public from short and long-term exposure to endosulfan residues, occupational health and safety, trade and the environment. In 1998, the OCSEH completed a focussed review entitled "*Review of the Mammalian Toxicology and Metabolism - Toxicokinetics of Endosulfan*". This review was published on the APVMA website as part of its Interim Review Report. The OCSEH evaluated new data submissions on the toxicology of endosulfan following a data call in process, along with all previously submitted data. This report also included a chemistry, agricultural, environmental and OHS assessment of Endosulfan. This report recommended a number of changes to the use of endosulfan to reduce the risks to worker safety, the environment and to reduce residues in commodities including a recommendation to change the current ADI of 0.007 mg/kg bw/day based on a NOEL of 0.7-0.75 mg/kg bw/day to 0.006 mg/kg bw/day, based on the lowest NOEL of 0.6 mg/kg bw/day from short and long term studies in mice rats and dogs.

In May 2004, the APVMA released its Preliminary Review Findings which imposed mandatory buffer zones for spraying and required neighbourhood notification before endosulfan application. The APVMA cancelled the registration of ultra-low volume endosulfan products to help reduce long-distance drift of very fine spray mists.

In 2005, the APVMA released the final review report on endosulfan with the following recommendations in order to mitigate workers safety and residue concerns:

- Cancellation of uses on leafy vegetables, berry fruits (including grapes), bananas, sorghum, maize, peanuts, legume vegetables, bulb vegetables, sweet corn or cole vegetables (except cabbage (head), broccoli and cauliflower).
- Endosulfan cannot be used post-emergence on cereals, pulses and oil seeds (except cotton).
- Endosulfan cannot be used on any pasture, forage or fodder.
- Limiting the number of endosulfan applications each growing year.
- New maximal residue limits and withholding periods.
- No re-entry into treated areas until the spray has dried.
- Amended safety instructions on product labels.
- All users of endosulfan products must keep records of endosulfan use for up to 2 years.
- Before using endosulfan on cotton, users must: notify neighbours of spraying, observe downwind no-spray zones, only apply crop using techniques as specified on the new labels, and only use in the period of time specified on the new labels.
- Cattle producers who use endosulfan or are neighbours of endosulfan users must pay particular attention to Question 8 on the National Vendor Declaration (NVD) and Question 7 on the European Union Vendor Declaration (EUVD).

1.3 Australian review of endosulfan

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 foetuses, 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 short-term administration, and progressive chronic glomerulonephrosis or toxic nephropathy after long-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 (ARfD) 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 with a LOEL of 6.0 mg/kg bw/day. This value is supported by an acute neurotoxicity study with a NOEL of 1.5 mg/kg bw/day, based on clinical signs and increased mortality at the next highest dose of 3 mg/kg bw/day.

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 in the table below.

No-observed-effect-level (NOEL) seen in a range of endosulfan studies
• 0.58 mg/kg bw/day in female mice in a 78-week dietary study, the highest dose tested; • 0.64 mg/kg bw/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 bw/day; • 0.57 mg/kg bw/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 bw/day; • 0.66 mg/kg bw/day in female rats in a developmental study, based on decreased body weights at 2 mg/kg bw/day. • 0.6 mg/kg bw/day in a 2-year rat dietary study, based upon reduced body weights and kidney pathology at 2.9 mg/kg bw/day.

Several other studies of interest include an oral study in rats with a NOEL of 0.3 mg/kg bw/day (LOEL 3 mg/kg bw/day) based on decreased body wight gain, testis weight, sperm count, sperm motility, and sperm abnormalities (Rao et al 2005), and a series of neurobehavioural studies in rats (Paul et al 1993, 1994, 1995) with a NOEL of 0.2 mg/kg bw/day (LOEL 2 mg/kg bw/day) based on reduced body weight, reduced food consumption, increased mortality, increased tremor intensity and increased liver enzyme activity. However, these studies were not considered adequate for regulatory purposes as they were published studies and therefore the OCSEH was not able to assess the original data. Also, the most recent study (developmental neurotoxicity) conducted in accordance with standard toxicological guidelines did not confirm any of the concerns raised in the above journal articles.

OH&S Issues

On the basis of NOHSC advice the APVMA review concluded that there were some concerns 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 APVMA mandated that endosulfan be classified as a Restricted

Chemical, with supply and use restricted to Farmcare accredited personnel (or equivalent) and/or licensed operators; worker/operator training was to be upgraded for those using this chemical. Labels were to be modified to require record keeping, new re-entry periods, restriction of aerial application and the use of closed cabs for ground application equipment and flaggers. Data were to be generated to enable assessment of worker exposure for a variety of Australian agricultural practices including greenhouses and establishment of safe re-entry periods for crops.

Following evaluation of requisite worker exposure studies that had been identified in the 1998 review, the 2005 occupational health and safety evaluation concluded "that the APVMA could be satisfied that the continued use of products containing endosulfan 350 g/L in EC formulation in all situations as currently permitted (except for turf and hides) would not be an undue hazard to the safety of workers exposed to it during its handling. The evaluation has determined that instructions on product labels be varied by deleting the use on turf and hides. The occupational health and safety evaluation also recommended that labels be varied to include new safety direction, re-entry periods and PPE requirements. The occupational health and safety evaluation concludes that provided the labels are varied as proposed then the APVMA could be satisfied that continued use and other dealings of products containing endosulfan would not be an undue hazard to the safety of people exposed to it during handling."

2 ASSESSMENT OF ENDOSULFAN BY OTHER REGULATORY AGENCIES AND THE JMPR

2.1 US EPA Re-registration Eligibility Decision (RED)

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 product spectrum 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.

In 2007, the US EPA released its updated risk assessment of the potential human health effects of endosulfan based on the review of two submitted studies, a chronic neurotoxicity study and a developmental neurotoxicity study. These studies were identified as data gaps in the 2002 Endosulfan RED report. Based on the review of these studies the US EPA revised the endpoint used to evaluate short- and intermediate-term dermal exposure for occupational handlers.

In April 2009, a petition requesting the cancellation of all uses of endosulfan was open for public comment until June 29, 2009. Also, public comment is requested on the results of the recent impact assessment on endosulfan for eight crops (apple, cotton, cucumber, melon, potato, pumpkin, squash and tomato).

Summary conclusions of endosulfan re-registration 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 bw/day in a combined chronic toxicity/carcinogenicity study in rats.

The US EPA has recently revised the use of an endosulfan Food Quality Protection Act (FQPA) Safety Factor of 10 for the protection of children (US EPA 2007a). The safety factor of 10 was applied following the conclusions of the RED report. The RED report concluded that the weight-of-the-evidence 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. 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/day. The US EPA also recently evaluated a subchronic neurotoxicity study (Sheets et al 2004, cited in Cal DPR 2008) and a developmental neurotoxicity study (Gilmore et al 2006), concluding that there was no evidence that endosulfan induced developmental neurotoxicity in rats. In addition, there were no adverse effects in sperm parameters, testes weights or histopathology of the testes, ovary weights or other reproductive organs (Sheets et al 2004; Gilmore et al 2006). It was also concluded that increases in pituitary and uterine weights seen in a 2-generation reproductive study in rats were not of concern as these effects only occurred at the highest dose tested, 6.2 mg/kg bw/day (US EPA 2007a). Based on these results, the US EPA removed the FQPA safety factor of 10.

Using the new FQPA safety factor of 1, the US EPA re-assessed acute and chronic dietary risk from endosulfan and concluded that residues of endosulfan in drinking water and food were not of concern for either the acute or the chronic PAD. It was also noted that to date, none of the cancellations or other mitigations methods proposed in the 2002 RED report have been imposed (US EPA 2007b).

Occupational health and safety issues

The EPA review of 2002 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.

Based on the review of the chronic neurotoxicity study and the developmental neurotoxicity study, in 2007 the US EPA revised the endpoint used to evaluate short- and intermediate-term dermal exposure for occupational handlers. Previously, the endpoint used to evaluate short- and intermediate dermal exposure was based on two 21-day dermal studies in rats with a NOAEL of 10 mg/kg bw/day. Following the evaluation of the developmental neurotoxicity study, a LOAEL of 3.7 mg/kg/day was determined based on decreased pup body weights. The use of this endpoint for regulatory purposes was considered appropriate as it takes into account the most sensitive population, female workers.

The subsequent revised occupational assessment for endosulfan indicates that short- and intermediate-term risks for workers during mixing and loading and application for the majority of uses is concerning, even with the use of personal protective equipment (PPE). It was also determined that the current re-entry intervals for most activities would need to be extended.

2.2 Environmental Risk Management Authority (ERMA), New Zealand

The ERMA New Zealand completed a reassessment report on endosulfan in December 2008 using a risk/benefit analysis to determine whether use of endosulfan posed unacceptable risks for workers, the public and the environment. As a result of this

reassessment report, the ERMA New Zealand revoked approvals for endosulfan and prohibited it importation, manufacture and use in New Zealand. This decision was based on substantial risk to the environment (aquatic species, earthworms, bees and other non-target terrestrial invertebrates, and birds) and to human health, specifically to operators and bystanders during specific use applications (citrus applications).

2.3 Pest Management Regulatory Agency (PRMA), Canada

Endosulfan is currently being reviewed in Canada by the PMRA. The PMRA has proposed to implement measures in advance of completing a full review as a precautionary approach to mitigate potential dietary and occupational risks.

2.4 European Chemicals Agency (ECHA), European Union

Endosulfan was not included in Annex 1 of Council Directive 91/414/EEC (EU 2005) at the European Commission meeting on 2 December 2005 and the authorisations for plant protection products containing the endosulfan were withdrawn. This decision was based on the environmental fate and behaviour of endosulfan as well as unacceptable risks to workers in indoor conditions. Greece, Spain, Italy and Poland were granted authorisation for continued use of endosulfan on selected crops until 30 June 2007. Annex 1 is a 'positive' list of active substances that are authorised for use in plant protection products within the community.

2.5 The Rotterdam and Stockholm Convention's

In March 2007, the Chemical Review Committee of the Rotterdam Convention on the Prior Informed Consent Procedure agreed to forward to the Conference of the Parties of the Convention the recommendation for inclusion of endosulfan in Annex III. Annex III is the list of chemicals that have been banned or severely restricted for health or environmental reasons by Parties and the exporting of which requires prior informed consent from the proposed recipient country.

In July 2007, the council of the European Union made the decision to propose endosulfan for listing in the Stockholm Convention on Persistent Organic Pollutants for global elimination.

2.6 The Joint FAO/WHO Meeting on Pesticide Residues (JMPR), United Nations

JMPR has evaluated the toxicology of endosulfan on several occasions with the most recent review completed in 1998. JMPR set an ADI of 0.006 mg/kg bw/day based on a two-year dietary study in rats (NOEL of 0.6 mg/kg bw). This study was also the basis of the Australian ADI, as detailed in section 1.3.1. An ARfD of 0.02 mg/kg bw was established based on a NOEL of 2 mg/kg bw/day in a rat neurotoxicity study.

Part II: IS ENDOSULFAN AN ENDOCRINE DISRUPTOR?

1 BACKGROUND

In the APVMA interim report on the review of endosulfan (1998), a comprehensive toxicity data package was evaluated and it was concluded that there was no evidence that endosulfan cause's disruption to the endocrine hormonal system.

In the recent review of endosulfan completed by the APVMA in 2005, the APVMA re-examined the endocrine disrupting potential of endosulfan and it was concluded that despite effects seen in wildlife that indicate that endosulfan may have endocrine disrupting potential in some species, the overall weight-of-evidence indicates that endosulfan has limited endocrine disrupting potential in mammals. The 2005 review also concluded that the endocrine disrupting potential of endosulfan is not a significant risk to public health under the current health management controls and health standards. It was also noted in this 2005 review that further testing of endosulfan using validated assays would be valuable and might help to further characterise effects related to endocrine disruption.

The US EPA RED (2002) identified endosulfan as "a potential endocrine disruptor" based on the weight-of-evidence from all studies in both non-target animals (amphibians, fish and birds) and mammals. The US EPA agrees, however, with the other regulatory agencies, including the APVMA, that more information is needed before any conclusions can be made.

1.1 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 it progeny or (sub)populations" (OECD 1998).

The working definition used in the final report of the US EPA's 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 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 which 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 Federal Food, Drug, and Cosmetic Act (FFDCA) 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.

1.2 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 oestrogenic 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 report 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.

1.3 The position of other regulatory agencies on endosulfan as an endocrine disruptor

California Department of Pesticide Regulation (Cal DPR) (2008) risk assessment concluded that endosulfan has not been proven to be an endocrine disruptor in humans and like the APVMA also conclude that the current health management controls (NOELs) set for neurotoxicological effects are protective for all other adverse health effects in all human subpopulations.

In their recent review of endosulfan, ERMA New Zealand also concluded that the weight-of-evidence suggested that endosulfan did not act as a strong endocrine disruptor.

2 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.

Summary table of unpublished studies considered.

Study type	Species	Duration	Clinical signs of Toxicity	NOEL mg/kg bw/d	LOEL mg/kg bw/d	Primary toxicity	Author
Acute neurotoxicity	Rat - Wistar	Acute	Mortality ↑ Clinical signs	12.5 (male) 1.5 (female)	25 (male) 3 (female)	Systemic	Bury 1997
Subchronic neurotoxicity	Rat- Wistar	13 weeks	Body weight ↓	37.2 (male) 16.6 (females)	13.7 (males) 2.88 (females)	Systemic	Sheets et al 2004
Chronic	Mouse – B6C3F1	78 weeks	Nil	0.58	<1.0	Nil	Powers et al 1978
Chronic	Mouse - NMRI	104 weeks	Body weight ↓ Mortality ↑	0.84 (male) 0.97 (female)	2.86	Systemic	Donaubauer 1988, 1989
Chronic	Rat – Osborne-M	78 weeks	Body weight ↓ Mortality ↑ Nephropathy Pituitary hyperplasia Testicular atrophy	-	10.0 (female) 20.0 (male)	Systemic Renal	Powers et al 1978
Chronic	Rat – SD	104 weeks	Body weight ↓ Renal toxicity	0.6 (female) 0.7 (male)	2.9 (female) 3.8 (male)	Systemic Renal	Ruckman et al 1989
Chronic	Rat – Wistar	104 weeks	Renal toxicity	1.5	5	Renal	Hazelton Laboratories 1959a
Chronic	Dog – Beagle	52 weeks	Body weight ↓ Mortality ↑	0.65 (male) 0.57 (female)	2.3	Systemic	Brunk 1989, 1990
Chronic	Dog – mongrel	52 weeks	Nil	-	0.75	Nil	Hazelton Laboratories,1959b
Reproduction	Rat – SD	36 weeks	Renal Liver	Parental: 1.1 Offspring: 1.0	Parental: 6.0 Offspring: 6.0	Systemic Renal	Edwards et al 1984; Offer 1985
Developmental	Rat – Wistar	10 d	Body weight ↓ Mortality ↑	Maternal: 2 Foetal: 2	Maternal: 6 Foetal: 6	Maternotoxicity	Albrecht & Baeder 1993
Developmental	Rat – SD	14 d	Body weight ↓	Maternal: 0.66 Foetal: 2	Maternal: 2 Foetal: 6	Maternotoxicity	MacKenzie 1980
Developmental	Rabbit – NZW	23 d	Maternal: Convulsions Foetal: Delayed development	Maternal: 1.8 Foetal: 2	Maternal: 1.8 Foetal: 6.0	Maternotoxicity	MacKenzie 1981
Developmental	Rabbit - NZW	22 d	Maternal: Convulsions, mortality ↑ Foetal: none	Maternal: 0.7 Foetal: 1.8	Maternal: 1.8 Foetal: -	Maternotoxicity	Nye 1981
Developmental neurotoxicity	Rat - Wistar		Maternal: Body weight gain↓ Food consumption ↓ Foetal: Body weight gain↓	Maternal: ND Foetal: 3.75	Maternal: 3.75 Foetal : 10.8	Maternotoxicity	Gilmore et al (2006)

ND = not determined

2.1 Chronic toxicity studies

Male and female B6C3FI mice were dosed with endosulfan at <1 mg/kg bw/day 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 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 bw/day) for females, and 0, 408 and 952 ppm (0, 20, 40 mg/kg bw/day) 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).

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 bw/day, and in females was 0.32, 0.97, and 2.86 mg/kg bw/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).

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 bw/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 bw/day) 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 bw/day), based on kidney effects at 100 ppm (5 mg/kg bw/day) (Hazelton Laboratories 1959a).

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 bw/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 gelatin 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 bw/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).

2.2 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 bw/day for males, and 0.24-0.26, 1.23-1.32, and 6.18-6.92 mg/kg bw/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).

2.3 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 bw/day, and 22.8% at 10 mg/kg bw/day). 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 bw/day. No clinical signs of toxicity were reported in females at 0.66 or 2 mg/kg bw/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 bw/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 bw/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 bw/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 bw/day (MacKenzie 1980).

In another study, pregnant Druckrey rats (3/dose) were orally dosed with endosulfan at 0, 1 or 2 mg/kg bw/day 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) and sorbitol dehydrogenase (SDH) were observed. Treatment at both doses also induced a decrease in spermatid count in testis and sperm count in cauda epididymis, along with a significant decrease in testis, epididymis and seminal vesicle weights (Sinha et al 2001). However, there are several study limitations including the very small group sizes (3/dose) used, the use of an uncommon laboratory rat strain (Druckrey), and a lack of information on clinical observations in pregnant females. Consequently, the significance that can be attached to the findings from this non-standard and poorly reported study is limited.

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 deceased at 3.0 mg/kg bw/day but litter size and mean birth weight were not affected. Treatment had no effect

on the weight of reproductive and accessory sex organs 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 bw/day, and at 3.0 mg/kg bw/day in adults (Dalsenter et al 1999).

Female Wistar rats were dosed with endosulfan orally at 0, 0.5 or 1.5 mg/kg bw/day 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).

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 bw/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 bw/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 bw/day (MacKenzie 1981).

In a developmental toxicity study, mated New Zealand White rabbits (20/dose) were given endosulfan at doses of 0, 0.3, 0.7 or 1.8 mg/kg bw/day by gavage during days 6-28 of gestation. At 1.8 mg/kg bw/day an additional 6 dams were added (total = 26 dams) due to an unexplained high mortality. The maternal NOEL was 0.7 mg/kg bw/day based on increased mortality (4/20 dams died; one a day on day 7, 10, 21 and 29) and on clinical signs of toxicity observed during treatment: convulsions/thrashing (3/26), noisy/rapid breathing (2/26), hyperactivity (1/26), salivation (1/26), and nasal discharge (3/26) at 1.8 mg/kg/day. Clinical signs of toxicity were observed from day 6 at 1.8 mg/kg bw/day (thrashing, phonation, coughing, cyanotic), from day 14 at 0.7 mg/kg bw/day (nasal congestion: 2/20) and from day 18 in control animals (congestion / nasal congestion, 2/20). No signs of developmental toxicity were observed at the top dose of 1.8 mg/kg bw/day, a dose that produced severe maternal toxicity (Nye 1981, cited in Cal DPR 2008).

2.4 Testicular toxicity

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 bw/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 (G6PD) 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 bw/day, respectively. In the absence of historical control data, it is unclear if the decrease in sperm count at 2.5 mg/kg bw/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 (Sinha et al 1995). There are several study limitations including the use of an uncommon laboratory rat strain (Druckrey) and the absence of historical control data. Consequently, the significance that can be attached to the findings from this non-standard and poorly reported study is limited.

In a later study by the same author (Sinha et al 1997), weanling male Druckrey rats (prepubertal sexual maturity at 3 weeks old, 5/dose) were gavaged with endosulfan at doses of 0, 2.5, 5.0 or 10 mg/kg bw/day for 90 days (5 days/week) to determine the effect of endosulfan on testicular maturation. Results showed statistically significant decreased sperm count (cauda epididymis), increased sperm abnormalities, decreased spermatid counts and decreased daily sperm production, as well as increased lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PD), gamma glutamyl transpeptidase (GGT), and decreased sorbitol dehydrogenase (SDH) at doses of 2.5 mg/kg bw/day and above. These effects were similar to those observed by the same authors in an earlier study in mature rats at 2.5 mg/kg bw/day and above (Sinha et al 1995) at doses of 2.5 mg/kg bw/day and above. However, the effects observed in weanling rats were dose-related, whereas in mature rats they were not. Again, numerous study deficiencies were identified including, the small number of animals treated, the use of an uncharacterized rat strain, and no adult male comparison group included. There were also no clinical signs of neurotoxicity reported in this study. Consequently, the significance that can be attached to the findings from this non-standard and poorly reported study is limited.

Endosulfan was administered orally male Wistar rats (10/group) from postnatal day 7-60 at doses of 3, 6, 9 and 12 mg/kg bw/day. Sub-sets of rats were treated with L-ascorbic acid (20 mg/kg bw/day) alone or in combination with either 9 or 12 mg/kg bw/day endosulfan. In endosulfan treated rats, there was a statistically significant decrease in body weight gain, testis weight, sperm count, sperm motility and sperm abnormalities at doses of 3 mg/kg bw/day and above. Rats treated with endosulfan in combination with L-ascorbic acid reduced the effects of endosulfan on sperm count, sperm motility and sperm abnormalities. Furthermore, there was a statistically significant increase in body weight and testis weight in rats treated with the combination of L-ascorbic acid and endosulfan compared to rats treated with endosulfan alone, however the effects of treatement with endosulfan alone were not completely reversed by L-ascorbic acid (Rao et al 2005).

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 bw/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 bw/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 bw/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 bw/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).

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 ethoxyresorfurin-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 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 bw/day 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, 7.5 mg/kg bw/day) 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 & Pandey 1989).

In a later study by the same authors (Singh & Pandey 1990), 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-βHSD), and 17-β hydroxysteroid dehydrogenase (17-βHSD) 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).

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 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 (Murono et al 2001).

Male Wistar prepubertal rats (45 days old) were treated by gavage with endosulfan (35%; possibly a formulated product) at 0 and 1 mg/kg bw/day (6/dose) for 30 days. At study termination, statistically significant decreases were seen in body, testes, epididymal, ventral prostate and seminal vesicle weights at 1 mg/kg bw/day compared to controls. Biochemical parameters showed statistically significant increases in protein and decreases in DNA, RNA, ascorbic acid, lactate, pyruvate, 3-βOH-steroid dehydrogenase, acid phosphatase (AP) and alkaline phosphatase (ALP) at 1 mg/kg bw/day. The changes in DNA, RNA and protein suggest a shift in synthetic activity in the testis. The decrease in pyruvate (necessary for Sertoli cell function) along with lactate indicates a possible decrease in testicular metabolism, and decreased 3-βOH-steroid dehydrogenase indicates a decreased steroidogenesis. Ascorbic acid, AP and ALP decreases in the spermatogenic chamber, Leydig cells and semen acts to inhibit oxidative damage to sperm and this correlated with a decrease in testicular steroidogenesis. Clinical sings of toxicity were not reported. Major deficiencies in this study include, the composition of the dosing material was not described, a small number of animals per group and only one dose used. Therefore this study was not considered adequate for regulatory purposes (Chitra et al 1999).

Endosulfan (0.1 mM) has been shown to inhibit the mammalian sperm acrosome reaction (AR) *in vitro*, which is essential to fertilization. In human sperm, AR is imitated *in vitro* by progesterone or glycine, resulting in the activation of sperm $GABA_A$ or glycine receptor/chloride channels, respectively (Turner et al 1997).

2.5 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 ^{3}H-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 µM, and at higher concentrations cytoxicity 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 µM (but not at lower concentrations), and showed no oestrogenic 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 oestrogen-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 µM. 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 activity 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 (*Cyprinodin 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).

The oestrogenicity of endosulfan (98% pure) was determined using a combination of *in vitro* and *in vivo* assays. In the competitive binding assay, female CD-1 mice were ovariectomized at 10-12 weeks of age and sacrificed 2 weeks later. The uteri was removed and processed and used in for the cytosol receptor binding assay in which the ability of endosulfan to bind with the oestrogen receptor was assessed. In a second *in vitro* assay, transcription activation of endosulfan in HeLa cells transfected with plasmids containing an oestrogen receptor as a responsive element was assessed. Finally, the uterotrophic assay was used to assess the effects of endosulfan on an oestrogen-responsive tissue *in vivo*. In this assay, 17 day old female mice (5/dose) were subcutaneously injected for 3 consecutive days with varying doses of endosulfan for four days before being sacrificed. Body weights and uterine weights were determined. Endosulfan showed no evidence of oestrogenicity in any of the assays (Shelby et al 1996).

Endosulfan (98% pure) was administered to hemicastrated (right ovary removed) virgin Swiss albino mice by gavage at doses of 0, 1.5, 3, 6 and 9 mg/kg bw/day for 15 consecutive days. As expected, control hemicastrated mice showed 40.5% increase in the weight of the remaining left ovary. In endosulfan treated mice, there was a statistically significant decrease in absolute and relative ovary weights at 3 mg/kg bw/day and greater compared to the hemicastrated controls. In endosulfan treated animals there was also a decrease in the duration of the oestrus cycle at 6 mg/kg bw/day and above. Endosulfan treatment had no effect on body weight or the weights of the uterus, kidney, adrenal, liver, thymus or thyroid (Hiremath & Kaliwal 2002).

In another study by the same authors (Hiremath & Kaliwal 2003), endosulfan (98%) pure was administered orally to ovariectomized virgin Swiss albino mice (10/group) at a dose of 4 mg/kg bw/day for 30 days. The antiestrogenic activity of endosulfan was also assessed in ovariectomized mice by treating mice with a combination of endosulfan, 4 mg/kg bw/day, and estradiol-17β, 5 µg/g bw/day. Endosulfan treated mice showed no change in uterine weight compared to controls. In contrast, mice treated with both endosulfan and estradiol-17β or with estradiol-17β alone showed a statistically significant increase in uterine weight compared to controls. Mice treated with endosulfan and estradiol-17β, alone and in combination, showed an increase in liver glycogen levels, while treatment with estradiol-17β alone or in combination with endosulfan showed an increased in uterine protein, glycogen and lipid levels. These results indicate that endosulfan did not have oestrogenic or antiestrogenic effects in mice.

Ovariectomized albino rats treated with endosulfan at 0 or 1.5 mg/kg bw/day for 30 days showed no effects on uterine, cervical, vaginal or pituitary weights. However, combined treatment with endosulfan and estradiolpropionate for 30 days produced significant increases in uterine, cervical, vaginal or pituitary weights compared to controls. The increase in organ weight seen with combined treatment was similar to those seen with estradiolpropionate alone (Raizada et al 1991).

2.6 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).

2.7 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).

2.8 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 of 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 oestrogen-inducible PRL by endosulfan and chlordane, possibly acting via second messenger-mediated cellular mechanisms instead of solely competing with estrogens for the nuclear estrogen receptor sites (Rousseau et al 2002).

2.9 Neurobehavioural effects

Three rat studies conducted by the one laboratory were complicated by poor reporting and systemic signs of toxicity (reduced body weight, reduced food consumption, increased mortality, increased intensity of tremors, and increased liver enzyme activity) at the doses used. A single dose level of 2 mg/kg/day for 90 days resulted in an increase in motor activity, an increase in the inhibition of the pole climbing escape response (learning) and the avoidance response (memory). An increase in the concentration of 5-HT in the brain of treated animals was also observed (Paul et al 1993, 1994). Paul et al 1995 also noted sex related difference in regard to motor activity, with a greater dose related increase observed in males compared to females.

There was no evidence of developmental neurotoxicity seen in rat developmental neurotoxicity study (see Section 4.3).

2.10 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 & Hussai 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).

2.11 Endocrine effect in humans

An epidemiological study by Saiyed et al (2003) assessed the potential effect of aerial spraying of endosulfan on sexual maturation in 117 male children. Endosulfan was sprayed 2-3 times per year for over 20 years on cashew nut plantations in India. Results showed that 78% of male children sampled had significant levels of endosulfan in their serum, as well as decreased testosterone levels and delayed sexual development. The authors of this study noted that to understand the implications of these results a larger sample size needs to be tested and a follow-up on all children involved should be performed.

In an epidemiological study by Damgaard et al (2006), the association between exposure to organochlorine pesticides via human breast milk and cryptorchidism in male children was examined. This longitudinal birth cohort study, conducted in Finland and Denmark between 1997 and 2001 examined the regional prevalence rates and risk factors for cryptorchidism using questionnaires and biological samples including blood samples from both mother and child, as well as breast milk and placenta samples. The presence of organochlorine pesticides was determined in 62 breast milk samples from mothers of cryptorchidism boys and 68 from mothers of healthy boys. The study showed that 8 different organochlorine pesticides (including endosulfan) were quantifiable in all breast milk samples (control and case samples). It was shown that 17 pesticides were measured in slightly higher media concentrations in milk from mothers giving birth to cryptorhid boys than in mother giving birth to health boys, however these results did not reach statistical significance (except for trans-chlordane). Combined statistical analysis (a Monte Carlo permutation test) of the 8 most prevalent pesticides demonstrated that pesticide levels in breast milk were significantly higher in boys with cryptorchidism. From these results it can be concluded that although there is no association between exposure to individual organochlorine pesticides alone and cryptorchidism, there may be an association between exposure to more than one pesticide and cryptorchidism.

Exposure to xenoestrogens during pregnancy and the development of male sexual organs was further examined in a case-control, nested mother-child cohort (n = 702) study by Fernandez et al (2007). In this study, the concentration of 16 organochlorine pesticides was measured in the placenta of 50 newborn boys with cryptorchidism and 114 newborn boys without malformations. Results showed that mothers working in agricultural settings, occupational exposure of fathers to xenoestrogens and a history of previous stillbirths were associated with an increased risk of urogenital malformations in boys. All placentas studied were positive for at least one pesticide. However a higher

number of pesticides were detected in cases than in controls. Endosulfan was found in 52.4% of placentas examined, however there was no statistically significant difference in the mean concentration of endosulfan in control and case placentas. The authors of this study concluded that the combined effect of environmental estrogens is a risk factor for male urogenital malformations.

The levels of organochlorine pesticide residues were measured in samples of maternal and cord blood collected from 68 healthy women with full term pregnancies in Delhi, India between 2006 and 2007. In India, pesticide residues have been detected above tolerance levels in approximately 20% of food products and the aim of the study was to gain insight into the burden of organochlorine pesticides in newborns. Endosulfan residues were detected at mean levels of 3.70 ± 4.20 ng/mL and 2.27 ± 2.44 ng/mL in maternal and cord blood respectively. These results indicate that organochlorine pesticides such as endosulfan are transferred from mothers to foetuses with a transplacental transfer rate of ~60% (Pathak et al 2008).

2.12 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 bw/day 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 bw/day. 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. Testicular atrophy in treated male rats (20 mg/kg bw/day and above) was reported in a chronic dietary study. However, similar effects were not observed in other repeat-dose studies at higher dose levels and were accompanied by significant renal and liver toxicity, as well as increased mortality, suggesting that the testicular effects were a result of systemic toxicity. 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.

In the rabbit, no foetal effects were seen up to the highest dose tested, 1.8 mg/kg bw/day, which produced clinical signs of neurotoxicity and death in dams. In rat studies that were reliable for regulatory purposes, foetal effects (i.e. decreased foetal body weight and percentage of live foetuses) were only seen at doses greater than 2.0 mg/kg bw/day in the presence of marked maternal toxicity. Additionally, maternal toxicity was also observed at doses lower than 2.0 mg/kg bw/day in some studies.

Therefore, no evidence that endosulfan is a developmental toxicant was observed in either species.

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 disrupters 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/day 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*.

A rat developmental neurotoxicity study (see Section 4.3) also showed no indication of endocrine effects in rats exposed to endosulfan *in utero* and during lactation. Dams, foetuses and pups showed a decrease in body weight gain during treatment (3.75 mg/kg bw/day) and male pups had a slight delay (4-5%) in preputial separation at 10.8 mg/kg bw/day and greater.

Testicular toxicity

Testicular toxicity is clearly demonstrated in a number of relatively high-dose studies in mice and rats. In studies considered adequate for regulatory purposes, effects such as decreased testicular weight, decreased testicular testosterone levels, reduced sperm count and sperm production were seen at concentrations of 5 mg/kg bw/day and above. However, the testicular effects observed are 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.

In one study, rats exposed to endosulfan *in utero* showed decreased sperm production at 3 mg/kg bw/day and decreased spermatogenesis at 1.5 mg/kg bw/day and above

(Dalsenter et al 1999). However, a subsequent study by the same authors showed no treatment related effects in sperm production, sperm count and serum testosterone levels (Dalsenter et al 2003). Furthermore, no effect was observed on any sperm parameters assessed in a developmental neurotoxicity study in rats where the highest dose tested was 29.8 mg/kg bw/day (see Section 4.3).

Thyroid toxicity

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

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

Endosulfan exhibited only weak oestrogenic activity in *in vitro* assays. 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.

Bystander exposure

Air monitoring studies by PAN North America found endosulfan in every air sample collected at a Florida elementary school over 8 days in December 2006. The school was surrounded by fields of cabbage known to be sprayed with pesticide. The maximum level of endosulfan found (626 ng/m^3) was 1.8 times the 24-hour acute and subchronic 1-year child Reference Exposure Level (REL) of 340 ng/m^3. The REL is an air concentration in ng of pesticide/cubic meter of air (ng/m^3) equivalent to a dose in mg of pesticide/kg bw (mg/kg) below which no adverse effects are anticipated from exposure to a single pesticide. The REL was calculated from the US EPAs inhalational NOEL. All samples contained α-endosulfan and 88% also contained β-endosulfan. 38% of samples were above the 24-hour acute and subchronic 1-year old child REL (PANNA 2007). In a second study performed at the same location, sampling over a longer period of time (between 1 October and 6 December 2007), found detectable levels of endosulfan in 87% of air samples. 23% of samples were above the child REL of 340 ng/m^3. The highest concentration of total endosulfan observed for a 24-hour period was 1376 ng/m^3, which is 4 times the 24-hour acute 1-year old REL and 2.8 times the 7-year old REL (PANNA 2008). These results are concerning given recent published studies that have linked aerial spraying of endosulfan and delayed sexual development and decreased testosterone levels in male children in India (Saiyed et al 2003). The results of these air monitoring studies also indicate that people in the area are regularly exposed to multiple pesticides at the same time. The synergistic effects resulting from exposure to these four pesticides in simultaneously or cumulatively is unknown, however, as detailed above (Section 3.4), exposure to multiple pesticides

during gestation or *in utero* is associated with conditions such as cryptochidism (Damgaard et al 2006; Fernandez et al 2007).

Bioaccumulation

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.

PART III: NEUROTOXICITY

As detailed above (see Section 4.9), the OCSEH evaluated several neurobehavioral studies performed with endosulfan in 1998 and 2005. In these evaluations, endosulfan was found to have neurobehavioral effects in rats. A dose of 2 mg/kg bw/day administered orally for 90 days resulted in an increase in motor activity, an increase in the inhibition of the pole-climbing escape response (learning) and avoidance response (memory) to electrical shock and an increase in the concentration of 5-HT in the brain (Paul et al 1993, 1994).

Additional neurotoxicity studies not previously seen by the OCSEH are discussed below.

1.1 Acute neurotoxicity

In a study evaluated and reported by JMPR 1998 and Cal DPR 2008, endosulfan technical grade (98.6% pure) was administered by oral gavage in a single dose to fasted Wistar rats (10/sex/dose) at 0, 6.25, 12.5, 25, 50 or 100 mg/kg bw/day (males) and 0, 0.75, 1.5, 3, 6 or 12 mg/kg bw/day (females). The vehicle was 2% starch mucilage (potato starch in deionized water). Neurotoxicological screening (FOB and motor activity) was performed 7 days prior to treatment initiation, 8 hours post-dosing (time of peak effect) and at 7 days and 14 days post-dosing. Three weeks post-dosing, controls and treated animals were terminated for neuropathological examination. The systemic NOEL was 12.5 mg/kg bw (males) and 1.5 mg/kg bw (females), based on an increase in clinical signs (mortality, tonoclonic convulsions, coarse tremor, uncoordinated gait, increased salivation, stupor, prone position, increased fright reaction, squatting posture, stilted gait, irregular respiration, straddled hind limbs, decreased spontaneous activity, panting, bristled coat, flanks drawn in and narrowed palpebral fissure) at ≥ 25 mg/kg bw in males, and ≥ 3 mg/kg bw in females. These clinical signs of toxicity were observed for up to 1 day after dosing (Bury 1997, cited in JMPR 1998 and Cal DPR 2008).

1.2 Sub-chronic neurotoxicity

In a study evaluated and reported by Cal DPR (2008), endosulfan (96.5-98.1% pure) was administered via the diet to Wistar Crl:WI[Glx/BRL/Han]IGS BR rats (12/sex/dose) at 0, 40, 225 or 600 ppm (equivalent to 0, 2.11, 13.7 and 37.2 mg/kg bw/day for males, and 0, 2.88, 16.6 and 45.5 mg/kg bw/day for females) for 13 weeks. Neurobehavioral assessment (functional observational battery (FOB) and motor activity testing) was performed at pre-treatment and at 4, 8 and 13 weeks. At 45.5 mg/kg bw/day, one female had clonic convulsions during week 1 of exposure and died in week 8, and 3 females were reported to have red nasal stain. Decreased body weights were seen in female rats on day 7 only, at doses of 16.6 mg/kg bw/day and greater, however this was possibly due to palatability, as food consumption was decreased at week 1 only in females at doses of 16.6 mg/kg bw/day and greater. Food consumption also decreased during week 1 in males at 37.2 mg/kg bw/day. It is reported that plasma ChE activity was decreased in females at 16.6 mg/kg bw/day and above, though the data were not provided. Absolute and relative kidney and liver weights were increased in both sexes at the mid-dose and greater. At all dose levels, the kidneys in both sexes had an amorphous brown-to-yellow pigment in the cytoplasm of the proximal convoluted tubular epithelium, with pigment occasionally present in the lumen of proximal tubules. There

were no treatment related effects on FOB or motor activity in either sex at any dose level. The systemic NOELs were 37.2 mg/kg bw/day (males) and 16.6 mg/kg bw/day (females) based on decreased body weights in females at 16.6 mg/kg bw/day (Sheets et al 2004, cited in Cal DPR 2008).

1.3 Developmental neurotoxicity

A developmental neurotoxicity study by Gilmore et al (2006) was evaluated by the OCSEH. The results of this study are summarised below and a full evaluation can be found in Appendix III.

Technical grade endosulfan (99.1% pure) was administered via the diet to Wistar rats (30/group) at dose levels of 0, 50, 150 or 400 ppm (equivalent to 0, 3.74, 10.8 or 29.8 mg/kg/day) from gestation day (GD) 6 to postnatal day (PND) 21. The concentration of endosulfan in dietary preparations was adjusted during the lactation period to ensure a constant dosage throughout exposure.

The maternal LOEL was 50 ppm (3.75 mg/kg bw/day). A maternal NOEL could not be established as significant decreases in body weight, weight gain and food consumption were seen at the lowest dose tested.

At 50 ppm, there was a statistically significant decrease in body wight gain has seen in both sexes on PND 11 (9%), in males only on PND 17 (7%) and in females only on PND 21 (7%). However, the study authors did not consider these findings to be treatment related since the changes were moderate and inconsistent, and were within the historical control range for the laboratory. In contrast, a statistically significant and dose realted decrease in body wight gain was seen at 150 ppm and above, the magnitude of which was statistically significant and of toxicological significance: ≥13% in both sexes on PND 11; and ≥10% in both sexes on PND 17. There were no treatment related effects on offspring survival, clinical signs, functional observational battery (FOB) tests, motor activity, auditory startle response, learning and memory, neuropathology, brain weight, or brain morphometrics at any dose level. Sperm parameters were evaluated in control and high-dose males on PND 75. No treatment related effects were seen in any of the sperm parameters evaluated (sperm and spermatid counts, and sperm morphology) or testes and epididymis weights. Furthermore, no histopathological lesions were seen in the male reproductive organs. However, the onset of preputial separation was significantly delayed in males at 150 ppm and above (day 47.1 and 46.8 at 150 and 400 ppm respectively, compared to day 44.9 in controls). Consequently, a NOEL of 50 ppm (3.75 mg/kg bw/day) was established for offspring, based on the observation of decreased body weight gain in both sexes and a delay in preputial separation in males at 150 ppm.

In conclusion, dietary administration of endosulfan to pregnant rats at the highest tolerated dose of 400 ppm (29.8 mg/kg bw/day) from GD 6 to lactation day (LD) 21 (a dose which produced marked maternal toxicity) does not produce evidence of neurotoxicity in the offspring.

In another study, weanling rats (6/dose) were treated by gavage with endosulfan technical (96% pure) at 0 and 6 mg/kg bw/day during postnatal days 2-25. Pups were sacrificed on day 10 and 25 (6/dose/group). At day 10, there was an increase in

noradrenalin in the olfactory bulb, brainstem and in hippocampus. At day 25, there was an increase in noradrenaline in the cerebellum. Dopamine was decreased in the hippocampus at both 10 and 25 days. 5-HT was increased in olfactory bulb, hippocampus and brain stem at day 10, but at day 25 levels were decreased in the brain stem and cerebellum. There were no treatment related effects on brain acetylcholinesterase. There was no change in brain or body weight gain in treated rats compared to controls. These changes in neurotransmitter levels were associated with changes in learning and memory. There was a statistically significant increase in the time treated rats took to learn a task. Treated rats were also less able to retain the acquired task than controls (Lakshmana & Raju 1994).

The possible neurotoxic effects of endosulfan were evaluated on male offspring rats exposed to endosulfan *in utero* and during lactation. Dams were treated by gavage with 0.61 or 6.12 mg/kg bw/day of endosulfan from the beginning of gestation until weaning (PND 21). Male offspring were sacrificed at post-natal day (PND) 15, 30 and 60 and the content and metabolism of biogenic amines and amino acids were determined in the prefrontal cortex using high-performance liquid chromatography (HPLC). The body weight gain of offspring was decreased at PND 21 (weaning) at 0.61 mg/kg bw/day endosulfan, while at 6.12 mg/kg bw/day body weight gain was decreased at PND 15, 21 and 30. At PND 0 and PND 60, bodyweight of offspring was not affected by treatment. Endosulfan, 6.12 mg/kg bw/day, induced an increase in amino acid content in the prefrontal cortex at PND 15 (GABA, aspartate, glutamate, glutamine and taurine). At PND 30, the levels of aspartate, glutamate and taurine were increased at both dose levels, while glutamine and GABA were increased at the high dose level only. At PND 60, a significant reduction in the content of GABA and taurine was observed at both dose levels, while the concentration of glutamate, aspartate and glutamine were unchanged. Norepinephrine and dopamine were unchanged, but the concentration of 5-HT was increased at PND 30 and 60 at both dose levels. Serotoninergic and dopaminergic metabolism were also modified at all time points examined. These results suggest that pre- and post-natal exposure to endosulfan affects biogenic amine and amino acids in prefrontal cortex (Cabaleiro et al 2008).

It was proposed that pesticide exposure (endosulfan and dicofol) to pregnant women living near agricultural applications induces neurotoxicity in foetuses when exposure occurred during gestation weeks 1-8 (period of central nervous embryogenesis) (Roberts et al 2007). Exposure was proposed to result in an increased incidence in autism spectrum disorder. However, the study authors concluded that there were many flaws to the study including, no knowledge of diet, actual duration of exposure, or if the selected population was exposed at all and, consequently there is no cause and effect between endosulfan and autism spectrum disorder.

1.4 Other neurotoxicity studies

Endosulfan was administered intraperitoneally (ip) to groups of lesioned and non-lesioned adult female albino rats at 3 mg/kg bw/day for 10 days (9/group). Lesions were performed in the amygdala, septum and substantia nigra regions of the brain which are areas of the brain high in dopaminergic neurones and are involved in movement and emotions. Lesioned and non-lesioned rats treated with endosulfan showed increased dopamine levels and decreased 5-HT levels in the brain compared to untreated lesioned

and non-lesioned rats. There was a statistically significant increase in foot-shock fighting behaviour in septal and nigral lesioned rats. However in amygdaloid-lesioned rats, there was a decrease in aggressiveness. Increased convulsions and locomotor activity was seen in endosulfan treated rats (Anand et al 1985).

Mice (C57Bl/6) were treated with endosulfan at 0.15 mg/kg bw/day ip from postnatal days 5-19 (8/group). At 8 months of age, mice were re-exposed to 1.55 mg/kg bw/day endosulfan ip for seven days. There were no treatment related changes in body weight gain or the brain weight compared to control. When exposed as juveniles (postnatal day 5-19), levels of dopamine and its metabolite dihydroxyphenylacetic acid (DOPAC) and acetylcholinesterase (AChE) in the brain, were not different to controls. When re-exposed to endosulfan as adults, there was a statistically significantly decrease in the level of dopamine and DOPAC, and an increase in AChE in the brain compared to controls. Neither juvenile nor adult exposure to endosulfan resulted in changes in the level of α-synuclein in the brain, however, exposure to a combination of endosulfan and zineb as juveniles and again during adulthood, resulted in a statistically significant increase in α-synuclein levels. A loss of dopamine producing cells and an accumulation of α-synuclein in the brain are pathological hallmarks of Parkinson's disease (Jia & Misra 2007b).

SH-SY5Y human neuroblastoma cells (dopaminergic neurons) were exposed to endosulfan (99.9% pure), zineb (a dithiocarbamate fungicide) or a combination of the two chemicals in vitro. Endosulfan and zineb (100 μM) in combination exhibited significantly higher toxicity to human cells than either pesticide by itself. Both pesticides were found to cause apoptotic cell death that was concentration dependent (50-400 μM). The type of cell death (apoptotic or necrotic) was determined using flow cytometry. It was determined that exposure to 100 μM endosulfan caused an increase in apoptotic cells while combined treatment with endosulfan and zineb caused and increase in necrotic cell death. These in vitro findings suggest that the cytotoxicity of endosulfan and zineb, both individually and in combination are associated with the occurrence of apoptotic/necrotic processes in human neuroblastoma cells. However, while there is current evidence available to suggest that apoptotic cell death may contribute to various pathological conditions such Parkinson's disease and Alzheimers disease, the relevance of these *in vitro* findings is unknown (Jia & Misra 2007a).

It has been suggested that oxidative stress is an important pathway leading to neuronal cell death, therefore, the role of oxidative stress caused by exposure to the pesticides endosulfan and zineb in human neuorbastoma cells (SH-SY5Y) was examined. There was a statistically significant increase in H_2O_2, reactive oxygen species (ROS) and superoxide anion (O^{2-}) in cells exposed to endosulfan. A combination of endosulfan and zineb invoked a significantly higher level of H_2O_2 and O^{2-} production in cells than with endosulfan alone. SH-SY5Y cells exposed to endosulfan (100 μM) showed a significant decrease in the specific activity of the antioxidant enzyme catalase, however superoxide dismutase (SOD), and glutathione peroxidase (GPX) were not affected. Combined pesticide exposure significantly decreased the activities of SOD, catalase and GPX compared to controls. MDA levels, measured as an indicator of oxidative stress, in SH-SY5Y cells were significantly increased following endosulfan exposure (100 μM). Cells treated with a combination of endosulfan and zineb showed significantly higher levels of

MDA than cells treated with endosulfan alone. Caspase-3 activity (an enzyme involved in neuronal apoptosis and whose activation is implicated in Parkinson's disease) in SH-SY5Y cells treated with endosulfan (100 μM) was significantly higher than in control cells, while combined pesticide exposure caused a significant decrease in caspase-3 activity compared to controls. The activity of NFκB, a ubiquitous transcription factor was measured as an indicator of oxidative stress. Exposure to endosulfan (100 μM) showed significantly higher levels of NFκB than controls, however, when exposed to both endosulfan and zineb, no differences were observed compared to cells treated with endosulfan alone (Jia & Misra 2007c).

1.5 Discussion

Evaluation of the developmental neurotoxicity study found that exposure to endosulfan in utero and during lactation resulted in no treatment related effects on offspring survival, clinical signs, sexual maturation or developmental neurotoxicity parameters at the highest dose tested (29.8 mg/kg bw/day).

Rats exposed to endosulfan by gavage at doses of 0.61 mg/kg bw/day and above, in utero or postnatally, showed increases in the levels of amino acids (aspartate, glutamate, glutamine, taurine and GABA) and decreases in dopamine levels in the brain (Lakshmana & Raju 1994; Cabaleiro et al 2008). 5-HT was also shown to be decreased on PND 25 in rats (Lakshmana & Raju 1994).

Adult rats exposed to endosulfan at a dose of 3 mg/kg bw/day also showed changes in brain dopamine and 5-HT levels, and this was associated with an increase in aggressive behaviour (increased foot-shock fighting behaviour) (Anand et al 1985). However in this study endosulfan was administered by ip injection, which does not represent a normal route of exposure in humans and therefore the relevance of this study to human exposure situations (ie. dermal, oral or inhalation) is unknown.

More recently, endosulfan has been reported to be linked to an increased risk of Parkinson's disease. Mice treated postnatally with endosulfan (0.15 mg/kg bw/day, ip) and then re-exposed during adulthood (1.55 mg/kg bw/day, ip) in combination with other pesticides, showed an increase in α-synuclein levels in the brain, a pathological hallmark of Parkinson's disease (Jia & Misra 2007b). In human neuroblastoma cells studies *in vitro*, endosulfan caused apoptotic cell death (Jia & Misra 2007a) and oxidative stress (Jia & Misra 2007c).

1.6 Conclusion

The recent Australian APVMA (1998, 2005) and US EPA reviews (2002) 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 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 page 10 of this report for the NOEL table) is adequately sensitive and protective against potential endocrine disruption by endosulfan. Furthermore, the recently evaluated developmental neurotoxicity study reported no effects on sperm parameters

The US EPA evaluation noted the effects seen in test animals and argued additionally that the effects seen in amphibians, fish, birds and hormone receptor studies are indicative of potential endocrine disruption. Recently Cal DPR (2008) stated that the uncertainty regarding endosulfan as an endocrine disruptor has been reduced following the submission of the developmental neurotoxicity study in rats where there were no treatment related effects on sexual maturation of offspring at the highest dose tested (29.8 mg/kg bw/day).

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.

Summaries of two neurotoxicity studies (Bury 1997; Sheets et al 2004) and one developmental toxicity study (Nye et al 1981) have been included in this report, however, the original study reports were not evaluated by the OCSEH.

Evaluation of the new developmental neurotoxicity study found that exposure to endosulfan in utero and during lactation was not associated with neurotoxic effects on the offspring at doses up to 29.8 mg/kg bw/day.

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OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

EXECUTIVE SUMMARY

Endosulfan was selected for review under the Australian Pesticides and Veterinary Medicines Authority (APVMA's) Existing Chemical Review Program (ECRP) in 1996. An Interim Review report on endosulfan was published in 1998. Since that time, the registrations of all ultra low volume (ULV) products have been cancelled. Based on the OHS assessment that formed part of the APVMA review of endosulfan in 1998 there were concerns regarding the likely extent of occupational exposure for some end-use and re-entry scenarios when using surrogate exposure data. Although there was exposure data available for cotton chippers re-entering treated fields 7 and 24 hours post-application it was not suitable to permit the calculation of a re-entry period beyond 24 hours. As an interim regulatory measure certain uses of endosulfan were permitted to allow the necessary Australian worker exposure data to be generated.

In the 2005 OHS review, supplementary studies, which included human *in vitro* percutaneous absorption data, indicated that a lower dermal absorption factor could be used to assess the occupational risk. When coupled with new Australian occupational exposure data, and the use of PHED, PPE was recommended. For re-entry interval estimations in cotton, melon, peach and grape crops, an acceptable margin of exposure was obtained on day 0. Recommendations for First Aid Instructions, Safety Directions, re-entry intervals and precautionary statements are included in this report.

In this amended report (2009), the dermal absorption factor used in the 2005 OHS review has been slightly revised following re-interpretation of the *in vitro* percutaneous absorption data. Subsequently, worker exposures for mixing, loading and applying EC endosulfan products, as well as re-entry activities were recalculated. The recommendations for PPE reamin basically unchanged. The re-entry interval estimations in cotton, melon, peach and grape crops, also reamins unchanged.

1. BACKGROUND

The major use of endosulfan in Australia is in cotton production (70%) and vegetables (20%) with the balance (10%) being divided among oilseeds, pome and stone fruits, exotic fruits and other crops, such as pulses and ornamentals. Label instructions also permit the use of endosulfan in cereal crops, turf and lawn, tobacco, and nursery crops. Current labels include instructions for application by ground and by air, with endosulfan being applied aerially in significant quantities since the major crop is cotton. Ground applications are either by boom spray, air blast, air shear or knapsack with hand wand/nozzle.

Following the interim review of endosulfan, the APVMA requested that worker exposure data be generated under Australian use conditions in order to generate specific data/information on the extent of exposure to endosulfan. Work practices that were identified by the APVMA for consideration were:

- Mixer/loaders in ground and aerial applications
- Manual flaggers for aerial applicators
- Orchard ground spray applicators (including re-entry)
- Broadacre ground spray applicators (including re-entry)
- Greenhouse workers
- Workers using hand-directed spray applicators

The worker exposure studies were conducted in broadacre cotton industries and were based on a protocol approved by the APVMA and OCSEH (OHS), and in accordance with standards prescribed by the New England Health Research and University of Sydney Research ethics committees. All studies used the same formulation of endosulfan containing 350 g ai/L, which was considered representative of each of the EC products under review. End-use exposure and risk were determined for workers treating broadacre (cotton), horticultural and nursery crops.

Re-entry exposure and risk were determined for workers conducting various re-entry activities for cotton, e.g., cotton chipping, crop checking and irrigating. No re-entry exposure data were provided for tree crops (orchards) or nurseries. In the absence of these data the DFR values from a cotton study were extrapolated to other crops e.g., citrus, pecans, fruit and nut trees etc. by considering the relative application rates and generic transfer coefficients identified in the US Occupational Post-Application Risk Assessment Calculator (US EPA Policy 003.1). Application rates for the various crops were used (where provided). Using DFR data for cotton, generic transfer coefficients and standardised application rates the re-entry intervals were calculated for the various crops. During the public consultation phase in 2004 questions were raised regarding the dermal absorption factor (10%) used in the OHS risk assessment and the use of cotton DFR data to determine re-entry intervals for other broadacre and tree crops. Supplementary data which includes a new *in vitro* dermal absorption study (Davies, 2002) and a re-entry study on melons, peaches and grapes (Singer, 1995) were also submitted for consideration in the 2005 review of endosulfan. The dermal absorption

factor used in the 2005 worker exposure evaluation has been amended based on a re-interpretation of the dermal absorption studies.

2. Dermal Absorption

To date there is a lack of international consensus regarding the derivation of appropriate dermal absorption factors for endosulfan. However, all of the available data suggests that the concentrated material is less well absorbed (on a percentage basis) through the skin than spray mixes and that human skin is less permeable to endosulfan than rat skin.

Endosulfan risk assessments performed by the United States Environmental Protection Agency (US EPA) (2007), California Department of Pesticide Regulation (Cal DRP) (2008), Pest Management Regulatory Agency (PMRA) (2007) and the Environmental Risk Management authority New Zealand (ERMA New Zealand) (2008) all use the same *in vivo* rat studies by Craine (1986 & 1988) as the basis for their dermal absorption factors. The US EPA, PMRA and Cal DPR derived dermal absorption factors for rat skin of 45%, 47% and 47.3% respectively, while ERMA New Zealand (2008) has derived dermal absorption factors for rat skin of 46% for diluted spray mix and 20% for concentrates. In contrast, the European Union has used dermal absorption factors for humans of 0.8% for concentrates and 2.2 % for the dilute spray.

In the 2005 review of endosulfan, to estimate a dermal absorption factor for human skin, the OCSEH used both the *in vivo* rat study by Craine (1988) and an *in vitro* study by Davies (2002) which compares the rates of endosulfan penetration through rat and human skin samples. In accordance with the method proposed in the EC Draft Guidance on Dermal Absorption, the rat *in vivo* absorption values are adjusted by the ratio of the human and rat *in vitro* absorption values obtained in the Davies (2002) study to derive dermal absorption factors for humans of 0.5% for concentrates and 1.52 % for spraying and re-entry activities.

In this present review of endosulfan, the Davies (2002) study was reassessed and revised dermal absorption factors of 0.8% for concentrates and 2.2% for spraying and re-entry activities were established. In the 2005 report, the calculations of the total absorbed dose of endosulfan on human skin did not include the percent of endosulfan present on tape strips. The endosulfan on the tape strips should be included in the absence of evidence that this material would not be absorped. *In vivo* studies showed that absorption continues for > 7 days and that the majority of endosulfan found in the skin at 24 hours is eventually absorbed. Therefore the dermal absorption factors have been recalculated to include the percentage endosulfan on tape strips together with the percentage remaining on the epidermis plus the percentage in receptor fluid. These revised dermal absorption factors are the same as those used by the European Union.

The US EPA, Cal DPR, PRMA and ERMA New Zealand have not used this approach to calculate their dermal absorption factors. As detailed in the ERMA New Zealand (2008) review, the *in vitro* study by Davies (2002) was not considered adequate to estimate a human dermal absorption factor from the rat data. The ERMA New Zealand (2008) raised several concerns regarding the adequacy of the Davies (2002) study: the study does not include results for other test substances of similar lipophilicity to endosulfan, the source of human skin is not given, which raises questions around the relevance for

persons exposed, and the comparison of data for the absorption in rats *in vitro* (Davies 2002) and *in vivo* (Craine 1988) supposedly shows differences that call into question the validity of the *in vitro* results. The last point is touched on below (Section 2.3), and the OCSEH does not consider that such concerns invalidate the use of these studies.

The available database on endosulfan contains four studies relevant to estimation of a dermal absorption factor: two *in vivo* studies in rats, and two *in vitro* studies which generated comparative data in rat and human skin.

2.1 *In vivo* studies

Craine (1986) applied radiolabelled endosulfan in an EC formulation to a 10.8 cm^2 area of the skin of male rats (260 g bw) at 0.026, 0.20 and 2.6 mg/animal, equating to doses of 0.10, 0.76 and 10.13 mg/kg bw or 2.4, 18.5 or 240 $\mu g/cm^2$. Recovery of radiolabel was essentially complete. Absorption of endosulfan into the skin was rapid and extensive at all doses, as skin washings removed generally only 20% of the applied dose. However, movement through the skin was slow and up to 67% of the absorbed radiolabel remained bound to the skin at 24 h, by which time absorption was 21.5% of the dose at 2.4 and 18.5 $\mu g/cm^2$, and 8.4% at 240 $\mu g/cm^2$.

In the second rat study (Craine, 1988), radiolabelled endosulfan in an EC formulation was applied to the skin (10.8 cm^2) of female rats (mean bw 240 g) at 0.09, 0.98 and 10.98 mg/kg (equal to 22, 235, 2640 µg/animal or 2.0, 22, 244 $\mu g/cm^2$). The test compound was then washed off after 10 hours. Animals were sacrificed at 24, 48, 72 hours or 7 days after the dose application to determine absorption and distribution of endosulfan. Mean recovery of radiolabel ranged between 96 – 108%. Initial absorption into the skin was related inversely to dose, with skin washings removing 30, 45 and 66% of the applied radiolabel at 2, 22 and 244 $\mu g/cm^2$, respectively. Movement through the skin was slow. In the 2, 22 and 244 $\mu g/cm^2$ groups respectively, penetration of radiolabel reached 22, 16 and 4% of the applied dose by 24 hours, when 41, 39 and 33% of applied radiolabel was still bound to the skin. At 48 hours, penetration of radiolabel had attained 35, 36 and 11% in the three respective groups. Penetration attained 45, 46 and 20% by 7 days, by which time only 1 – 2% of the dose remained bound to the skin.

2.2 *In vitro* studies

Noctor & John (1995) applied radiolabelled endosulfan in an EC formulation to skin slices from rats and humans, and measured the extent of penetration over 72 hours. Additional studies were performed where the skin surfaces were washed 10 hours after application. However, this study is considered unreliable due to methodological deficiencies including low total recovery of applied radioactivity, inadequate verification of membrane integrity, and potential loss of viability (and hence enhanced permeability) of the skin sections over the 72 hour incubation period.

In the definitive study, Davies (2002) applied ^{14}C-endosulfan for 8 or 24 hours to unoccluded intact rat and human epidermal membranes at 3580, 1710 or 10 $\mu g/cm^2$. At the highest dose, the radiolabel was applied in an EC formulation containing endosulfan at 358 g/L. Aqueous dilutions of the formulation containing 171 and 1 g endosulfan/L were applied at the mid and low doses. The lowest concentration was equivalent to spray mixture. Membranes were washed at 8 and 24 hours to remove unabsorbed radiolabel. After washing, tape stripping was performed on human epidermal

membranes, but not those from rats. Membrane integrity was verified by electrical resistance.

Recovery of radioactivity ranged from 94 – 113%. Washing removed the majority of the high dose (64-79%), mid dose (58-91%) and low dose (49-98%, except 12-23% on rat skin at 24 h). Tape stripping removed an additional 0.08 – 0.35% of applied radiolabel from the outer layers of human epidermis. Penetration of endosulfan was essentially linear over 24 hours, and was much slower through human epidermis than rat epidermis (ratio human : rat 0.03 – 0.05 : 1). There was an inverse relationship between the proportion of radiolabel absorbed and the dose and concentration of endosulfan applied. Results obtained with the undiluted formulation and spray mixture are summarised below.

	Sampling time (h)			
	8	**24**	**8**	**24**
	Undiluted EC formulation (358 g/L)		**Spray mixture (1.0 g/L)**	
	Human epidermis: Percent of applied dose detected in sample matrix			
Tape strips	0.30	0.35	1.34	1.16
Epidermis	0.40	0.28	1.17	0.79
Receptor fluid	0.13	0.33	1.18	1.90
Total absorbed	**0.82**	**0.96**	**3.69**	**3.85**
	Rat epidermis: Percent of applied dose detected in sample matrix			
Tape strips	-	-	-	-
Epidermis	21.2	14.4	30.8	15.9
Receptor fluid	7.17	10.2	42.9	65.8
Total absorbed	**28.4**	**24.6**	**73.7**	**81.7**
Ratio human:rat	**0.03**	**0.04**	**0.05**	**0.05**

2.3 Dermal absorption factor for exposure to concentrates and spray mixtures

It is apparent that endosulfan is absorbed at a comparable rate across rat skin *in vivo* and *in vitro*. However, under identical experimental conditions, human epidermis is at least 30-fold less permeable to endosulfan than rat epidermis. Probably due to saturability at high concentrations, absorption of endosulfan from spray mixture across isolated human epidermis is several-fold more extensive (on a percentage basis) than from the undiluted concentrate. Similarly, there was about 2-fold and 3-fold more absorption from spray mixture (on a percentage basis) than from concentrate across whole rat skin and isolated rat epidermis, respectively. Therefore, separate dermal absorption factors should be used for estimation of systemic exposure to endosulfan

arising from dermal contamination by undiluted products and spray mixture.

Consistent with the EC Guidance Document on Dermal Absorption, factors for endosulfan can be calculated by adjusting the rat *in vivo* absorption values by the ratio of the human to the rat *in vitro* absorption. The dermal absorption factor for concentrate exposure will be 20% x 0.04 = 0.8%, while the factor for exposure to spray mixture will be 46% x 0.05 = 2.2%.

2.4 Dermal absorption factor for re-entry exposure

In addition to being potentially exposed to endosulfan during mixture and application of products, workers may also be exposed following re-entry into treated fields or other areas. Exposure would be predominantly via the dermal route, through making contact with endosulfan residues on foliage, fruit or soil. Clarke & Churches (1992) measured exposure to endosulfan among cotton chippers re-entering endosulfan-treated fields 7 or 24 hours post-application. The heaviest exposure occurred to workers at 24 hours, probably because the cotton height was greater than the crop re-entered after 7 hours (50 vs 30 cm). Following a 1-hour work period, the heaviest mean exposure was 3.0 μg endosulfan/cm^2 skin, detected on the hands.

Given the comparatively short time interval between treatment and re-entry, an endosulfan deposition rate of 3.0 μg/cm^2/h is likely to be approaching the maximum rate at which exposure would occur. If endosulfan accumulated on the skin at a constant rate throughout an 8-hour workday, a peak dermal concentration of 24 μg endosulfan/cm^2 would be attained. This is similar to the mid concentration used in the *in vivo* dermal absorption study of Craine (1988) (at which endosulfan penetration attained 46%) and to the lowest concentration used in the *in vitro* absorption study of Davies (2002). Therefore, the extent of dermal absorption arising from re-entry exposure would be closely similar to that which has been estimated for endosulfan in diluted spray mixture (ie. 2.2%; see discussion above), rather than the extent of absorption from exposure to concentrated formulations. A dermal absorption factor of 2.2% will be used for re-entry exposure assessment.

NOELs used for occupational health and safety assessment.

3. OCCUPATIONAL EXPOSURE

The following main groups of studies were conducted:

i) Worker exposure following application to tree crops
ii) Worker exposure following application to nursery crops
iii) Worker exposure following aerial application to cotton (broadacre crops)
iv) Worker exposure following re-entry in cotton cropping activities (broadacre crops).

Mixer/loader and applicator exposure was estimated using a variety of application methods for the treatment of tree, broadacre, and nursery crops. The EC formulation of endosulfan (350 g ai/L) was used in all the studies. Application rates were generally in accordance with label instructions for the various crops/situations. Estimation of inhalation exposure was not included in the study protocol because it had been previously shown (see interim report) that the contribution to overall exposure from spray inhalation during application was minimal compared with dermal exposure. For ground rig applications, inhalation contributed only 1% to total endosulfan exposure for both mixing/loading and application. For hand spraying, inhalation contributed only 2% of exposure to applicators.

For the purpose of measuring dermal exposure chromatographic paper patches attached to cloth pads were fixed (using velcro) either on singlets (under the overalls) on the body of the worker, or externally on overalls of workers. The distribution of the patches are described below:

<u>Internal patches (patches fixed with velcro) on the singlet, under the overalls</u>

a) Two patches, (one on either side) on the top of the external shoulders (dorsal side)
b) One patch on the back of the neck (dorsal side) below the lower edge of the collar
c) One patch on the upper chest (ventral side) near the jugular notch

<u>Internal patches (patches fixed with velcro) on the body of the worker</u>

a) Two patches, one on each forearm (at the back)
b) Two patches, one on each thigh (in front)
c) Two patches, one on each knee (in front)
Cotton gloves were used to measure residue deposition on hands.

<u>External patches (on the overalls)</u>

a) Two patches, (one on either side) on the top of the internal shoulder (ventral side)

Estimation of total endosulfan exposure based on the surface area of the different body parts is outlined in Table 1.

Table 1: Estimation of total endosulfan exposure based on surface area of different body parts.

Body parts	Deposition dosimeter quantity x surface area (cm^2) of body part
Head and face	(Mean of ext. shoulder, chest and back patches) x 1300
Back of neck	(Back patch) x 110
Front of neck	(Chest patch) x 150
Chest/stomach	(Chest patch) x 3550
Back	(Back patch) x 3550
Upper arms	(Mean int shoulder and forearm) x 1210
Forearm	(Arm patch) x 2910
Hand	(Glove result) x 2
Thigh	(Thigh patch) x 3820
Lower leg	(Low leg patch) x 2380
Feet	(Foot patch) x 1310

For the purpose of study control, a member of the field monitoring team for each study session was "patched" with three field blanks. This member remained outside the paddock for the duration of each session, in an area that was apparently free from direct exposure to endosulfan. Some patches and gloves were also 'spiked' with endosulfan and exposed to similar weather conditions. The field blanks were used for the purpose of estimating the cross-contamination of the patches while handling them. Exposure samples were sealed (in test tubes and jars) and transported under cool conditions to the laboratory for analysis. Meteorological conditions during the sessions were recorded. Section 2.1 outlines the parameters of each study. Section 2.2 summarises the dermal exposure data generated for the various occupational scenarios studied.

3.1 Parameters used in exposure studies

3.1.1 Worker exposure by application to tree crops

The following six studies were undertaken to estimate exposure for workers using endosulfan in tree crops. A cleaning down study, though not requested as part of the initial requirements for additional data, was also provided for assessment.

Study H-1-1: Mixing/Loading

Study H-1-2-U: Spraying air-assist spray, no cabin

Study H-1-2-C: Spraying air-assist, with cabin

Study H-2-2-C: Spraying air-shear, with cabin

Study H-5-2-C: Oscillating boom spray

Study H-1-4: Cleaning down

Applications of endosulfan were made in the course of actual pest control under a range of differing weather conditions. Any chemical spills or other incidents were reported, and exposure values were adjusted accordingly. In the above studies endosulfan was poured from 20 L steel drums either into mixing tanks or directly into spray tanks of capacity 1200 L-3000 L. Dilution was an average of 150 mL/100 L of water, with

dilutions varying for different applications. The total amount of spray volume handled per session during mixing/loading and application ranged from 100 L - 4800 L, with 0.05 kg –1.58 kg ai handled per study session.

In air-assisted sprayers (tractors with and without cabins), the spray droplets were generally produced by standard hydraulic nozzles, with air blown over the nozzle or spray plume to direct the spray into the tree canopy. Exposure to endosulfan while cleaning the mixing/spraying equipment was also measured. To estimate worker exposure during cleaning down spray equipment, subjects were re-patched after they completed spraying. It should be noted however that in practice, all tasks (mixing/loading, spraying and cleaning down) are often undertaken by the same operator. Therefore, worker exposure may not be adequately measured by separating these activities.

During cleaning down operations, work was carried out (where possible) so that the wind directed any spray or fumes away from the worker, thus minimising airborne contamination and contamination of equipment. Connection and disconnection of hoses to and from the container, pump and mixing tanks was undertaken with care to avoid coming in contact with contaminated surfaces. Care was also taken to avoid touching the face and exposed skin when wearing gloves. The parameters of the above studies are outlined in Table 2.

Table 2: Parameters of studies conducted for measuring exposure to endosulfan in the course of application to tree crops

Parameters	**Mixing/Loading** **(Study H-1-1)**	**Air-assist spray [no cabin]** **(Study H-1-2-U)**	**Air-assist [with cabin]** **(Study H-1-2-C)**	**Air-shear [with cabin]** **(Study H-2-2-C)**	**Oscillating boom spray** **(Study H-5-2-C)**	**Cleaning down** **(Study H-1-4)**
Number of subjects/replicates	16/19	7/15	14/15	2/5	8/14	9/15
Duration of study (days)/ No. of sites/No. of sessions	7/7	3/3/3	8/8/8	1/1/1	4/4	8/8/9
Time taken for procedure (minutes) [(1)]	5-65	16-50	20-55	25-55	20-40	5-35
Spray volume handled (L)[(2)]	750-3000	100-1500	500-2100	1000-2400	1500-4800	100-9600[(3)]
Total active ingredient (kg) handled[(1)]	0.13-1.58	0.05-0.79	0.05-1.10	0.53-1.26	0.16-0.51	0.05-2.36[(3)]
Tasks/procedures	Transport of pesticide drums, transferring chemicals to and from the storage area, pouring and mixing the chemical, loading the spray unit, removing empty containers from the working area and cleaning up spills.	Spraying tree crops, recording details of chemical prepared and loaded	Moving spray equipment to spray site, applying chemical to tree crop		Towing trailer to site, cleaning nozzles, applying chemical to tree tops	Rinse drums and mixing tanks, wash spray equipment, hose down handling area, remove empty containers from the working area, clean up spills
PPE used	Waterproof or cotton overalls done up to neck and wrist, washable cotton hat, elbow-length gloves, full face-shield or goggles, half face piece respirator, and water-resistant footwear/boots, worn beneath the overalls.					

(1 per session
(2) No data were provided on application volume (L/ha), however, studies H-1-2-C; H-2-2-C and H-5-2-C were assumed to be high volume studies

(3)Amount of endosulfan and total volume sprayed before the cleaning operation. The amount of ai. handled during cleaning operations is not known.

3.1.2 Worker exposure by application to nursery crops

The following three studies were undertaken to estimate exposure to workers using endosulfan in nursery crops. A cleaning down study, though not requested as part of the initial requirements for additional data, was also provided for assessment.

H-3-1: Mixing/loading

H-3-2: Spraying

H-3-3: Cleaning down

The above studies were conducted to define levels of worker exposure to endosulfan when mixing/loading, cleaning equipment and applying endosulfan products to nursery crops. The workers mixed endosulfan by first pouring the concentrate from 10/20 L steel drums into cylinder measuring jugs and then poured into 200 L spray tanks with water. The pad and mixing area were considered to be contaminated areas. Where possible, mixing/loading was carried out in conditions where the wind directed spray or fumes away from the workers, thereby minimising airborne contamination and contamination of equipment.

The typical operation for spraying in nurseries is by use of a spray tank on a trailer, with retractable hose and hand gun permitting coverage of the whole nursery. The two types of spray systems used in nursery applications are high and low pressure systems. The high-pressure system tends to produce fine mister spray, whereas the low-pressure system tends to produce larger droplets. For both systems nozzles can be adjusted to regulate the spray pressure. It was not identified in the study (H-3-2) which system was used.

In the cleaning down study, the spray tank was filled with clean water, which was then used to clean hoses and nozzles. The 'wash residue' drained into a sump while some was washed onto a concrete area (without a drainage sump). No information was provided as to whether the amount of wash residue was measured. Potential for worker exposure was touching contaminated spray unit and hoses, contamination from leaking clamps and lines while connecting and disconnecting hoses, splashes from pad/work area and contaminated surfaces of empty containers. The duration of the cleaning-down operation depended on the size of the nursery to be treated (7-20 min), but was assumed to be up to one hour for larger nurseries. The parameters of the above studies are presented in Table 3.

Table 3: Parameters of studies conducted for measuring exposure to endosulfan in the course of application to nursery crops

Parameters	**Mixing/Loading (Study H-3-1)**	**Application (Study H-3-2)**	**Cleaning down (Study H-3-3)**
Number of subjects/replicates	8/12	12/18	10/11
Duration of study (days)/ No. of sites/No. of sessions	5/5/5	6/6/6	5/5/5
Time taken for procedure (minutes)[(1)]	4-16	15-76	7-20
Spray volume (L)[(1)]	25-300 L	25-200 L	30-300 [(2)]
Total ai handled/day (kg) [(1)]	0.03-0.2	0.03-0.13	0.03-0.20 [(2)]
Tasks/procedures	Transport of pesticide drums, transferring chemicals to and from the storage area, pouring and mixing the chemical, loading the spray unit, removing empty containers from the working area and cleaning up spills.	Towing trailer to site, unrolling spray hose, spraying nursery beds, rolling up hose to move to new area	Spraying cleaned residue from spray unit, hosing down the outside of spray unit
PPE	Waterproof or cotton overalls done up to neck and wrist, washable cotton hat, elbow-length gloves, full face-shield or goggles, and half face piece respirator, and water-resistant footwear/boots, worn beneath the overalls		

[(1)] per session

[(2)] Amount of endosulfan and total volume sprayed before the cleaning operation. The amount of a.i. handled during cleaning operations is not known.

3.1.3 Worker exposure by aerial application in broadacre cropping industries

The following six studies were undertaken to estimate exposure for workers in the course of aerial application in broadacre crops.

A-1-1: Mixing/Loading Bulk and Mini Bulk (closed base)

A-1-2: Mixing/Loading small containers (open/remote)

A-1-3: Aerial applicators

A-1-4: Support workers (vehicles)

A-1-5: Support workers (ATVs)

A-1-6: Cleaning down

The above studies were conducted to define levels of exposure to endosulfan for workers mixing and loading endosulfan products for aerial application to cotton, using bulk and mini bulk (closed/base and open/remote) containers, aerial application and assessment of exposure for support workers and those involved in cleaning down operations.

Mixing/loading was done at three different airbases. Where possible, mixing was carried out so that the wind directed any spray or fumes away from the worker, minimising airborne contamination and contamination of equipment. Connection and disconnection of hoses to and from the container, pump and mixing tanks/aircraft tanks was undertaken with care to avoid undue contact with contaminated surfaces. Loaders were directed not to approach aircraft until the aircraft was stationery, and until they had received a clear signal from the pilot to proceed with loading the aircraft. The mixer/loader vacated the pad while the aircraft was taxiing to minimise airborne contamination.

Dermal contamination with endosulfan during mixing and loading was measured. Application rates for the studies were made generally in accordance with label specification for cotton. The average rate of application of endosulfan was 2.1 L/ha, with a range of 2.09 L to 2.11 L/ha. The total volume of spray applied was either 30 or 40 L/ha, however, the amount of endosulfan used per hectare was maintained at 2.1 L/ha, irrespective of spray volume.

Leaking equipment was attended to immediately, and spills of concentrate were cleaned up by workers wearing full waterproof clothing. In the studies conducted on ATVs (All Terrain Vehicles) and vehicle support workers, it was noted that the points of potential exposure to markers were spray drift (from aircraft), contaminated surfaces of vehicles and splashes from contaminated puddles. The workers were advised to observe safe marking procedures (detailed in the Chemical Handling Manual for Agricultural Aviation, AAAA, Operation Spray Safe, 1998) and to move away from the aircraft's flight path quickly after marking. If unable to move away, support workers were advised to lie face down on the ground. If contaminated by spray, they were advised to cease marking activities, wash themselves and change into clean clothes before resuming work. However no such incidents were reported.

Cleaning down operations following aerial application were estimated to be one hour, with potential exposure to endosulfan being, splashes from spills and wet surfaces, contact with contaminated surfaces of mixing/loading and spray equipment, and contamination from residues and rinsings from drums. Workers were required to use the recommended PPE before touching any contaminated surface. Other specific label instructions were observed during the study. The parameters of the above studies are presented in Table 4.

Table 4: Parameters of studies conducted for measuring exposure to endosulfan in broadacre cropping industries using aerial application

Parameters	Mixing/loading bulk and mini bulk (closed base) (Study A-1-1)	Mixing/Loading small containers (open/remote) (Study A-1-2)	Aerial applicators (Study A-1-3)	Support workers (vehicles) (Study A-1-4)	Support workers (ATVs) (Study A-1-5)	Cleaning down (Study A-1-6)
Number of subjects /replicates	9/13	9/13	10/16	11/14	6/7	10/11
Duration of study (days)/No. of sites/No. of sessions	7/6/11	8/6/9	7/9/15	7/8/13	5/5/6	8/7/8
No. of airbases/airstrips	3	3	3/3	7	4	6
Area sprayed (ha)[1]	NA[2]	NA[2]	37.66-459.76	38.5-496.20	38.50-393.93	47.77-1150
Time taken for procedure (minutes)[1]	40-255	20-220	25-220	65-370	45-385	10-50
Spray volume (L) [4]	1461-13792	1500-19261	1155-13792	1155-15759	1155-15759	1911-34500[3]
Total ai handled per session (kg)[1]	27.69-337.90	32.17-353.87	27.69-337.90	28.28-364.68	28.28-289.55	35.11-845.25[3]
Tasks/procures	Transport of pesticides, transferring chemicals to and from store room/storage area, mixing chemicals (dilution) to pilot's instructions, loading of chemical into aircraft, removal of empty containers from the working area, cleaning up significant spills, re-fuelling the aircraft, cleaning aircraft lights and windscreen, recording details of chemical prepared and loaded		General instructions regarding mixing/ loading/spraying and flagger procedures. Pre-flight inspection of aircraft and spray equipment, supervision of loading/refilling, carrying out the spraying. Checking nozzles/micronairs/filters/flow rates, checking wind speed, drift etc, cleaning and adjusting nozzles, cleaning boom filter, supervising changes to spray configuration, maintenance of flight/application and maintenance records	Indicate the paddock to be sprayed by waving a 1 m^2 white, yellow or red flag, or activating a flashing light		Decontamination and cleaning of mixing/filling systems, rinsing and disposal of containers, crushing and removal of drums, general clean up of aircraft and equipment, wash down mixing and loading area

Parameters	**Mixing/loading bulk and mini bulk (closed base)** **(Study A-1-1)**	**Mixing/Loading small containers** **(open/remote)** **(Study A-1-2)**	**Aerial applicators** **(Study A-1-3)**	**Support workers (vehicles)** **(Study A-1-4)**	**Support workers (ATVs)** **(Study A-1-5)**	**Cleaning down** **(Study A-1-6)**
PPE	Cotton overalls done up to the neck and wrists, full length waterproof bib apron, elbow-length gauntlet gloves cuff folded outwards, washable cotton hat, full face-shield/or goggles, and half-face piece respirator, water-resistant footwear/ boots, worn beneath the overalls, and hearing protection for work conducted around 'working aircraft'.		Cotton overalls, flying helmet, flying glasses during the day, nitrile gloves for adjusting CP (pressure control) nozzles, fire protective or waterproof boots, and hearing protection (optional)	White full length cotton overalls buttoned to the neck and wrist, mask/respirator, goggles, washable broad-brimmed hat, PVC gloves, and water resistant boots		Cotton overalls done up to the neck and wrists, full length waterproof bib apron, elbow-length gloves, cuff folded outwards, full face-shield/or goggles, and half-face piece respirator, water-resistant footwear/boots, worn beneath the overalls, and hearing protection for work conducted around 'working aircraft'.

(1)Per session; (2)not applicable

(3)Amount of endosulfan and total volume sprayed before the cleaning operation. The amount of ai. handled during cleaning operations is not known.

(4) Data on acreage sprayed indicates low volume spraying 30-50 L/ha)

3.1.4 Worker exposure by re-entry in broadacre cropping industries

The following five studies were provided to estimate exposure to endosulfan for workers when re-entering treated areas or to measure residues following endosulfan applications:

RC-1-1: Cotton chipping

RC-1-2: Crop checking

RC-1-3: Irrigating

RC-1-4: Siphon residue

RC-1-5: Foliar residue

Re-entry studies involved in cotton chipping, crop checking and foliar residue estimation from areas treated with endosulfan. Studies were conducted to define levels of exposure and to set a safe re-entry interval(s) for workers entering treated cotton fields.

For the study on cotton chipping, 10 dosimeter-patched and gloved workers wearing full PPE (refer Table 5) were allowed to enter the field 48 hours after spraying endosulfan,(interim re-entry interval on label) for 2 hours work. The study was set up to investigate re-entry following both ground rig and aerial application of endosulfan to crops of varying heights 26 cm (short) and 82 cm (high).

Similarly, for the crop checking study, 10 dosimetry-patched workers were allowed to enter the field 48 hours after endosulfan application, for their normal work, which included checking the crops for pests, counting flowers, bolls, number of nodes and measuring plant height (per linear metre of crop). The crop checkers spent 30 minutes in the field. These activities were repeated at random within the sprayed block.

For the irrigating study, 10 dosimetry-patched workers were allowed to enter the field immediately after endosulfan application, to simulate the starting of 10 siphons (pumping each 5 times and then laying each back on the head ditch). Crop irrigators were monitored for 10 minutes during these activities. The points/areas of potential contamination during re-entry activities were identified as contact with contaminated leaves, plants and soil while moving around sprayed sites. Irrigators are also expected to be exposed from siphon contamination.

In a Dislodgeable Foliar Residue (DFR) study, endosulfan residue deposition and the dissipation pattern in foliar samples was measured. Sixty 22 mm leaf discs (total surface area was 228.17 cm^2) were cut from leaves sampled at random from the first fully expanded leaf on primary and secondary plant terminals. The leaf discs were then placed in 350 mL jars, sealed and sent for analysis. This procedure was repeated for each of three blocks at the selected site. The parameters used in the above studies are described in Table 5.

Table 5: Parameters of studies conducted for measuring exposure during re-entry/re-handling broad acre crops treated with endosulfan

Parameters	Cotton Chipping (Study RC-1-1)			Crop checking (Study RC-1-2)			Irrigating (Study RC-1-3)		Siphon residue (Study RC-1-4)		Foliar residue (DFR) (Study RC-1-5)	
	RC-1-1A	RC-1-1B	RC-1-1C	RC-1-2A	RC-1-2B	RC-1-2C	RC-1-3A	RC-1-3B	RC-1-4A	RC-1-4B	RC-1-5A	RC-1-5B
Date of endosulfan application	15/01/00	9/12/00	9/12/00	15/01/00	9/12/00	9/12/00	12/12/00	7/03/01	12/12/00	7/03/01	15/01/00	9/12/00
Application method	Ground rig	Aerial	Aerial	Ground rig	Aerial	Aerial	Aerial	Aerial	Aerial	Aerial	Ground rig	Aerial
Crop height	82 cm	26 cm	26 cm	82 cm	26 cm	26 cm	26 cm	NA	26 cm	NA	82 cm	26 cm
Application rate (kg ai/ha)	0.735	0.735	0.735	0.735	0.735	0.735	0.735	0.735	0.735	0.735	0.735	0.735
Post application re-entry days	2, 3, 4, 5, 7, 13	2, 3, 4	2, 3, 4	2, 3, 4, 5, 7, 13	2, 3, 4	2,3,4	-1, 0, 1	-1, 0, 1, 2	-1, 0, 1, 2, 3, 4, 5, 7, 13	-1, 0, 1, 2, 3, 4, 5	-1[(1)], 0, 1, 2, 3, 4, 5, 7, 13	-1[(1)], 0, 1, 2, 3, 4, 5
Tasks involved	Hand weeding, or weeding using a hoe			Checking crops for pests, counting flowers, bolls, number of nodes and measuring plant height			Picking up siphon, pumping siphon and laying back on head ditch		NA			
PPE	Full length light cotton trousers, long-sleeved light cotton shirt, washable cotton hat, cotton gloves and comfortable boots						Shorts, short sleeved shirt and work boots		NA		NA	

[(1)] refers to the day before endosulfan application

NA = Not applicable

3.1.5 Worker exposure by re-entry in melon, peach and grape crops

Dissipation of foliar dislodgeable residues of endosulfan following application of Phaser EC and Phaser WP to Melons, Peaches and Grapes, USA, 1995, AgrEvo USA Company, AgrEvo Research Center, Residue Chemistry Department, Pikeville, NC 27863.

Introduction

The foliar residue dissipation study in melons, peaches and grapes was conducted according to the Good Laboratory Practices Standard Guidelines. The field phase of the foliar dislodgeable residue study was conducted in-house while the analytical phase was conducted by a contract laboratory. There were protocol and standard operating procedure deviations recorded that were general modifications in techniques to fit the needs of the study. According to information provided by the study author, none of the amendments or deviations had a negative impact on the study. Environmental data was collected on-site using an automated weather station.

Study details & analysis

Field phase

The study was designed as an unreplicated large plot, single site field trial with replicated sampling. Each crop was planted in separate plots with a treated plot and an untreated control plot. Endosulfan formulated as the end use products (Phaser) was applied twice at one week intervals on melons and once on peaches at application rates of 1.0, 1.5 and 3.0 lb ai/A (metric conversion; kg/ha = lb/acre, divide by 0.89), ie. 1.12, 1.68 and 3.37 kg ai/ha respectively. Samples were collected as 5 cm^2 leaf punches representing 400 cm^2 of leaf surface area. Samples were collected into glass jars and placed in ice. Endosulfan residues were washed from the leaf punches on the same day as the sample collection. Two of the washing solutions from the untreated control punches were fortified with the equivalent of 0.01, 0.50 or 1.5 $\mu g/cm^2$ endosulfan.

Analytical phase

Field samples sent to the laboratory included leaf punches generated at the field site. The samples included treated and untreated punches. Additional internal laboratory fortifications of dislodging solution from untreated leaf punches and fresh dislodging solution as quality control samples (QC) were prepared. These QC samples were included with each batch of samples analysed.

The analysis method was validated using 7 samples of solutions fortified at LOQ (limit of quantitation) of 0.01 $\mu g/cm^2$ and 100 x the LOQ. The overall recovery for the validation samples ranged between 84% and 95% ($\pm$8-9%) for alpha- and beta-endosulfan and endosulfan sulfate respectively. The leaf punches were washed three times with 50 mL of 0.012% aerosol OT. The analyte was extracted from the pooled wash solution using hexane. Samples were stored under refrigeration at about 4°C until quantified.

The dissipation of foliar dislodgeable residues of endosulfan was initially analysed by linear regression of natural log transformed data. When two applications were made, only samples from the second application and sampling were analysed. A summary of measured dislodgeable foliar endosulfan residues is presented in Table 6.

Table 6: Dislodgeable endosulfan residues from the leaves of melons, peaches and grapes

Days after application	Dislodgeable foliar residues (μg/cm^2)		
	Melons	**Peaches**	**Grapes**
	Application rate		
	1.12 kg ai/ha	3.36 kg ai/ha	1.68 kg ai/ha
0	1.23	0.46	0.71
1	0.54	0.16	0.31
3	0.15	0.09	0.11
5	0.09	0.07	0.09
7	0.06	0.04	0.03
10	0.05	0.03	0.02
14	0.05	0.03	0.04
17	0.03	0.03	0.05
21	0.02	0.05	0.02
24	0.02	0.02	0.04
28	0.02	0.01	<0.01

3.1.6 End use exposure (tree crops, nursery and broadacre crops)

Dermal exposure values in workers were estimated for the various crops/situations based on the geometric mean of the total endosulfan handled per day and standardised to normal working conditions (average crop sizes and work rates) and a body weight of 70 kg. The dermal exposure for workers conducting ground application to broad acre crops was not included in the worker exposure studies as the margin of exposure was found to be acceptable based on PHED and the use of a 29% dermal absorption factor (Interim Report, 1998).

The dermal absorption rate has been revised following a reassessment of supplementary dermal data. The revised dermal absorption rates were 0.8% for mixer/loader exposure and 2.2% for applicators and re-entry workers. The amended worker exposure data are presented in Tables 8-11 of this report. The PHED exposure data have also been amended, and are presented in Table 7.

To estimate exposure for workers using ground application for broadacre crops the Pesticide Handlers Exposure Database (PHED) Surrogate Exposure Guide (1998) was used. The following scenarios were assessed:

PHED surrogate scenario 3: All liquids, open mixing and loading

PHED surrogate scenario 13: Ground boom application, open cab

PHED surrogate scenario 28: All liquids, open pour, ground boom, open cab

Table 7: Absorbed endosulfan doses for workers mixing/loading & applying product to broad acre crops using ground boom open cab (PHED)

Scenarios	Absorbed doses following exposure to endosulfan* (mg/kg bw/day)

		Dermal		Inhalation		Total
	Gloves	*Mixer/Loader M/L*	*Applicator A*	*Mixer/Loader M/L*	*Applicator A*	M/L M/L/A
Scenario 3- all liquids open mixing and loading	N	0.0264	-	0.0014	-	0.0291
	Y	0.0002	-	0.0014	-	0.0028
Scenario 13: Ground boom application, open cab	N	-	0.0002	-	0.0009	0.0011
	Y	-	0.0002	-	0.0009	0.0011
Scenario 28- liquid/open pour/ground boom/open cab	N	0.0094		0.0015		0.011
	Y	0.0014		0.0015		0.0029

*Based on an application rate of 2.1 L/ha, handling 36.75 kg ai/day, 70 kg bw person, dermal absorption factor (0.8% M/L; 2.2% A) and 100% inhalation absorption.

Table 8: Absorbed dermal endosulfan dose for workers engaged in mixing/loading/applying & cleaning equipment following application to trees and crops

Studies	Mean Exposure [1] (mg/kg bw/kg ai)	Mean Exposure [2] (study rates) (mg ai /kg bw/day)	Mean Exposure [3] (standardised to amount of ai handled/day (mg ai/kg bw/ day)	Absorbed dermal dose [4] (mg ai/kg bw/day)			
				M/L	A	C	M/L/A/C
Mixing/Loading (H-1-1)	0.0005	0.0076	0.0200	0.00016			
Air-assist spray-no cabin (H-1-2-U)	0.0048	0.0730	0.1920		0.0042		0.0048
Air-assist-with cabin (H-1-2-C)	0.0014	0.0213	0.0560		0.0012		0.0019
Air-shear-with cabin (H-2-2-C)	0.0005	0.0076	0.0200		0.00043		0.0010
Oscillating boom spray (H-5-2-C)	0.0013	0.0198	0.0520		0.00158		0.0073
Cleaning down (H-1-4)	0.0005	0.0076	0.0200			0.00043	

[1] Geometric mean of exposures standardised for 70 kg body weight;
[2] Mean exposure based on 15.2 kg ai handled/day (study rates)
[3] Mean exposure based on 40 kg ai handled/day (190 mL/100 L; spray volume 2000 L/ha, work rate 30 ha/day, (standardised work rates);
[4] Mean dermal absorbed dose (mg ai/kg bw/day)= mean dermal exposure x dermal absorption factor (0.8% M/L; 2.2% A & C)
M/L=mixing/loading; A=application; C=cleaning down; M/L/A/C=mixing/loading/application/cleaning down

Table 9: Absorbed dermal endosulfan dose for workers engaged in mixing/loading/applying & cleaning equipment following application to nursery crops

Studies	Mean Exposure [1] (mg/kg bw/kg ai)	Mean Exposure [2] (study rates) (mg ai/kg bw/day)	Mean Exposure [3] (standardised to amount of ai handled /day (mg ai/kg bw/day)	Absorbed dermal dose [4] (mg ai/kg bw/day)			
				M/L	A	C	M/L/A/C
Mixing/Loading (H-3-1)	0.0043	0.0022	0.0022	0.000016	-	-	-
Application (H-3-2)	0.0082	0.0041	0.0041		0.000087	-	-
Cleaning down (H-3-3)	0.0024	0.0012	0.0012		-	0.000029	-
							0.00013

[1] Geometric mean of exposures, standardised for 70 kg body weight
[2] Mean exposure based on 0.5 kg ai handled/day and 2 hours spraying/day (study rates)
[3] Mean exposure based on 0.5 kg ai handled/day with 2 hours spraying/day (no standardisation required, current work rates)
[4] Mean dermal absorbed dose (mg ai/kg bw/day) = mean dermal exposure x dermal absorption factor (0.8% M/L; 2.2% A & C)
M/L=mixing/loading; A=application, hand-held); C=cleaning down; M/L/A/C=mixing/loading/application/cleaning down

Table 10: Absorbed dermal endosulfan dose for workers engaged in mixing/loading/applying & cleaning equipment following aerial application to broadacre crops

Studies	Mean Exposure [1] (mg/kg bw/kg ai)	Mean Exposure [2] (study rates) (mg ai/kg bw/day)	Mean Exposure [3] (standardised to amount of ai handled /day (mg ai/kg bw/day)	Mean dermal absorbed dose [4] (mg ai/kg bw/day)			
				M/L	A	C	S
Mixing/Loading Bulk and Mini bulk (closed base) (A-1-1)	0.00012	0.097	0.176	0.0014			
Mixing/Loading small containers (open/remote) (A-1-2)	0.00011	0.089	0.162	0.00128			
Aerial applicators (A-1-3)	0.00003	0.024	0.044		0.001		
Support workers (vehicles) (A-1-4)	0.00001	0.008	0.015				0.00029
Support workers (ATVs) (A-1-5)	0.00005	0.041	0.074				0.0016
Cleaning down (A-1-6)	0.00002	0.016	0.029			0.00058	

[1] Geometric mean of exposures, standardised for 70 kg body weight
(2) Based on 811 kg ai handled/day (study rates),
(3) Based on 1470 kg ai handled/day, application rate of 2.1 L/ha; work rate 2000 ha/day (standardised work rates)
(4) Mean dermal absorbed dose (mg ai/kg bw/day) = mean dermal exposure x dermal absorption factor (0.8% M/L; 2.2% A & C)
M/L=mixing/loading; A=application; C=cleaning down; S= support workers

3.1.7 Worker exposure to re-entry/rehandling activities (ground and aerial application)

According to information provided in Study No. RC 1-2, crop checkers usually spend 1/3 of the working day (assumed to be 8 hours) in the field checking crops for pests, and the remaining time in other activities such as data entry, traveling etc in their work schedule. Cotton chippers usually perform 8 hours work/day (Study RC 1-1) in the field. Therefore, exposure for crop checkers was estimated based on a 3-hour/day work period, and cotton chippers based on a 8 hour/day work period. The study authors indicated that irrigation workers spend 8 hours at work but that not all of this time is spent in the field (no time estimate was provided). Therefore exposure for crop irrigators was estimated based on a 2-hour/day work period. To determine a safe re-entry interval(s) for workers entering treated fields for various activities, the following data were used:

- measured (mean) dermal exposure dosimetry data provided in the ground rig and aerial studies, and
- exposure calculated from DFR data (from foliar sampling).

The mean measured dermal exposure values for workers (wearing PPE) conducting crop checking, cotton chipping, and crop irrigation at different time intervals (following ground and aerial application of endosulfan) are presented in Table 11.

Table 11: Mean dermal exposure values for workers conducting crop checking, cotton chipping, and crop irrigation at different time intervals following ground and aerial application of endosulfan

Re-entry (day)	Mean measured dermal exposure (mg/kg bw/day)[(1)] (dosimeter data) (with PPE)							
	Cotton chipping			Crop checking			Irrigating	
	Ground application (RC-1-1A)	Aerial application (RC-1-1B)	Aerial application (RC-1-1C)	Ground application (RC-1-2A)	Aerial application (RC-1-2B)	Aerial application (RC-1-2C)	Aerial application (RC-1-3A)	Aerial application (RC-1-3B)
0	ND	ND	ND	ND	ND	ND	0.0103	0.0175
1	ND	ND	ND	ND	ND	ND	0.0050	0.0128
2	0.0075	0.0013	0.0008	0.0038	0.0007	0.0014	ND	0.0069
3	0.0016	0.0007	0.0007	0.0016	0.0007	0.0008	ND	ND
4	0.0014	0.0004	0.0004	0.0012	0.0004	0.0006	ND	ND
5	0.0005	ND	ND	0.0007	ND	ND	ND	ND
7	0.0006	ND	ND	0.0006	ND	ND	ND	ND
13	0.0002	ND	ND	0.0003	ND	ND	ND	ND

[(1)] geometric mean measured (dosimeters) dermal exposure (mg/kg bw/day) based on 3 hours of crop checking and 8 hours of cotton chipping and 2 hours crop irrigation. These values are based on the author's raw exposure data (µg/cm^2, uncorrected for field blanks) and 70 kg bw per person
ND: not determined

Table 12: Transfer coefficients calculated from the dislodgeable foliar residues and dermal exposure data for workers (wearing PPE) following ground and aerial application of endosulfan

Application method /crop height	Sampling days	DFR [1] µg/cm^2	Study dermal exposure estimates (mg/kg bw/day) (with PPE)			Transfer coefficient (cm^2/hr)[2] (calculated)		
			Cotton chipping	Crop checking	Irrig.	Crop checking	Cotton chipping	Irrig.
Ground rig (82 cm crop)		*RC 1-5A*	*RC 1-1A*	*RC 1-2A*				
	-1[3]	0.0011	ND	ND	ND	ND	ND	ND
	0	2.826	ND	ND	ND	ND	ND	ND
	1	4.927	ND	ND	ND	ND	ND	ND
	2	2.526	0.0075	0.0038	ND	26	13	ND
	3	0.444	0.0016	0.0016	ND	32	32	ND
	4	0.480	0.0014	0.0012	ND	26	22	ND
	5	0.278	0.0005	0.0007	ND	16	22	ND
	7	0.332	0.0006	0.0006	ND	16	16	ND
	13	0.150	0.0002	0.0003	ND	12	18	ND
						Average 21	***Average 20***	
Aerial (26 cm crop)		*RC 1-5B*	*RC 1-1B*	*RC 1-2B*	*RC 1-3A*			
	-1	0.0019	ND	ND	ND	ND	ND	ND
	0	3.003	ND	ND	0.0103	ND	ND	30
	1	3.407	ND	ND	0.0050	ND	ND	13
	2	0.929	0.0013	0.0007	ND	12	7	ND
	3	0.582	0.0007	0.0007	ND	11	11	ND
	4	0.381	0.0004	0.0004	ND	9	9	ND
	5	0.263	ND	ND	ND	ND	ND	ND
		RC 1-5B	*RC 1-1C*	*RC 1-2C*	*RC 1-3B*			
	-1	0.0019	ND	ND	ND	ND	ND	ND
	0	3.003	ND	ND	0.0175	ND	ND	51
	1	3.407	ND	ND	0.0128	ND	ND	33
	2	0.929	0.0008	0.0014	0.0069	8	13	65
	3	0.582	0.0007	0.0008	ND	11	12	ND
	4	0.381	0.0004	0.0006	ND	9	14	ND
	5	0.263	ND	ND	ND	ND	ND	ND
						Average 10	***Average 11***	***38***

(1) measured DFR values for endosulfan from 2 study sites and 2 crop heights provided in the submitted studies, with sampling starting from the day before endosulfan was sprayed until day 13 for RC 1-5A, and day 5 for RC 1-5B; [2] Transfer coefficient (cm^2/hr) calculated using measured dermal exposure values for cotton chipping and crop checking and measured DFR following aerial application of endosulfan, TC (cm^2/hr) = dermal exposure (mg/day) ÷ time spent for activity (hrs/day) x DFR (µg/cm^2); [3] refers to the day before endosulfan was sprayed; ND no data

(2) Exposure for workers (with PPE) re-entering treated areas was estimated from DFR data. The transfer coefficients (TC) for crop checking and cotton chipping were determined from the DFR using the measured dermal exposure values for these activities (refer to equation in Table 12 footnote). Results are outlined in Table 12. From Table 12 it is noted that DFR varied on the different days with values higher on day 1 when compared to day 0, and days 4 and 7 having higher residues when compared to days 3 and 5. According to the study author, this variation in residues may have been due to incomplete settling of residue following endosulfan application.

Table 12 shows that TC determined from dermal exposure estimates and DFR data (both provided in the study) were low; i.e. TCs 21, & 20 for crop checking & cotton chipping (ground rig application), and TCs 10 and 11 for crop checking and cotton chipping (aerial application). TC for irrigation following aerial application was 38. No data were provided for irrigation following ground rig application. These TCs were determined from workers using PPE (i.e., from dosimeters placed underneath gloves and protective clothing. To determine actual TC (i.e. amount transferred to a workers' skin), the data were recalculated assuming 90% protection is provided to workers using PPE. The results are presented in Table 13.

Table 13: Transfer coefficients calculated from the dislodgeable foliar residues and dermal exposure data for workers not wearing PPE following ground and aerial application of endosulfan.

Application method /crop height	Sampling days	DFR [1] (µg/cm^2)	Study dermal exposure estimates [2] (mg/kg bw/day) (without PPE)			Transfer coefficient [3] (cm^2/hr) (calculated)		
			Cotton chipping	Crop checking	Irrig.	Crop checking	Cotton chipping	Irrig.
Ground rig (82 cm crop)		*RC 1-5A*	*RC 1-1A*	*RC 1-2A*				
	-1[4]	0.0011	ND	ND	ND	ND	ND	ND
	0	2.826	ND	ND	ND	ND	ND	ND
	1	4.927	ND	ND	ND	ND	ND	ND
	2	2.526	0.075	0.038	ND	260	132	ND
	3	0.444	0.016	0.016	ND	315	315	ND
	4	0.480	0.014	0.012	ND	255	219	ND
	5	0.278	0.005	0.007	ND	157	220	ND
	7	0.332	0.006	0.006	ND	158	158	ND
	13	0.150	0.002	0.003	ND	117	175	ND
					Average	***210***	***203***	
Aerial (26 cm crop)		*RC 1-5B*	*RC 1-1B*	*RC 1-2B*	*RC 1-3A*			
	-1	0.0019	ND	ND	ND	ND	ND	ND
	0	3.003	ND	ND	0.103	ND	ND	30
	1	3.407	ND	ND	0.050	ND	ND	13
	2	0.929	0.013	0.007	ND	122	66	ND
	3	0.582	0.007	0.007	ND	105	105	ND
	4	0.381	0.004	0.004	ND	92	92	ND
	5	0.263	ND	ND	ND	ND	ND	ND
		RC 1-5B	*RC 1-1C*	*RC 1-2C*	*RC 1-3B*			
	-1	0.0019	ND	ND	ND	ND	ND	ND

	0	3.003	ND	ND	0.175	ND	ND	510
	1	3.407	ND	ND	0.128	ND	ND	33
	2	0.929	0.008	0.014	0.069	75	132	65
	3	0.582	0.007	0.008	ND	105	120	ND
	4	0.381	0.004	0.006	ND	92	138	ND
	5	0.263	ND	ND	ND	ND	ND	ND
					Average	***99***	***109***	***380***

(1) measured DFR values for endosulfan from 2 study sites and 2 crop heights provided in the submitted studies, with sampling starting from the day before endosulfan was sprayed until day 13 for RC 1-5A, and day 5 for RC 1-5B

(2) dermal exposure (without PPE) = dermal exp (with PPE) x 100%/10%

(3) Transfer coefficient (cm^2/hr) calculated using measured dermal exposure values for cotton chipping and crop checking and measured DFR following aerial application of endosulfan, TC (cm^2/hr) = dermal exposure (without PPE (mg/day) ÷ time spent for activity (hrs/day) x DFR (µg/cm^2)

(4) refers to the day before endosulfan was sprayed

ND no data

Dermal doses (on different re-entry days) were estimated using the mean DFR (µg/cm^2), and the average TCs estimated for workers using PPE with work rates of 3 hrs/day for crop checking, 8 hrs/day for cotton chipping and 2 hours/day for irrigation, and a 1.5% dermal absorption rate. For comparison, generic transfer coefficients available in the US EPA Re-entry risk calculator were also used to estimate dermal doses. These values are presented in Table 15 together with dermal dosimetry data.

Dermal doses for crops other than cotton were estimated using the mean DFR (µg/cm^2) provided in the re-entry study for melons, peaches and grapes and specific TCs for these crops provided in the US EPA Re-entry risk calculator. These are presented in Table 14:

Table 14: Summary of total dislodgeable endosulfan from melons, peaches and grapes crops and dermal absorbed dose calculated using generic TC values

Crop	Appl. Rate used in the study* (kg ai/ha)	Label Appl. Rate (L/ha)	Days after applic.	DFR (μg/cm^2)			Dermal absorbed dose (mg ai/kg bw/day)**		
				Melons	Peaches	Grapes	Melons	Peaches	Grapes
Melons	1.12	2.1	0	1.23	0.46	0.71	0.0049	0.00058	0.0039
			1	0.54	0.16	0.31	0.0022	0.00029	0.0017
			3	0.15	0.09	0.11	0.00058	0.00014	0.00058
			5	0.09	0.07	0.09	0.00043	0.00014	0.00043
			7	0.06	0.04	0.03	0.00029	0.000043	0.00014
			10	0.05	0.03	0.02	0.00014	0.000043	0.00014
Peaches	3.36	2.1	14	0.05	0.03	0.04	0.00014	0.000043	0.00029
			17	0.03	0.03	0.05	0.00014	0.000043	0.00029
			21	0.02	0.05	0.02	0.00014	0.000072	0.00014
			24	0.02	0.02	0.04	0.00014	0.000029	0.00029
			28	0.02	0.01	<0.01	0.00014	0.000014	<0.000043
Grapes	1.68	2.1							

LOQ Limit of quantitation = 0.01 μg/cm^2

*Appl. rates used in the DFR study in melons, peaches and grapes (Singer, 1995), (see Section 2.1.5 for details)

**Dermal absorbed dose = DFR (study) ÷1000 (μg/mg) x application rate (crop) ÷ application rate (study) x TC (crop) x 8 hr working day ÷ 70 kg (bw) x dermal absorption factor (2.2%)
(TC melons 2500, peaches 3000, grapes 5000)

Table 15: Standardised dermal absorbed doses for workers (without PPE) conducting re-entry activities (crop checking, cotton chipping and irrigating) determined from foliar residue data (using calculated and generic transfer coefficients and dosimetry data

Re-entry day	Dermally absorbed dose (mg ai/kg bw/day)[1] (without PPE)											
	Cotton chipping				Crop checking				Irrigating			
	Calculated [2]			Measured exposure [3]	Calculated [2]			Measured exposure [3]	Calculated [2]			Measured exposure [3]
	Study TC (average) (203)	Generic TC - low exposure (100)	Generic TC – medium exposure (1500)		Study TC (average) (210)	Generic TC - low exposure (100)	Generic TC – medium exposure (1500)		Study TC (average) ND	Generic TC - low exposure (100)	Generic TC – medium exposure (1500)	
Ground rig (82 cm crop)												
0	0.0013	0.00072	0.0106	ND	0.00058	0.00029	0.0039	ND	ND	ND	ND	ND
1	0.0025	0.00012	0.0184	ND	0.001	0.00043	0.00698	ND	ND	ND	ND	ND
2	0.0013	0.00058	0.0094	0.0016	0.00043	0.00029	0.0035	0.00087	ND	ND	ND	ND
3	0.0043	0.00014	0.0016	0.00029	0.00014	0.000043	0.00058	0.00029	ND	ND	ND	ND
4	0.00029	0.00014	0.0017	0.00029	0.00014	0.000043	0.00072	0.00029	ND	ND	ND	ND
5	0.00014	0.000058	0.001	0.00014	0.000072	0.000029	0.00043	0.00014	ND	ND	ND	ND
7	0.00014	0.00014	0.013	0.00014	0.000072	0.000029	0.00043	0.00014	ND	ND	ND	ND
13	0.00014	0.000043	0.00058	0.000043	0.000029	0.000029	0.00029	0.000072	ND	ND	ND	ND
Aerial (26 cm crop)	**(109)**				**(99)**				**(383)**			
0	0.00087	0.00072	0.0011	ND	0.00029	0.00029	0.0042	ND	0.00072	0.00014	0.0028	0.0022
1	0.00087	0.00087	0.0127	ND	0.00029	0.00029	0.0048	ND	0.00087	0.00029	0.0032	0.0012
2	0.00029	0.00029	0.0035	0.00029	0.00014	0.00014	0.0013	0.00014	0.00029	0.000072	0.00087	ND
3	0.00014	0.00014	0.0022	0.00014	0.000043	0.000043	0.00087	0.00014	0.00014	0.000043	0.00058	ND
4	0.00014	0.00014	0.0013	0.00014	0.000043	0.000043	0.00058	0.00014	0.00014	0.000029	0.00029	ND
5	0.000072	0.000072	0.001	ND	0.000029	0.000029	0.00043	ND	0.000072	0.000029	0.00029	ND

[1] dermal absorbed dose (mg ai/kg bw/day) = mean dermal exposure x dermal absorption (2.2%).

[2] dermal absorbed dose calculated using average study TC (calculated from measured dermal exposure data) or generic TC (100 for low exposure and 1500 for medium exposure, USEPA Re-entry calculator TC values for Transfer Coefficient Group: Field/row crop, low/medium) and measured DFR; dermal absorbed dose (mg/kg bw/day) = TC (cm^2/hr) x time spent for activity (hr/day) x DFR (µg/cm^2) ÷1000 (µg /mg)÷ 70 kg x 2.2% (dermal absorption).

[3] data derived from measured worker exposure (dosimeters) following ground and aerial applications; ND no data

4. OCCUPATIONAL RISK ASSESSMENT

In the absence of exposure data for the proposed mode of application, the OCSEH used the Pesticide Handler Exposure Database (PHED) Surrogate Exposure Guide (2002) to estimate exposure to endosulfan.

The OHS risk assessment used the margin of exposure (MOE) approach to quantify the risk to workers from dermal and inhalational exposure to endosulfan. Appropriate PPE for the purpose of protecting workers from any possible eye and skin irritancy effects of products was based on a consideration of the hazard only. Since the likely exposure duration of workers, ie. seasonal use, it was concluded that the most appropriate animal study on which to base the OHS risk assessment for dermal and inhalational exposure should have a duration of about three months. This duration of exposure in rats was considered to be suitable based on a comparison of the longevity of a rat relative to humans (ie. approximately 2 years compared with 70 years). As there was a suitable 3-month dietary animal study in the toxicological database and the likely routes of exposure for workers will be dermal and inhalational, it is necessary to take into account differences in the extent of absorption for the dermal and inhalation routes of exposure. For vapours and aerosols it is assumed that absorption across all regions of the respiratory tract is 100%. For percutaneous absorption the submitted supplementary studies indicated that in humans it was relatively low, ie. 0.8% for the concentrate and 2.2% for dilute sprays (see sections 2.3 and 2.4).

The principal toxicological effects observed in a 13-week dietary rat study were related to adverse changes in the kidneys. These kidney effects are considered to be relevant for a human occupational risk assessment and a NOEL for these effects was established at 1.92 mg/kg bw/day. Since the selected NOEL derived from repeat dose study in experimental animals, a margin of exposure (MOE) of approximately 100 or more is considered acceptable. The MOE takes into account both intra-species variability (10x) and inter-species extrapolation (10x).

4.1 NOELs used in international OHS risk assessments

A number of different studies have been used by overseas regulators to derive NOELs for occupational exposure assessment:

ERMA New Zealand chose a NOEL of 1.92 mg/kg bw/day from a 13-week dietary study in rats for occupational exposure of workers on a short-term and seasonal basis with a safety factor of 100. The OCSEH also uses a NOEL of 1.92 mg/kg bw/day was for the occupational exposure assessment.

The US EPA and Cal DPR both separate exposure by route, and use different NOAELs for dermal and inhalational exposure. For short-term and intermediate dermal and oral exposure, the US EPA (2007) uses a LOAEL of 3.7 mg/kg bw/day from a rat developmental neurotoxicity study. This study was used as it was protective for the most sensitive population (female workers). An extra safety factor of 3 was used to account for using a LOAEL instead of a NOEL. For short-term and seasonal inhalational exposure, a NOAEL of 0.2 mg/kg bw/day from a 21-day rat inhalational study was used.

For short-term dermal exposure, Cal DPR uses a NOEL of 0.7 mg/kg bw/d from a rabbit developmental study was used with a safety factor of 100. For seasonal dermal exposure, a NOEL of 1.18 mg/kg/day from a 2-generation reproduction study was used with a safety factor of 100. For both short-term and seasonal inhalational exposure, a NOEL of 0.194 mg/kg/day from a sub-chronic rat inhalational study was used with a safety factor of 100.

4.2 Margin of Exposure

The MOE calculated for the various crops/situations and application methods from dermal exposure values determined from the studies (Table 16). These values have been recalculated based on exposure data using dermal absorption factors of 0.8% (concentrates) and 2.2% (dilute sprays). MOE calculated for broad acre crops using ground application equipment were determined from PHED data. These are presented in Table 17.

Table 16: Margins of exposure (MOE) for workers mixing/loading (M/L) and applying (A) endosulfan to tree, nursery and broad acre crops by ground application and to broad acre crops by aerial equipment.

Studies	**MOE[1]**				
	M/L	**A**	**S[2]**	**C**	**M/L/A/S/C [3]**
Tree crops (40 kg ai/30 ha/day)					
Mixing/loading (H1-1)	12000	-		-	-
Spraying air-assist, no cabin (H-1-2-U)	-	457		-	400
	-	1600		-	1011
Spraying air assist, with cabin (H-1-2-C)	-	4465		-	1920
	-	1215		-	263
Spraying air-shear, with cabin (H-2-2-C)	-	-		4465	-
Oscillating boomspray (H-5-2-C)					
Cleaning down (H-1-4)					
Nursery crops (0.5 kg ai/2 hours/day)					
Mixing/loading (H-3-1)	120000	-	-	-	-
Spraying (H-3-2)	-	22069	-	-	14769
Cleaning down (H-3-3)	-	-	-	66207	-
Broad acre crops (Aerial application) (1470 kg ai/2000 ha/day)					
Mixing/loading, bulk and mini bulk, closed base (A1-1)	1333	-	-	- -	
Mixing/loading, small containers, open/remote (A1-2)	1500	-	-	-- -	- -
Aerial application (A1-3)		1920	-	-	-
Support workers, vehicles (A1-4)		-	6621		-
Support workers, ATVs (A1-5)		-	1200		-

Cleaning down (A1-6)			-	3310	NA[4]

M/L= Mixer/Loader; A=Applicator; S =Support worker; C=Cleaner; M/L/A/S/C; Mixer/Loader/Applicator/Support workers/Cleaner.
[1] MOE= NOEL (1.92 mg/kg bw/day) ÷ mean dermal absorbed dose (mg ai/kg bw/day).
[2] only aerial application has support workers.
[3] exposure to workers performing all tasks.
[4] not applicable as each activity is usually undertaken by different workers
ND not determined

Table 17: Margins of exposure (MOE) for workers mixing/loading and applying endosulfan to broad acre crops by ground application using PHED data

PHED Estimates	**MOE[1]**					
	Dermal			Inhalation		
	Gloves	*Mixer/Loader*	*Applicator*	*Mixer/Loader*	*Applicator*	*Total*
PHED Surrogate Scenarios 3 and 13: all liquid/open mixing/loading and ground boom application/open cab	N	73	5299	1382	2242	66
	Y	9023	5299	1382	2242	682
PHED Surrogate Scenario 28: All liquids, open pour, ground boom, open cab	N	203		1276		88
	Y	1330		1276		326

[1] based on a NOEL of 1.92 mg/kg bw/day

4.2.1 Ground application to tree crops

In the case of mixing and loading, tree crop workers handling 40 kg of endosulfan per day, the resultant MOE of 12000 (Table 16) (when head/face exposure was included) is above the minimum acceptable MOE of 100 when working without gloves. That is when the exposure is compared with the relevant NOEL of 1.92 mg/kg bw/day. In the case of application using air-assist with no cabin the resultant MOE is 457 and for application using air assist with cabin, the MOE is 1600. In the case of application using air shear with cabin the MOE is 4465, and for oscillating boom spray application, the MOE is 1600. In the case of cleaners, the MOE is above the minimum acceptable level (4465). They all are above the minimum acceptable level.

In the combined scenario of mixing/loading with application using air-assist with or without cabin, the MOE is above the minimum acceptable level, 1600 and 582, respectively. In the combined scenario of mixing and loading with air shear application, the MOE is 4465 (with cabin) and with oscillating boom spray equipment, the MOE is 1215.

When head/face exposure was excluded, the resultant MOEs for all scenarios (single and combined) are also above the minimum acceptable level.

4.2.2 Ground application to nursery crops

In the case of mixing and loading, nursery workers handling 0.5 kg of endosulfan per day, the resultant MOE 120000 (table 16) (when head/face exposure was included) is above the minimum acceptable MOE of 100 when working without gloves. That is when the exposure is compared with the relevant NOEL of 1.92 mg/kg bw/day. In the case of applicators and cleaners, the resultant MOE is 22069 and 66207, respectively.

In the combined scenario of mixing and loading with any of the activities, the resultant MOE of 14769 is above the minimum acceptable level.

Similar results were seen when head/face exposure was excluded.

4.2.3 Aerial application to broadacre crops

In the case of mixing and loading, broadacre workers handling 1470 kg of endosulfan per day, the resultant MOE 1333 for closed mixing (Table 16) and 1500 for open mixing (when head/face exposure was included) are above the minimum acceptable MOE of 100 when working without gloves. That is when the exposure is compared with the relevant NOEL of 1.92 mg/kg bw/day. In the case of aerial application, vehicle support workers, ATV support workers and cleaners, the resultant MOEs are 1920, 6621, 1200 and 3310, respectively. Similar results were seen when head/face exposure was excluded.

The combined scenarios of mixing and loading with any of the activities for broadacre crops (aerial) were not estimated as these activities are usually undertaken by different workers.

4.2.4 Ground application to broadacre crops (from PHED data)

In the case of mixing and loading, broadacre workers handling 36.75 kg of endosulfan per day without gloves, the resultant MOE, as indicated by PHED (Table 7) is 66 (Table 17), compared with the minimum acceptable MOE of 100. However, wearing gloves increases the MOE to 682, above the minimum acceptable level. In the case of applicators using open cab with or without wearing gloves, the resultant MOE 5299, is above the minimum acceptable level when treating broadacre crops using ground equipment.

In the combined scenario of mixing and loading with open cab application without gloves, the resultant MOE of 88, is below the minimum acceptable level of 100. Wearing gloves, however, increased the MOE to 326, which is above the minimum acceptable MOE of 100.

4.3 Re-entry risk assessment

MOE were determined for workers conducting various re-entry activities (cotton chipping, crop checking and irrigating) determined for workers from DFR (using calculated and generic transfer coefficients) and from measured worker exposure data (dosimeters). These MOE were based on the dose derived from actual TC (i.e. amount transferred to workers' skin, on the assumption that 90% protection is provided to workers using PPE). These are presented in Table 18.

Table 18: Margins of exposure (MOE) for re-entry activities (cotton chipping, crop checking and irrigating) determined for workers from DFR (using calculated and generic transfer coefficients) and from measured worker exposure data (dosimeters).

Re-entry day	MOE[1]											
	Cotton chipping				Crop checking				Irrigating			
	Calculated			Measured Exposure[2]	Calculated			Measured exposure[2]	Calculated			Measured exposure[2]
	Study TC (average) (203)	Generic TC – low exposure (100)	Generic TC – medium exposure (1500)		Study TC (average) (210)	Generic TC - low exposure (100)	Generic TC – medium exposure (1500)		Study TC (average) ND	Generic TC - low exposure (100)	Generic TC – medium exposure (1500)	
Ground rig (82 cm crop)												
0	1477	2667	818	ND	3310	6621	492	ND	ND	ND	ND	ND
1	768	1600	104	ND	1920	4465	278	ND	ND	ND	ND	ND
2	1477	3310	204	1200	4465	6621	549	2207	ND	ND	ND	ND
3	477	13714	1200	6621	13714	44651	3310	6621	ND	ND	ND	ND
4	6621	13714	1129	6621	13714	44651	2667	6621	ND	ND	ND	ND
5	13714	33103	1920	13714	26667	66207	4465	13714	ND	ND	ND	ND
7	13714	13714	1477	13714	26667	66207	4465	13714	ND	ND	ND	ND
13	13714	44651	3310	44651	66207	66207	6621	26667	ND	ND	ND	ND
Aerial (26 cm crop)	**(109)**				**(99)**				**(383)**			
0	2207	2667	175	ND	6621	6621	457	ND	2667	13714	686	873
1	2207	2207	151	ND	6621	6621	400	ND	2207	6621	600	1600
2	6621	6621	549	6621	13714	13714	1477	13714	6621	26667	2207	ND
3	13714	13714	873	13714	44651	44651	2207	13714	13714	44651	3310	ND
4	13714	13714	1477	13714	44651	44651	3310	13714	13714	66207	6621	ND
5	26667	26667	1920	ND	66207	66207	4465	ND	26667	66207	6621	ND

[1] MOE = NOEL (mg/kg bw/day) ÷ mean dermal absorbed dose (mg ai/kg bw/day)
[2] data from single study for ground application and two studies for aerial application

ND no data

The DFR values obtained in the re-entry study for melons, peaches and grapes were extrapolated to other crops (citrus, pecans, fruit and nut trees, vegetables, nursery crops, and broadacre crops – Table 19) by considering relative application rates and specific transfer coefficients for the crops identified in the US Occupational Post-Application Risk Assessment Calculator (US EPA Policy 003.1).

The dermal absoption dose was calculated from the DFR data using the following formula: Dermal absorbed dose = DFR (study) ÷ 1000 (μg/mg) x application rate (crop) ÷application rate (study) x TC (crop) x 8 hr working day ÷ 70 kg bw x dermal absorption factor (2.2%). The MOE were then determined using the dermal absorbed dose and NOEL of 1.92 mg/kg bw/day.

Table 19: MOE for various crops extrapolated from the re-entry DFR data on melons, peaches and grapes, standardised to relevant application rates and TC for the crops.

Re-entry day	MOE (Dermal absorbed dose/NOEL)							
	Melons	**Peaches**	**Grapes**	**Citrus**	**Nut trees (Pecans)**	**Fruit**	**Vegetables, Nursery crops**	**Broadacre Crops (other than cotton)**
	*TC:2500 (high exposure)***	*TC:3000 (high exposure)***	*TC 5000 (high exposure)****	*TC: 3000 (high exposure)***	*TC: 2500 (high exposure)***	*TC: 3000 (high exposure)***	*TC: 2500 (high exposure)***	*TC: 1500 (medium exposure)**
	Application rate: 2.1 L/ha	*Application rate: 2.1 L/ha*	*Application rate: 2.1 L/ha*	*Application rate: 2.8 L/ha*	*Application rate: 3.0 L/ha*	*Application rate: 3.0 L/ha*	*Application rate: 2.1 L/ha*	*Application rate: 2.1 L/ha*
0	392	3310	4492	343	392	325	565	914
1	873	6621	1129	800	873	914	1280	2133
3	3310	13714	3310	2743	3200	2743	4800	6400
5	4465	13714	4465	4800	4800	4800	6400	9600
7	6621	44651	13714	6400	9600	6400	9600	19200
10	13714	44651	13714	9600	9600	9600	19200	19200
14	13714	44651	6621	9600	9600	9600	19200	19200
17	13714	44651	6621	19200	19200	19200	19200	19200
21	13714	26667	13714	21333	19200	19200	19200	64000
24	13714	66207	6621	21333	19200	19200	19200	64000
28	13714	137143	<44651	21333	19200	19200	19200	64000

* irrigation, scouting, weeding mature plants
**harvesting, pruning, training, tying
***hand harvesting resulting in the greatest re-entry exposure

4.3.1 Risks to re-entry workers (cotton crop)

MOE for re-entry activities (cotton chipping, crop checking and irrigating) were recalculated for workers from DFR (using calculated and generic transfer coefficients) and from measured worker exposure data (dosimeters) (Table 18). No measured exposure data were provided for workers re-entering treated areas on day 0 and day 1 as the study authors observed the re-entry interval of 48 hours stipulated on the label. Measured data were only provided from day 2 onwards. Based on DFR data and using the study and generic TC for low and high exposure acceptable MOE were determined from day 0 for workers conducting the various re-entry activities i.e., cotton chipping, crop checking and irrigation .

4.3.2 Risks to re-entry workers (other crops)

DFR data from the re-entry study for melons, peaches and grapes provided by industry in July 2004 were extrapolated to determine re-entry intervals for orchard, broadacre and nursery crops. As the study was conducted on three crops, the DFR data for melons which had the highest DFR value was used to extrapolate and determine re-entry intervals for the crops outlined in Table 19 (Singer 1995). DFR values for peaches and grapes were used from the study. Based on the extrapolated data, acceptable MOE were obtained for workers conducting re-entry activities on day 0 for melons, peaches, grapes, citrus, nut trees (including pecans), vegetables, nursery, and other fruit and broadacre crops.

5. SUMMARY AND CONCLUSIONS

5.1 Orchard applications

With regard to mixing/loading and spraying endosulfan (using ground air assist application with and without the use of closed cabins, ground air-shear spray and ground boom oscillating spray), an *acceptable* MOE was determined for workers handling up to 40 kg ai/day with a work rate of 30 ha/day, when exposures for individual tasks were considered separately.

The MOE for combined exposures (M/L/A/C) were also *acceptable* for air assist with cabin, air shear with cabin, and oscillating boom spray applications. The MOE for combined exposures (M/L/A/C) were a*cceptable* for air assist applications without cabins, where head/face exposure was included in the determination (i.e. where workers were not wearing a respirator/hat). An *acceptable* MOE was determined for cleaning down operations following mixing/loading and spraying. No hand spraying, aerial application or re-entry studies were carried out for orchard applications.

5.2 Nursery crop applications

Studies were carried out for mixing/loading, hand-held spraying and cleaning down associated with nursery crops. It was not clear from the studies whether high or low-pressure systems were used. However, an *acceptable* MOE were determined for workers mixing/loading and cleaning down operations, where up to 0.5 kg endosulfan was handled per day. Combined M/L/A and cleaning down exposure provided *acceptable* MOE for workers

carrying out all activities. The MOE determined for applicators were *acceptable*. No application or re-entry studies were carried out for greenhouses and no re-entry studies were provided for outdoor nursery crops.

5.3 Broadacre applications

Studies were carried out for mixing/loading endosulfan for aerial application and exposure to support workers (markers etc) using vehicles (including ATVs) and cleaning down operations. Mixer/loader exposures were determined for bulk, mini-bulk and small containers in open and closed systems for aerial application of broad acre crops. The total endosulfan handled/day was 1470 kg ai based on an application rate of 2.1 L/ha and work rate of 2000 ha/day. An a*cceptable* MOE was determined for mixer/loaders using open/remote or closed base systems for aerial application. Similarly, an *acceptable* MOE was determined for aerial applicators (pilots), and support workers in vehicles and ATVs. The MOE for applicators and support workers were also *acceptable*.

PHED data for ground application (boom spray) were recalculated using 0.5% and 1.52% dermal absorption rates for mixing/loading and application. *Acceptable* MOE were determined for workers open mixing/loading endosulfan for treatment of broad acre crops by ground application, with the use of gloves. *Acceptable* MOE were also found for workers using open cab for ground application of endosulfan to broad acre crops, with and without the use of gloves. *Acceptable* MOE were determined for workers involved in open pour mixing, and ground boom open cab application (combined activity) to broad acre crops with the use of gloves.

5.4 Re-entry studies

Initially re-entry exposure data was submitted for cotton crops only following ground and aerial application. No measured exposure data were provided for workers re-entering treated areas on day 0 and day 1 as the study authors observed the 48 hour re-entry interval stipulated on the label. MOE for other crops (identified in labels) were extrapolated from the DFR data from a re-entry study on melons, peaches and grapes (2004). Transfer Coefficients determined from measured DFR data, dosimetry data, and generic TC for low and medium exposure were used to calculate the MOE and determine re-entry intervals for cotton, and for other crops using the extrapolated re-entry study DFR data.

Based on the DFR data from the cotton study, and extrapolating the DFR data from the re-entry study on melons, peaches and grapes to other non-cotton crops, acceptable MOEs were obtained on day 0 for workers re-entering cotton fields, orchards and broadacre crops for various re-entry activities.

5.5 First-Aid Instructions

At present, the following standard statements for endosulfan are specified in the *Handbook of First Aid Instructions, Safety Directions, Warning Statements and General Safety Precautions for Agricultural and Veterinary Chemicals* (TGA, 2009);

http://www.health.gov.au/tga/docs/pdf/faisd.pdf.

If poisoning occurs contact a doctor or poisons information centre. *Phone Australia 131126* a

5.6 Safety Directions, re-entry interval and precautionary statements

The current Safety Directions in the FAISD Handbook are:

Endosulfan

CS 330 g/L or less in liquid hydrocarbons 300g/L or less	130 131 132 133 161 162 164 210 211 220 223 279 280 281 290 292 294 297 298 300 279 282 290 292 294 298 300 350 360 361 363 364 366 370
EC 350 g/L or less with surfactant in hydrocarbon solvent 650 g/L or less	100 101 120 130 131 132 133 207 162 161 163 164 210 211 220 222 330 331 332 340 342 340 343 279 280 281 290 292b 294 301 (or 279 300) 279 282 290 292b 350 360 361 364 366

The above codes refer to the following safety directions,

Poisonous if absorbed by skin contact, inhaled or swallowed. Will irritate the eyes and skin. Avoid contact with eyes and skin. Do not inhale spray mist. When opening the container, preparing spray wear cotton overalls buttoned to the neck and wrist, a washable hat, elbow length PVC gloves, goggles, impervious footwear and half facepiece respirator. When using the prepared spray wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow length PVC gloves, impervious footwear and half facepiece respirator. After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use, wash gloves, respirator and if rubber wash with detergent and warm water, and contaminated clothing. Do not reuse footwear until thoroughly aired.

Very dangerous. Particularly the concentrate. Undiluted product poisonous if absorbed by skin contact, inhaled or swallowed. Will damage the eyes. Will irritate the nose, throat and skin. Avoid contact with eyes and skin. Do not inhale vapour. If clothing becomes contaminated with product or wet with spray remove clothing immediately. If product on skin, immediately wash area with soap and water. If product in eyes, wash it out immediately with water. When opening the container and preparing the spray wear cotton overalls buttoned to the neck and wrist (or equivalent clothing), elbow length PVC gloves and a full face respirator (or half face-piece respirator and goggles). When using the prepared spray wear cotton overalls buttoned to the neck and wrist (or equivalent clothing). After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use, wash gloves, respirator and if rubber wash with detergent and warm water, and contaminated clothing.

Acute toxicity 350 g/L EC formulations

Species	Sex	Route	LD_{50} (mg/kg bw) LC_{50} (mg/m^3) – inhal.	Reference
Mouse (HoeNMRKf)	M, F	Oral	39, 41	Ebert & Leist (1989a)
Rat (Wistar)	M, F		67, 17	Ebert & Leist (1989b)
Rabbit (NZW)	M, F		50, 34	Ebert & Leist (1990)
Rat (Wistar)	M, F	Dermal	412, 266	Ebert & Leist (1989c)
Rat (Wistar)	F	Inhal	84.4*	Hollander & Wiegand (1976)
Rat (Wistar)	M, F	Inhal	35†, 13†	Hollander & Weigand (1983)
Irritancy			**Rating**	
Eye			Severe	Ebert & Leist (1989d)
Skin			Moderate	Ebert & Leist (1989e)
Skin sensitisation			Not sensitising (Buehler)	

* Thiodan 25 ULV formulation; † Technical active

Based on a consideration of the hazard (intrinsic toxicity) of the product (see table above), endosulfan EC products are considered to have high acute oral and inhalational toxicity (aerosols) and moderate dermal toxicity. Irritancy to the skin is moderate but severe to the eyes. It is also likely that the hydrocarbon solvent vapour will irritate the nose and throat. None of the products are considered to be skin sensitisers. Considering the hazard of endosulfan and likely worker exposure (from exposure studies) the following amended safety directions are considered appropriate:

Amended entry

EC 35 g/L or less with surfactant in hydrocarbon solvent 650 g/L or less	
100 101	Very dangerous. Particularly the concentrate
120 130 131 132 133	Undiluted product poisonous if absorbed by skin contact, inhaled or swallowed
207 162	Will damage the eyes
161 163 164	Will irritate the noes, throat and skin
210 211	Avoid contact with eyes and skin
220 222	Do not inhale vapour
330 331 332	If clothing becomes contaminated with product or wed with spray remove clothing immediately
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
279 280 281 282 290 292a 294c 301 (or 297 300)	When opening the container, preparing the spray and using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat, elbow length chemical resistant gloves and a full face-piece respirator (or half face-piece respirator and goggles)
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.
360 361	After each day's use, wash gloves, respirator and if rubber wash with
364 366	Detergent and warm water, and contaminated clothing.

Although the inhalational hazard is high the exposure data considered in this review indicated that the risk during spraying is low. Therefore, under normal use a respirator should not be required. The amended dermal absorption data indicated that percutaneous absorption in humans in likely to be low. Hence the, risk assessment for dermal exposure confirmed that the PPE, as described in the safety directions, is suitable to provide acceptable margin of exposure for workers.

Re-entry interval

Since there was no data to consider worker exposure to an endosulfan spray which had not dried, a statement advising workers not to re-enter treated crops until the spray has dried is appropriate.

Precautionary statement

For aerial application, support workers/markers should be protected by enclosed cabs

5.7 Conclusions

The APVMA can be satisfied that the continued use of EC products containing 350 g/L of endosulfan would NOT pose an undue hazard to the safety of workers when used in accordance with the label instructions as described below.

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Worker exposure to endosulfan (EC) in the course of application in tree crops (Air-shear, with cabin)– Lyn Fragar, Report No. H-2-2-C (February, 2002).

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Appendix 1

QUALITY ANALYSIS OF THE ENDOSULFAN WORKER EXPOSURE STUDIES

Study guidelines

The endosulfan worker exposure studies were conducted by the Australian Centre for Agricultural Health and Safety (Moree) and the Centre for Pesticide Application Safety (Gatton). The studies were based on a protocol approved by the APVMA and OCSEH (OHS) and in accordance with standards prescribed by the New England Health Research and University of Sydney Research ethics committees. All studies used the same formulation of endosulfan containing 350 g ai/L, which was considered representative of each of the products under review.

For the purposes of measuring dermal exposure, the US EPA Occupational and Residential Exposure Test Guidelines were adopted to assess worker exposure to endosulfan, which includes guidance on estimating total body deposition for workers.

General reporting of data

The applicator raw data had many values with "nd" (not detected), but there was no mention of the limits of detection or quantitation. It is usual practice that levels below the limit of detection are included in the data at half the LOQ**.** No explanation was provided by the study authors for the high field blank values for aerial applicators. The location of the field blank patches appear to be the same for all studies, i.e. 3 internal patches (2 on shoulders one on back below neck). The authors did not say how the field blanks were conducted for aerial applicators. The re-entry raw data had many values with "nd" (not detected), but there was no mention of the limit of detection or the limit of quantitation. It is usual practice that levels below the limit of detection are included in the data at half the LOQ.

The field blanks for re-entry workers were based on 3 patches only and extrapolated to all body parts. The positioning of the field blank patches gave an overestimate of contamination for body parts which were better protected from exposure. The variability between field blanks conducted on different days and also on the same day suggested poor sample handling, and it was unclear whether t the field blanks represented 'background' contamination rather than handler error. Therefore the raw data uncorrected for field blanks was used in the OHS assessment. It was also noted that field blank levels were often far greater than test sample levels. Furthermore, field blanks were not available for all re-entry days and on these occasions the study authors used inappropriately high surrogate field blanks to correct the raw data.

When a sample was lost or not obtained, the authors used an average of the other samples for that body part as a surrogate. On the whole this was considered acceptable, however the authors also used surrogate data to replace 'high' values. In study RC-1-3-A Day 0 subject DV, the authors replaced the entire glove reading (alpha + beta-endosulfan + endosulfan sulphate) by a surrogate total glove reading. In this case the alpha-endosulfan and the endosulfan sulfate values were not excessive in comparison to other

readings for this worker group, only the beta-endosulfan reading was excessive (approximately 80 times that for alpha-endosulfan). For this particular reading it was considered more appropriate to use the alpha endosulfan value reading as a surrogate for the beta value. No field blanks or field fortification data were reported for the siphon residues or the foliar residues studies.

Number of replicates

The number of replicates used in the studies were generally in accordance with US EPA recommendations, except in certain instances when they were reduced (eg, air-shear with cabin for tree crops, cleaning down for nursery crops etc).

Positioning/type of dosimeters

The positioning of the dosimeters was unclear and not consistent. From information provided in the studies, “chromatographic patches were fixed either on the singlet or overall or fixed on the cloth pads which were stitched with velcro adhesive straps. These straps attached with chromatographic papers by pins on the cloth pads were placed on the forearms, thighs and knees of the workers”. Explanations for the varied positioning of dosimeters were later provided but were still not consistent.

The PPE worn by the workers was generally similar for all studies, applicator and re-entry, i.e. in relation to cotton coveralls. However, the re-entry workers did not wear coveralls, they wore their own clothing. It was assumed that dosimeters which would have been internal dosimeters had the workers worn coveralls would also be internal dosimeters for workers wearing shirts and pants. Cotton gloves were used as dosimeters, however the authors did not state whether they were worn with PVC gloves and if so, whether they were worn outside or inside the PVC gloves. It was assumed that cotton gloves were worn inside protective gloves for the purpose of the exposure estimates.

In the study, head and face exposure was calculated from internal patches placed under the overalls on the external shoulder (dorsal side), chest, and back x 1300 cm^2. However, according to US EPA Guidelines, head and face exposure is estimated from patches placed on the outside (i.e. externally) of the garments at the back, chest, and shoulders. In order to determine the necessity for PPE for head and face exposure, and in the absence of external patch data, internal patch data (from the studies) were used to determine the need for a respirator and hat during mixing/loading/application.

Duration of monitoring

A range of monitoring times was provided for all mixer/loader, and applicator studies. Although the US EPA recommends a minimum of 4 hours per activity, the exposure and risk assessments were based on the amount of active ingredient handled per day and standardised for local use conditions.

Sample Recovery

Field fortification data for applicator studies were not used to adjust the sample values. Field recovery rates ranged from just over 50% to over 120%. The authors did not adjust the sample values for field recovery rates, nor did they present method sensitivity data or sample chromatograms (as required

according to the US EPA guidelines). Field fortification data for re-entry studies were not reported. Field recovery rates were reported to be greater than 50%. The authors did not adjust the sample values for field recovery rates, nor did they present method sensitivity data or sample chromatograms (as required according to the US EPA guidelines).

Statistical analysis

The data presented in the studies in some instances appeared to be skewed (higher or lower than expected). Explanations for these “out layers” were provided by the study authors. However, when the data were plotted on a log-normal distribution, the so-called “out layers” were determined as acceptable values, with the geometric mean the most appropriate statistical technique for averaging the data. These are provided in Appendices 3 and 4.

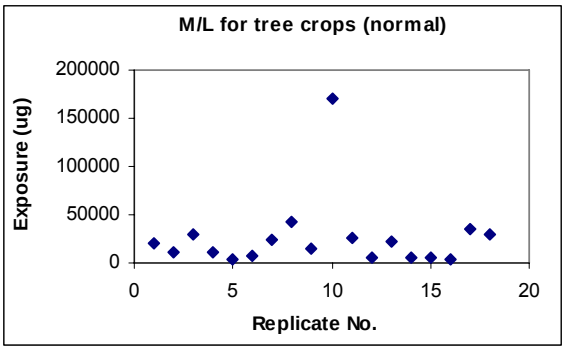
Summary and conclusions

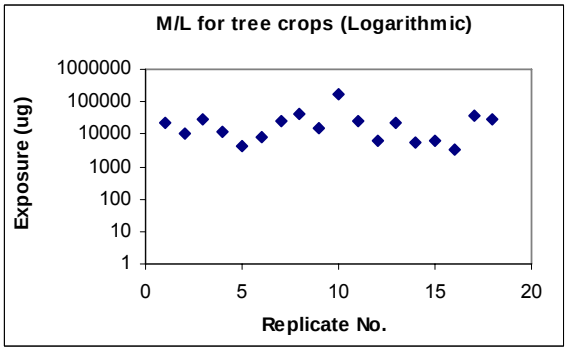
The studies covered a range of use pattern scenarios and application methods. However, data presentation was not clear and consistent in some studies, with reasons for missing data or high and low exposure values not justifiable due to lack of accurate reporting. In the case of support workers it was unclear whether enclosed ATV’s/vehicles were used, or in some instances whether the workers were outside the ATV’s/vehicles.. Sample values were not adjusted for field recovery rates. The field blanks for re-entry workers were based on 3 patches only and extrapolated to all body parts. In some instances, the positioning of the field blank patches gave an overestimate of contamination for body parts which were better protected from exposure.

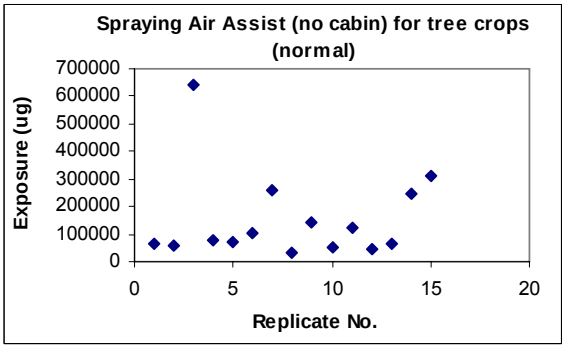
To overcome the deficiencies in data presentation, surrogate (minimal) values for missing data, were used to estimate exposure, adjusting for high and low exposure values by log transformation of data, and standardising to local conditions.

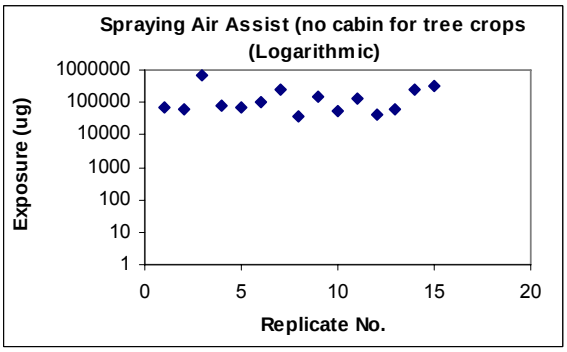
APPENDIX 2

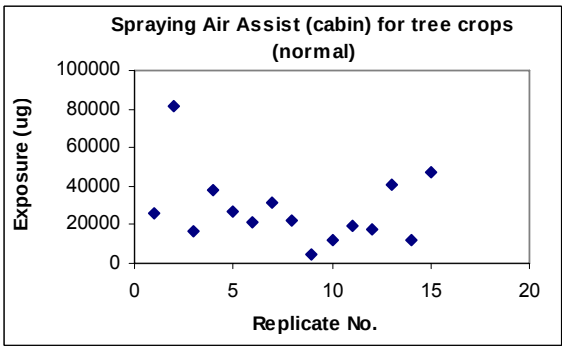
Comparison of normal and logarithmic distribution of mixer/loader/applicator/cleaner/support worker data for tree, nursery and broadacre crops.

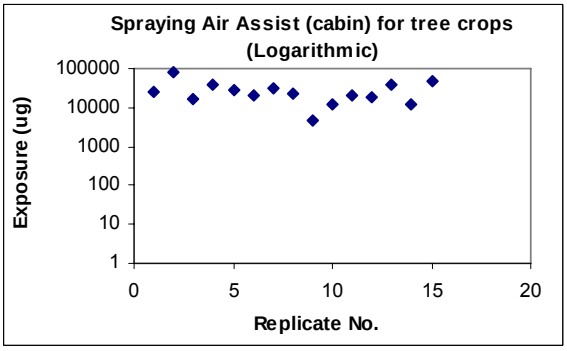


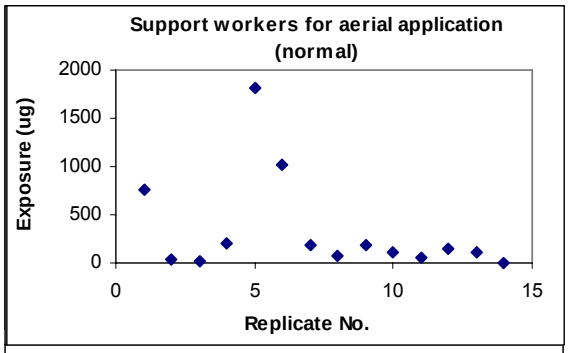








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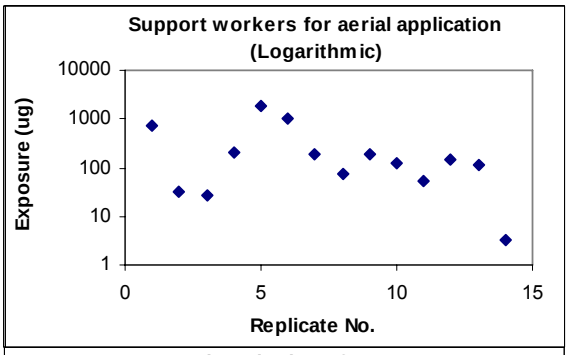
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Exposure (ug)
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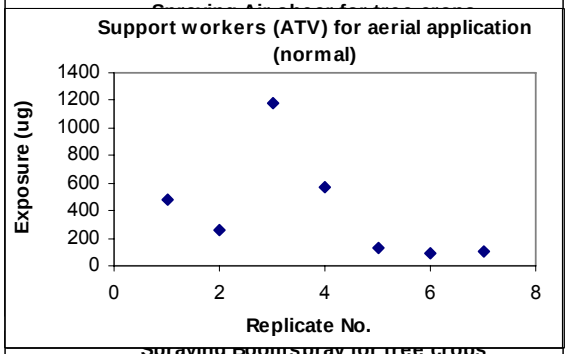
Support workers (ATV) for aerial application (normal)
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6
8
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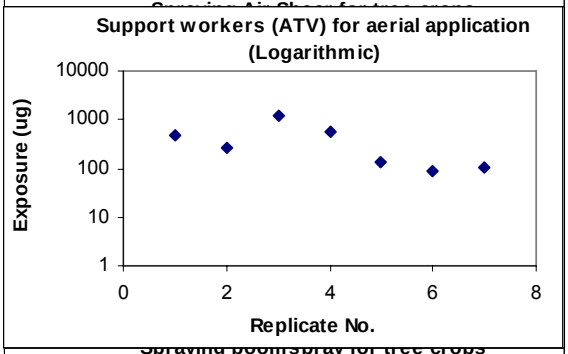
Support workers (ATV) for aerial application (Logarithmic)
Exposure (ug)
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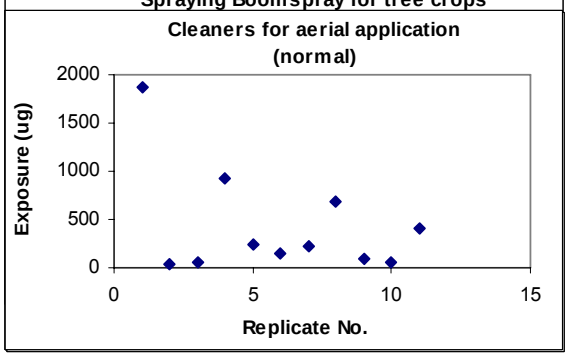
Replicate No.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Exposure (ug)
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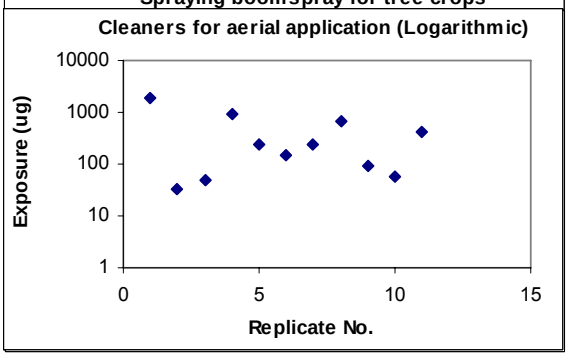
Replicate No.


Cleaners for aerial application (Logarithmic)
Exposure (ug)
10000
1000
100
10
1
0
5
10
15
Replicate No.

APPENDIX 3

Raw exposure data determined from the worker exposure studies for workers treating tree crops, nursery crops and broadacre crops (including and excluding head/face exposure).

Including head/face exposure

Tree crops: (H-1-1) Mixing/loading; (H-1-2-U) Air-assist spraying, no cabin; (H-1-2-C) Air-assist spraying, with cabin; (H-2-2-C) Air shear spraying; (H-5-2-C) Oscillating Boomspray; (H-1-4) Cleaning down

Replicate No.	Endosulfan handled (kg ai)	Total exposure (µg)	Exposure (mg/kg bw/kg ai)*
Mixing/Loading			
1	0.385	20.96	0.0008
2	0.385	10.41	0.0004
3	0.385	30.15	0.0011
4	0.385	11.33	0.0004
5	0.385	4.41	0.0002
6	1.050	8.27	0.0001
7	0.525	24.62	0.0007
8	1.575	42.19	0.0004
9	1.575	14.78	0.0001
10	1.575	169.95	0.0015
11	1.103	26.82	0.0003
12	0.210	6.30	0.0004
13	0.210	21.38	0.0015
14	0.131	5.83	0.0006
15	0.315	6.41	0.0003
16	0.315	3.23	0.0001
17	0.315	35.24	0.0016
18	0.315	29.44	0.0013
Geomean			**0.0005**
Air-assist spraying, no cabin			
1	0.385	67.37	0.0025
2	0.385	60.00	0.0022
3	0.385	642.42	0.0238
4	0.385	75.02	0.0028
5	0.385	71.10	0.0026
6	0.7875	104.68	0.0019
7	0.7875	260.92	0.0047
8	0.525	35.11	0.0010
9	0.525	142.74	0.0039
10	0.525	51.62	0.0014
11	0.525	125.40	0.0034
12	0.525	44.56	0.0012
13	0.0525	64.89	0.0177
14	0.0525	246.02	0.0669
15	0.0525	310.61	0.0845
Geomean			**0.0048**

Air Assist Spraying, with cabin			
1	0.8458	26.14	0.0004
2	0.8458	81.03	0.0014
3	0.8458	17.07	0.0003
4	0.3938	37.83	0.0014
5	0.6563	26.80	0.0006
6	0.525	21.14	0.0006
7	1.1025	31.70	0.0004
8	1.1025	21.82	0.0003
9	0.0525	4.53	0.0012
10	0.0525	12.09	0.0033
11	0.0525	19.74	0.0054
12	0.0525	17.69	0.0048
13	0.0525	40.46	0.0110
14	0.0525	12.27	0.0033
15	0.1313	46.90	0.0051
Geomean			**0.0014**
Air shear spraying, with cabin			
1	1.26	37.01	0.0004
2	0.63	39.45	0.0009
3	0.63	16.22	0.0004
4	0.525	13.68	0.0004
5	0.525	26.80	0.0007
Geomean			**0.0005**
Oscillating boomspray, with cabin			
1	0.16	53.46	0.0048
2	0.16	12.29	0.0011
3	0.16	18.16	0.0016
4	0.16	33.79	0.0030
5	0.16	28.36	0.0025
6	0.16	12.79	0.0011
7	0.16	28.23	0.0025
8	0.16	6.63	0.0006
9	0.16	65.29	0.0058
10	0.16	26.14	0.0023
11	0.51	55.55	0.0016
12	0.51	14.36	0.0004
13*	0.39	2.08	0.00008
14	0.39	8.73	0.0003
Geomean			**0.0013**
Cleaning Down			
1	1.925	19.53	0.0001
2	2.3625	30.17	0.0002
3	0.7875	60.37	0.0011
4	1.05	207.47	0.0028
5	2.3625	20.44	0.0001
6	1.54875	49.98	0.0005
7	2.205	25.54	0.0002
8	0.105	3.61	0.0005

9	0.13125	10.01	0.0011
10	0.7875	31.97	0.0006
11	0.5075	16.67	0.0005
12	1.015	16.14	0.0002
13	0.777	5.83	0.0001
14	0.0525	4.40	0.0012
15	0.105	14.93	0.0020
Geomean			
			0.0005

*based on 70 kg person

Nursery crops: (H-3-1) Mixing/loading; (H-3-2) Spraying; (H-3-3) Cleaning down

Replicate No.	Endosulfan handled (kg ai)	Total exposure (µg)	Exposure (mg/kg bw/kg ai)*
Mixing/Loading			
1	0.133	23.23	0.0025
2	0.133	43.92	0.0047
3	0.133	30.99	0.0033
4	0.200	18.06	0.0013
5	0.200	24.59	0.0018
6	0.200	9.53	0.0007
7	0.067	32.36	0.0070
8	0.067	29.44	0.0063
9	0.033	41.09	0.0177
10	0.035	32.04	0.0131
11	0.035	51.55	0.0210
12	0.070	17.18	0.0035
Geomean			**0.0043**
Spraying			
1	0.133	111.38	0.0120
2	0.133	71.28	0.0077
3	0.133	42.46	0.0046
4	0.100	37.25	0.0053
5	0.100	21.23	0.0030
6	0.100	30.98	0.0044
7	0.100	6.28	0.0009
8	0.100	25.73	0.0037
9	0.100	30.89	0.0044
10	0.067	110.87	0.0238
11	0.033	133.87	0.0575
12	0.067	18.52	0.0040
13	0.035	58.88	0.0240
14	0.035	31.16	0.0127
15	0.033	40.82	0.0175
16	0.033	29.67	0.0127
17	0.070	96.93	0.0198
Geomean			**0.0082**
Cleaning Down			
1	0.133	12.19	0.0013
2	0.133	4.40	0.0005

3	0.133	8.88	0.0010
4	0.200	3.02	0.0002
5	0.200	54.68	0.0039
6	0.200	11.69	0.0008
7	0.067	69.44	0.0148
8	0.067	25.24	0.0054
9	0.033	242.18	0.1048
10	0.100	21.66	0.0031
11	0.070	7.24	0.0015
Geomean			**0.0024**

*based on 70 kg person

Aerial application:

(A1-1) Mixing/Loading bulk and mini bulk (closed base);
(A1-2) Mixing/Loading small containers (open/remote);
(A1-3) Aerial applicators;
(A1-4) Support workers (vehicles);
(A1-5) Support workers (ATVs);
(A1-6) Cleaning down

Replicate No.	Endosulfan handled (kg ai)	Total exposure (µg)	Exposure (mg/kg bw/kg ai)*
Open/remote M/L for aerial application			
1	71.95	197.9	0.00004
2	71.95	4265.5	0.00085
3	163.49	902.6	0.00008
4	32.17	115.9	0.00005
5	353.87	4997.0	0.00020
6	41.53	545.6	0.00019
7	110.99	167.9	0.00002
8	36.75	186.7	0.00007
9	73.5	2988.1	0.00058
10	102.21	169.3	0.00002
11	73.5	609.9	0.00012
12	73.5	1143.4	0.00022
13	220.03	1017.4	0.00007
Geomean			**0.00011**
Close/base M/L for aerial application			
1	337.9	241.9	0.00001
2	84.48	502.6	0.00008
3	27.69	1264.0	0.00065
4	49	250.8	0.00007
5	35.81	2003.7	0.00080
6	107.42	4694.9	0.00062
7	35.11	233.4	0.00009
8	103.12	285.6	0.00004
9	103.12	365.7	0.00005

10	103.12	448.3	0.00006
11	103.12	596.0	0.00008
12	84.67	5281.2	0.00089
13	138.23	567.5	0.00006
Geomean			**0.00012**

Applicator			
1	71.95	176.4	0.00004
2	196.82	61.3	0.00000
3	337.9	42.0	0.00000
4	84.48	61.0	0.00001
5	160.86	390.9	0.00003
6	160.86	168.6	0.00001
7	27.69	2630.2	0.00136
8	49.21	319.8	0.00009
9	59.5	99.3	0.00002
10	35.81	191.4	0.00008
11	107.42	173.4	0.00002
12	35.11	236.1	0.00010
13	103.12	103.6	0.00001
14	103.12	132.2	0.00002
15	92.11	122.1	0.00002
16	28.28	33.0	0.00002
Geomean			**0.00003**

Support Workers (vehicles)			
1	196.18	756.6	0.00006
2	84.48	32.5	0.00001
3	337.9	27.0	0.00000
4	289.55	209.5	0.00001
5	143.22	1815.7	0.00018
6	35.11	1021.4	0.00042
7	103.12	188.6	0.00003
8	28.28	76.4	0.00004
9	204.42	193.3	0.00001
10	103.12	120.0	0.00002
11	83.06	52.7	0.00001
12	127.01	143.7	0.00002
13	364.68	109.6	0.00000
14	364.68	3.3	0.00000
Geomean			**0.00001**

Support Workers (ATVs)			
1	289.55	479.6	0.00002
2	49.21	265.6	0.00008
3	49.21	1179.3	0.00034
4	35.11	570.9	0.00023
5	28.28	131.4	0.00007
6	204.42	90.3	0.00001
7	83.06	108.4	0.00002
Geomean			**0.00005**

Cleaners

1	457.76	1877.25	0.00006
2	845.25	32.62	0.00000
3	49.25	49.75	0.00001
4	59.54	920.85	0.00022
5	582.86	238.18	0.00001
6	35.11	152.63	0.00006
7	111.79	230.22	0.00003
8	184.52	677.79	0.00005
9	36.75	93.5	0.00004
10	36.75	57.37	0.00002
11	115.75	405.22	0.00005
Geomean			**0.00002**

*based on 70 kg person

Excluding head/face exposure

Tree crops:

(H-1-1) Mixing/loading;
(H-1-2-U) Air-assist spraying, no cabin;
(H-1-2-C) Air-assist spraying, with cabin;
(H-2-2-C) Air shear spraying;
(H-5-2-C) Oscillating Boomspray;
(H-1-4) Cleaning down

Replicate No.	Endosulfan handled (kg ai)	Total exposure (µg)	Exposure (mg/kg bw/kg ai)*
Mixing/Loading			
1	0.385	19.32	0.0007
2	0.385	9.89	0.0004
3	0.385	27.94	0.0010
4	0.385	11.03	0.0004
5	0.385	4.20	0.0002
6	1.050	7.68	0.0001
7	0.525	22.51	0.0006
8	1.575	39.53	0.0003
9	1.575	14.06	0.0001
10	1.575	167.31	0.0015
11	1.103	25.08	0.0003
12	0.210	6.06	0.0004
13	0.210	20.08	0.0014
14	0.131	5.60	0.0006
15	0.315	6.15	0.0003
16	0.315	3.01	0.0001
17	0.315	32.50	0.0015
18	0.315	28.76	0.0013
Geomean			**0.0005**
Air-Assist spraying, no cabin			
1	0.385	9.08	0.0003
2	0.385	23.99	0.0009
3	0.385	31.11	0.0012
4	0.385	57.03	0.0021
5	0.385	52.04	0.0019
6	0.7875	97.23	0.0018
7	0.7875	230.71	0.0042
8	0.525	26.25	0.0007
9	0.525	127.04	0.0035
10	0.525	41.15	0.0011
11	0.525	114.34	0.0031
12	0.525	36.87	0.0010
13	0.0525	17.65	0.0048
14	0.0525	18.45	0.0050
15	0.0525	17.39	0.0047
Geomean			**0.0019**

Air-Assist spraying, with cabin			
1	0.8458	23.56	0.0004
2	0.8458	80.23	0.0014
3	0.8458	16.20	0.0003
4	0.3938	35.00	0.0013
5	0.6563	24.84	0.0005
6	0.525	19.68	0.0005
7	1.1025	29.81	0.0004
8	1.1025	20.87	0.0003
9	0.0525	4.35	0.0012
10	0.0525	11.92	0.0032
11	0.0525	18.08	0.0049
12	0.0525	16.69	0.0045
13	0.0525	38.39	0.0104
14	0.0525	12.15	0.0033
15	0.1313	43.23	0.0047
Geomean			**0.0013**
Cleaning Down			
1	1.925	17.58	0.0001
2	2.3625	21.72	0.0001
3	0.7875	56.36	0.0010
4	1.05	205.58	0.0028
5	2.3625	19.03	0.0001
6	1.54875	47.54	0.0004
7	2.205	23.86	0.0002
8	0.105	3.40	0.0005
9	0.13125	9.54	0.0010
10	0.7875	29.42	0.0005
11	0.5075	15.55	0.0004
12	1.015	15.20	0.0002
13	0.777	5.63	0.0001
14	0.0525	4.14	0.0011
15	0.105	12.97	0.0018
Geomean			**0.0004**
Air-shear spraying, with cabin			
1	1.26	36.00	0.0004
2	0.63	38.73	0.0009
3	0.63	15.20	0.0004
4	0.525	12.64	0.0003
5	0.525	24.84	0.0007
Geomean			**0.0005**
Oscillating boomspray, with cabin			
1	0.16	49.44	0.0044
2	0.16	12.10	0.0011
3	0.16	17.19	0.0015
4	0.16	30.75	0.0027
5	0.16	25.93	0.0023
6	0.16	11.89	0.0011
7	0.16	26.55	0.0023
8	0.16	6.54	0.0006
9	0.16	61.37	0.0055

10	0.16	24.84	0.0022
11	0.51	49.98	0.0014
12	0.51	13.40	0.0004
13*	0.39	1.92	0.0001
14	0.39	8.46	0.0003
Geomean			**0.0012**

*Based on 70 kg person

Nursery crops: (H-3-1) Mixing/loading; (H-3-2) Spraying; (H-3-3) Cleaning down

Replicate No.	Endosulfan handled (kg ai)	Total exposure (µg)	Exposure (mg/kg bw/kg ai)*
Mixing/Loading			
1	0.133	22.71	0.0024
2	0.133	43.88	0.0047
3	0.133	30.38	0.0033
4	0.200	17.63	0.0013
5	0.200	21.68	0.0016
6	0.200	8.88	0.0006
7	0.067	31.06	0.0067
8	0.067	27.34	0.0059
9	0.033	39.28	0.0169
10	0.035	30.08	0.0123
11	0.035	49.12	0.0201
12	0.070	16.57	0.0034
Geomean			**0.0041**
Spraying			
1	0.133	105.22	0.0113
2	0.133	63.87	0.0069
3	0.133	32.80	0.0035
4	0.100	32.65	0.0047
5	0.100	8.53	0.0012
6	0.100	27.82	0.0040
7	0.100	4.16	0.0006
8	0.100	24.43	0.0035
9	0.100	29.37	0.0042
10	0.067	98.63	0.0212
11	0.033	87.60	0.0376
12	0.067	16.20	0.0035
13	0.035	53.83	0.0220
14	0.035	28.83	0.0118
15	0.033	39.20	0.0168
16	0.033	28.62	0.0123
17	0.070	90.16	0.0184
Geomean			**0.0068**
Cleaning Down			
1	0.133	11.93	0.0013
2	0.133	4.10	0.0004
3	0.133	8.27	0.0009
4	0.200	1.94	0.0001
5	0.200	37.83	0.0027
6	0.200	4.06	0.0003
7	0.067	68.55	0.0146
8	0.067	24.42	0.0052
9	0.033	222.11	0.0961
10	0.100	20.55	0.0029
11	0.070	6.95	0.0014
Geomean			**0.0020**

*Based on 70 kg person

Aerial application:

(A1-1) Mixing/Loading bulk and mini bulk (closed base);
(A1-2) Mixing/Loading small containers (open/remote);
(A1-3) Aerial applicators;
(A1-4) Support workers (vehicles);
(A1-5) Support workers (ATVs);
(A1-6) Cleaning down

Replicate No.	Endosulfan handled (kg ai)	Total exposure (µg)	Exposure (mg/kg bw/kg ai)*
Open/remote M/L for aerial application			
1	71.95	191.5	0.00004
2	71.95	4245.5	0.00084
3	163.49	886.9	0.00008
4	32.17	109.5	0.00005
5	353.87	4891.7	0.00020
6	41.53	529.7	0.00018
7	110.99	153.8	0.00002
8	36.75	174.2	0.00007
9	73.5	2975.6	0.00058
10	102.21	157.5	0.00002
11	73.5	587.7	0.00011
12	73.5	1133.8	0.00022
13	220.03	999.0	0.00006
Geomean			**0.00010**
Close/base M/L for Aerial application			
1	337.9	229.1	0.00001
2	84.48	480.8	0.00008
3	27.69	1156.1	0.00060
4	49	231.6	0.00007
5	35.81	1884.7	0.00075
6	107.42	4686.0	0.00062
7	35.11	215.0	0.00009
8	103.12	279.3	0.00004
9	103.12	355.7	0.00005
10	103.12	429.2	0.00006
11	103.12	567.5	0.00008
12	84.67	5255.8	0.00089
13	138.23	552.7	0.00006
Geomean			**0.00011**
Applicator			
1	71.95	173.0	0.00003
2	196.82	59.0	0.00000
3	337.9	39.8	0.00000
4	84.48	59.5	0.00001
5	160.86	368.7	0.00003
6	160.86	149.7	0.00001
7	27.69	2443.0	0.00126
8	49.21	300.1	0.00009
9	59.5	97.3	0.00002

10	35.81	187.4	0.00007
11	107.42	168.0	0.00002
12	35.11	216.8	0.00009
13	103.12	98.5	0.00001
14	103.12	125.4	0.00002
15	92.11	115.3	0.00002
16	28.28	30.7	0.00002
Geomean			**0.00003**
Support Workers (vehicles)			
1	196.18	742.5	0.00005
2	84.48	31.6	0.00001
3	337.9	26.1	0.00000
4	289.55	201.1	0.00001
5	143.22	1649.3	0.00016
6	35.11	927.5	0.00038
7	103.12	169.6	0.00002
8	28.28	73.5	0.00004
9	204.42	178.1	0.00001
10	103.12	107.2	0.00001
11	83.06	48.7	0.00001
12	127.01	123.7	0.00001
13	364.68	108.4	0.00000
14	364.68	3.3	0.00000
Geomean			**0.00001**
Support Workers (ATVs)			
1	289.55	466.9	0.00002
2	49.21	249.3	0.00007
3	49.21	1148.8	0.00033
4	35.11	546.8	0.00022
5	28.28	126.8	0.00006
6	204.42	78.6	0.00001
7	83.06	103.8	0.00002
Geomean			**0.00005**
Cleaners			
1	457.76	1877.25	0.00006
2	845.25	32.62	0.00000
3	49.25	49.75	0.00001
4	59.54	920.85	0.00022
5	582.86	238.18	0.00001
6	35.11	152.63	0.00006
7	111.79	230.22	0.00003
8	184.52	677.79	0.00005
9	36.75	93.5	0.00004
10	36.75	57.37	0.00002
11	115.75	405.22	0.00005
Geomean			**0.00002**

*based on 70 kg person

APPENDIX 4 – HAZARD CLASSIFICATION

Endosulfan is listed on the ASCC Hazardous Substances Information System *HSIS* (ASCC, 2005) with the following health effects risk phrases and cut-off concentrations:

R26/28	Very toxic by inhalation and in contact with skin
R21	Harmful in contact with skin
R23/25	Toxic by inhalation and if swallowed
R20/22	Harmful by inhalation and if swallowed

Conc ≥25%	R26/28; R21
≥ 7% Conc <25%	R26/28
≥ 1% Conc <7%	R23/25
≥ 0.1% Conc <1%	R20/22

The National Model Regulations and National Code of Practice (NOHSC, 1994a) for the Control of Workplace Hazardous Substances apply to all hazardous substances, as defined in the national model regulations, and extend to all workplaces in which hazardous substances are used or produced and to all persons (consistent with the relevant Commonwealth/State/Territory occupational health and safety legislation) with potential for exposure to hazardous substances in those workplaces.

APPENDIX 5: Evaluation of a Developmental neurotoxicity study

Gilmore RG, Sheets LP & Hoss HE (2006) A Developmental Neurotoxicity Study with Technical Grade Endosulfan in Wistar Rats. Study Number 05/D72/YF, Report Number 201563. September 26, 2006. Corp. 1062p. MRID# 46968301. (Bayer study).

Test Substance:	Endosulfan (Technical grade)
Purity	99.1%
Lot/Reference #:	EGPC400349
Test Species:	Wistar rat Crl:WI(Han). Charles River Laboratories.
Study Duration:	9 May 2005 – 23 August 2005
Laboratory:	Bayer CropScience LP
GLP & QA:	Not specified
Guidelines:	US EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870.6300, Developmental Neurotoxicity Study (August 1998).

Study design and dosage:

Technical grade endosulfan (99.1% pure) was administered at doses of 0, 50, 150 or 400 ppm (equivalent to 0, 3.75, 10.8 or 29.8 mg/kg bw/day) to groups of pregnant female Wistar rats via the diet from gestation day 6 (GD 6) through to day 21 of lactation (LD 21). Dosages were adjusted during lactation to maintain a consistent dosage throughout exposure. All test diets (including control) were provided *ad libitum* throughout the study, except during neurobehavioral testing. Physical observations, bodyweight and food consumption measurements were performed on all dams (P-generation) at selected intervals throughout the gestation and lactation periods. The concentration, stability and homogeneity of endosulfan in the feed were confirmed by analytical means.

Paternal males were sacrificed following co-habitation. Litters were culled to 8 pups (4 male and 4 female) on postnatal day (PND) 4. Subsets of surviving offspring, representing at least 20 litters per dietary level, were used for evaluation. On PND 21, pups were weaned and dams were sacrificed. The offspring (F1-generation) that remained in the study after weaning were sacrificed at study termination on PND 75 (±5 days).

Maternal animals were evaluated for clinical signs of toxicity, changes in body weight gain and food consumption and functional observational battery (FOB) examination.

The offspring (F1-generation) were evaluated using detailed clinical observations, body weight, food consumption, developmental landmarks for sexual maturation, automated measures of activity (figure-eight maze),

auditory startle habituation, learning and memory (passive avoidance and a water maze task), and an ophthalmic examination. Tissues were collected for morphometry (brain) and microscopic examination on PND 21 (brain) and at study termination on PND 75 (brain, other neural tissues and skeletal muscle). Sperm analysis was also performed on selected F1 control and F1 high-dose males at PND 75.

Results:

Maternal animals (P-generation):

No P-generation females were found dead during gestation or lactation. No treatment related clinical signs of toxicity were evident at any dose level during gestation or lactation. Nasal staining was seen in two high-dose females, and areas of alopecia were seen in five mid and four high dose females. These observations were not considered by the study authors to be treatment related.

Functional observational battery (FOB) examination (assessments of lacrimation, salivation, piloerection, exophthalmia, urination, defecation, pupillary function, palpebral closure, convulsions, tremor, abnormal movements, unusual behaviours and posture and gait abnormalities) showed no treatment related effects at any dose level.

A statistically significant reduction in food consumption was seen from GD 6-13 at all treated levels (an average of 12%, 35% and 52% in low-, mid- and high-dose females, respectively, see Table 1) compared to controls. There was also a statically significant reduction in food consumption from GD 13-20 in mid and high-doses dams (15% and 17%, respectively) compared to controls. Associated with decreased food consumption was a statistically significantly reduction in body weight gain from GD 0-20 for all dose levels (an average of 11%, 23% and 36% for low-, mid- and high-doses, respectively, see Table 2). Statistically significant differences in body weight gain were also seen on GD 13 (an average of 5%, 10% and 16%, in low-, mid- and high-dose females, respectively), and GD 20 (an average of 6%, 9% and 14% in low-, mid- and high-dose females, respectively). On LD 0, 4 and 7, there were statistically significant differences in body weight gain at the mid- and high-dose levels (6-9% and 8-13%, respectively) compared to controls. The magnitude of the decrease in food consumption during lactation was small but not statistically significant (3 and 5% for the low- and high doses, respectively).

The average daily intake of endosulfan during gestation and lactation was 0, 3.74, 10.8 and 29.8 mg/kg bw/day (see Table 3).

Table 1. Maternal food consumption during gestation and lactation.

Mean food consumption	Dose (ppm in diet)			
	Control	50 ppm	150 ppm	400 ppm
GD 6-13	19.8 ± 0.39 (28)	17.5** ± 0.54 (30)	12.8** ± 0.31 (28)	9.5** ± 0.32 (27)
GD 13-20	21.2 ± 0.43 (28)	19.7 ± 0.55 (29)	18.1** ± 0.53	17.5** ± 0.53

			(28)	(26)
LD 0-7	34.2 ± 0.95 (23)	32.1 ± 1.26 (23)	31.4 ± 0.82 (23)	32.2 ± 0.79 (21)
LD 7-14	50.6 ± 0.85 (23)	49.1 ± 1.03 (23)	48.3 ± 1.00 (23)	48.8 ± 0.75 (21)
LD 14-21	61.7 ± 0.86 (23)	58.5 ± 1.73 (23)	60.7 ± 1.11 (23)	60.5 ± 1.04 (21)

Values are mean ± standard error (n). *significantly different from control, $p \leq 0.05$. **significantly different from control $p \leq 0.01$.

Table 2. Maternal body weight during gestation and lactation.

Mean body weight /study week	Dose (ppm in diet)			
	Control	50 ppm	150 ppm	400 ppm
Gestation				
GD0	202.5 ± 2.44 (28)	196.5 ± 2.71 (30)	198.7 ± 2.91 (28)	198.5 ± 2.16 (27)
GD6	221.8 ± 3.99 (28)	213.9 ± 3.76 (30)	220.1 ± 3.16 (28)	220.0 ± 2.32 (27)
GD13	250.7 ± 3.16 (28)	238.3* ± 3.11 (30)	226.6** ± 3.00 (28)	209.7** ± 2.60 (27)
GD20	311.6 ± 4.25 (28)	293.6* ± 4.24 (30)	282.8** ± 4.11 (28)	268.2** ± 3.36 (27)
Mean body weight gain GD0-20	109.1 ± 3.10 (28)	97.1* ± 2.69 (30)	84.0** ± 3.14 (28)	69.7** ± 2.52 (27)
Lactation				
LD0	241.4 ± 3.74 (28)	231.2 ± 3.55 (30)	219.1** ± 3.27 (28)	210.7** ± 3.64 (27)
LD4	253.0 ± 3.61 (24)	241.4 ± 3.25 (25)	234.0** ± 4.0 (23)	226.8** ± 2.51 (21)
LD7	262.0 ± 3.62 (23)	255.7 ± 2.79 (23)	245.3* ± 4.04 (23)	241.6** ± 3.53 (21)
LD14	277.8 ± 5.35 (23)	273.9 ± 3.09 (23)	267.1 ± 4.33 (23)	264.2 ± 3.71 (21)
LD21	271.0 ± 3.81 (23)	264.9 ± 4.44 (23)	265.0 ± 4.22 (23)	263.5 ± 5.32 (21)
Mean body weight gain LD0-21	29.6	33.7	45.9	52.8

Values are mean ± standard error (n). *significantly different from control, p ≤ 0.05. **significantly different from control p ≤ 0.01.

Table 3. Mean maternal test substance intake (mg/kg bw/day).

Study week	Dose (ppm in diet)		
	50 ppm	150 ppm	400 ppm
Gestation			
GD 6-13	4.0 ± 0.15	8.3 ± 0.24	16.8 ± 0.67
GD 13-20	4.0 ± 0.11	11.3 ± 0.28	32.4 ± 0.40

GD 6-20	4.0	9.8	24.6
Lactation			
LD 0-7	3.3 ± 0.14	10.5 ± 0.26	32.3 ± 0.49
LD 7-14	3.8 ± 0.07	11.9 ± 0.17	34.8 ± 0.54
LD 14-21	3.6 ± 0.09	12.0 ± 0.18	32.8 ± 0.49
LD 0-21	3.57	11.5	33.3
Mean gestation and lactation (combined)	3.74	10.8	29.8

Values are mean ± standard error. Dietary concentrations were reduced during weeks 1-3 of lactation (by factors of 1.9, 2.3 and 2.8 respectively), based on estimated increases in feed consumption during lactation).

Reproduction parameters (mating, fertility index and gestation length) were not affected by endosulfan at any dose level (see Table 4).

No gross or microscopic post-mortem examination of maternal animals was conducted.

Table 4. Reproductive performance.

Observation	**Dose (ppm in diet)**			
	Control	**50 ppm**	**150 ppm**	**400 ppm**
Number mated	30	30	30	30
Number pregnant	28	30	28	27
Number of litters	23	23	23	21
Mating index	100	100	100	100
Fertility index	93.3	100	93.3	90.0
Mean gestation duration (days)	21.6 ± 0.1	21.6 ± 0.1	21.9 ± 0.09	22.0 ± 0.1

Values for mean gestation are mean ± standard error. *significantly different from control $p \leq 0.05$, **significantly different from control $p \leq 0.01$. Fertility index = No. of pregnant females/ No. of inseminated females x 100.

Offspring (F1-generation):

Litter size and viability (survival) were not affected by treatment at any dose level. There were no treatment related clinical signs of toxicity during lactation in males or females at any dose level. Incidental findings evident on occasion in several pups from various dose groups, including control, included bruising on the face, back and /or head and miscellaneous wounds, cuts, scratches or lacerations. These findings occurred randomly and were considered to be treatment related.

Clinical examination post-weaning showed no treatment related effects at any dose level. Incidental findings considered unrelated to treatment included, urine stain (2 control females, 2 low-dose males, 2 low-dose females and 1 mid-dose male), red nasal stain (1 control and 1 low-dose female), oral stain (1 low-dose male), thin body (1 low-dose male and female, 1 mid- dose male), and cool to touch body and unthrifty (1 control female). These findings are not considered treatment related as they occurred after treatment had been discontinued (i.e. after weaning), there was no dose related pattern and they occurred in control as well as treated animals. One control male was found dead on day 35, as well as 2 males from the low-dose group. One control female was found dead on day 30, as well as 1 female from the low-dose group.

Body weight (pre- and post-weaning)

There was no difference in birth weight at any dose level. On PND 4, no statistically significant effects on body weight gain were seen at 50 ppm and above compared to controls. In contrast, a statistically significant and dose related decrease in body weight gain was seen in both sexes on PND 11 at 50 ppm and above (≥ 9% in males and females). A statistically significant and dose related decrease in body weight gain was also seen in males at 50 ppm and above (≥ 7%) and in females at 150 ppm and above (≥ 9%) on PND 17, and PND 21 in males at 150 ppm and above (≥ 8%) as well as females at 50 ppm and above (≥ 7%). The study authors state that the differences in body weight gain seen in low-dose pups are not considered treatment related, as the differences were very modest, inconsistent, and within the range of historical controls (see Table 5 and 6).

Post weaning, a consistent and dose related decrease in body weight gain was seen in mid- and high-dose males on every PND of weighing from PND 42 (ie PND 42, 49, 56, 63 and 70). Compared to controls, decreases of 7-11% and 8-11% were seen from PND 42-70 in mid- and high-dose males respectively. In contrast, a statistically significant decrease in body weight gain was only see in high-dose females on PND 28, 35, 42 and 49 (7-12%, see Table 7).

Table 5. Pre-weaning pup body weights (grams).

Postnatal day	Dose (ppm in diet)							
	0	50 ppm	150 ppm	400 ppm	0	50 ppm	150 ppm	400 ppm
	Males				Females			

0	5.8 ± 0.09 (23)	5.8 ± 0.11 (23)	5.9 ± 0.09 (23)	5.9 ± 0.12 (21)	5.5 ± 0.08 (23)	5.5 ± 0.10 (23)	5.6 ± 0.08 (23)	5.6 ± 0.10 (21)
4	9.3 ± 0.18 (23)	9.1 ± 0.21 (23)	8.8 ± 0.18 (23)	8.5 ± 0.26 (21)	8.9 ± 0.17 (23)	8.7 ± 0.17 (23)	8.5 ± 0.18 (23)	8.2 ± 0.23 (21)
11	24.3 ± 0.42 (23)	22.3** ± 0.49 (23)	21.5** ± 0.50 (23)	21.1** ± 0.52 (21)	23.6 ± 0.36 (23)	21.7** ± 0.46 (23)	20.9** ± 0.54 (23)	20.4** ± 0.48 (21)
17	37.6 ± 0.67 (23)	35.0* ± 0.82 (23)	34.3** ± 0.68 (23)	33.3** ± 0.61 (21)	36.5 ± 0.63 (23)	34.1 ± 0.78 (23)	33.5** ± 0.70 (23)	32.5** ± 0.59 (21)
21	47.5 ± 0.78 (23)	44.5 ± 1.10 (23)	43.9** ± 0.81 (23)	42.5** ± 0.86 (21)	45.9 ± 0.62 (23)	43.0* ± 0.97 (23)	42.7* ± 0.90 (23)	41.3** ± 0.83 (21)

Values are mean ± standard error (n). *significantly different from control, $p \leq 0.05$, **significantly different from control $p \leq 0.01$.

Table 6. Pre-weaning historical control body weight ranges (grams).

Postnatal day	Male	Female
11	21.7-26.1	21.2-25.3
17	33.1-40.4	32.3-39.3
21	43.2-51.7	41.8-49.7

Values are mean ± standard error (n).

Table 7. Post-weaning pup body weights (grams).

Postnatal day	Dose (ppm in diet)							
	0	50 ppm	150 ppm	400 ppm	0	50 ppm	150 ppm	400 ppm
	Males				Females			
28	77.0 ± 10.4 (23)	75.0 ± 7.6 (23)	71.5 ± 6.9 (23)	69.1* ± 7.8 (21)	75.5 ± 10.3 (23)	73.3 ± 6.7 (23)	70.5 ± 6.6 (23)	67.5* ± 7.6 (21)
35	125.4 ± 13.0 (23)	117.7 ± 13.2 (23)	111.3 ± 10.5 (23)	110.1 ± 11.7 (21)	111.7 ± 9.8 (23)	108.5 ± 8.3 (23)	105.7 ± 7.7 (23)	102.2* ± 9.0 (21)
42	171.6 ± 14.1 (23)	162.2 ± 15.7 (23)	154.7* ± 12.8 (23)	154.0* ± 14.6 (21)	136.8 ± 9.4 (23)	134.6 ± 8.7 (23)	130.8 ± 7.2 (23)	126.6* ± 9.8 (21)
49	214.6 ± 15.6 (23)	203.5** ± 17.6 (23)	194.5** ± 14.2 (23)	193.2** ± 18.1 (21)	152.1 ± 9.9 (23)	149.1 ± 9.7 (23)	146.0 ± 8.4 (23)	142.6** ± 11.2 (21)
56	257.2 ± 17.9 (23)	245.7 ± 20.0 (23)	236.9* ± 16.6 (23)	234.9* ± 21.0 (21)	171.3 ± 11.6 (23)	167.2 ± 11.5 (23)	166.4 ± 8.9 (23)	161.9 ± 12.5 (21)
63	289.3 ± 19.3 (23)	277.8 ± 24.0 (23)	269.5* ± 17.0 (23)	267.2* ± 23.2 (21)	181.8 ± 11.5 (23)	178.2 ± 11.5 (23)	178.0 ± 9.4 (23)	172.9 ± 12.9 (21)
70	317.6 ±	304.8 ±	297.0* ±	294.0* ±	191.0 ±	187.6 ±	188.2 ±	182.9 ±

	22.7 (23)	26.7 (23)	19.1 (23)	25.1 (21)	11.4 (23)	11.4 (23)	10.2 (23)	13.7 (21)

Values are mean ± standard error (n). *significantly different from control, p ≤ 0.05; ** p ≤ 0.01.

Developmental landmarks

The onset of preputial separation was significantly delayed in mid- and high-dose males (day 47.1 and 46.8, respectively), compared to controls (day 44.9, see Table 8). Low-dose males were not affected. The average age of onset of vaginal patency was significantly delayed at the low- and mid-dose levels (day 34.2 at both dose levels) compared to controls (day 33.0). However, these vaginal patency findings are not considered treatment related, there was no dose response (i.e. did not occur in the high dose group), and the average age of onset for all treatment groups was within the historical control range for this laboratory (32.0-34.6 days).

There was no change in pupil constriction in response to a penlight in endosulfan treated animals compared to controls. No other landmarks of physical development were reported.

Table 8. Age of sexual maturation (days).

Parameter	**Dose (ppm in diet)**			
	Control	**50 ppm**	**150 ppm**	**400 ppm**
Number (M/F)	66/67	67/69	69/69	63/63
Preputial separation	44.9 ± 0.40 (66)	44.8 ± 0.29 (66)	47.1* ± 0.49 (69)	46.8* ± 0.43 (63)
% pups reaching criteria	100	98.5	100	100
Vaginal opening	33.0 ± 0.21 (66)	34.2* ± 0.30 (68)	34.2* ± 0.40 (69)	34.0 ± 0.40 (63)
% pups reaching criteria	98.5	98.6	100	100

Values are mean ± standard error (n). *significantly different from control, p ≤ 0.05.

Behavioural assessments:

FOB examination showed no treatment related effects at any dose level (Table 9). There was a statistically significant increase in the number of rears during open field observations in high-dose males (PND 45) and mid-dose females (PND 21). However, these observations were not considered a treatment related finding as statistical significance was only seen at a single observation point for each sex, and were within the historical control range for the laboratory (PND 45 males: 2.9-5.1 and PND 21 females: 4.1-6.4). Additionally, the increase seen in the number of rears was observed well after the treatment period had finished in males, and was not dose-related in females.

Table 9. Functional observational battery results in males (incidence)

Observation	Dose (ppm in diet)			
	control	50 ppm	150 ppm	400 ppm
Males				
Ease of removal, min. resistance and vocalization				
PND 4	0/16	0/16	1/16	3/16
PND 11	0/16	0/16	0/16	0/16
PND 21	0/16	0/16	0/16	0/16
PND 35	0/16	0/16	0/16	0/16
PND 45	NE	NE	NE	NE
PND 60	1/16	0/16	0/16	0/16
Rearing				
PND 4	NA	NA	NA	NA
PND 11	NA	NA	NA	NA
PND 21	4.6 ± 2.2	4.8 ± 2.6	6.6 ± 2.9	5.4 ± 2.8
PND 35	4.9 ± 3.0	3.8 ± 1.90	4.1 ± 1.7	3.4 ± 2.3
PND 45	3.4 ± 1.2	4.1 ± 2.2	4.4 ± 1.1	4.9* ± 1.4
PND 60	4.1 ± 2.2	4.4 ± 1.6	4.8 ± 3.4	3.9 ± 2.0
Females				
Ease of removal, min. resistance and vocalization				
PND 4	0/16	1/16	1/16	2/16
PND 11	0/16	1/16	0/16	1/16
PND 21	NE	NE	NE	NE
PND 35	NE	NE	NE	NE
PND 45	NE	NE	NE	NE
PND 60	NE	NE	NE	NE
Rearing				
PND 4	NA	NA	NA	NA
PND 11	NA	NA	NA	NA
PND 21	4.2 ± 1.8	4.4 ± 2.8	6.4* ± 2.4	5.5 ± 2.5

PND 35	3.9 ± 1.7	4.4 ± 1.8	5.2 ± 2.4	3.9 ± 2.0
PND 45	5.4 ± 2.1	4.9 ± 2.2	5.5 ± 2.2	6.1 ± 1.8
PND 60	7.2 ± 2.7	7.6 ± 2.2	6.4 ± 2.5	6.4 ± 2.9

* Statically different from control, $p<0.05$. NE = No effect seen. NA= No data available.

Motor and locomotor activity

There was a continuous increase in male and female motor activity and locomotor activity from PND 13 to 60. However, no difference was seen between control and treatment groups on any PND of measurement.

Auditory startle habituation

Startle amplitude, latency and habituation were not affected by treatment at any endosulfan dietary level compared to controls.

Learning and memory tests

In the post-weaning passive avoidance test performed on PND 22 and 29, there was no evidence of treatment related effects in males or females at any dietary level for acquisition and retention of the avoidance response. However, there was a statistically significant increase in males in the latency to cross for trial 2 of the learning phase in low- and high-dose males. There was also a statistically significant increase in latency for mid-dose males. These observed differences in males were not dose dependent, are not considered to be treatment related, and are considered to be due to the low mean trial 2 latency value seen in control males. Furthermore, an increased latency to cross during trial 2 reflects increased acquisition, relative to controls (i.e. none of the low- or high-dose males and only one mid-dose male crossed to receive the conditioning stimulus after trial 1).

In the adult offspring water maze test, there were no treatment related effects on acquisition (measured as the progressive decrease in the average time to escape) or retention in males or females at any dietary level.

Ophthalmology

There were no treatment related lesions in males or females at any endosulfan dietary level. Retinal degeneration was found in control and high-dose males and in low-dose females. These findings were not considered treatment related due to the lack of dose response, consistency by gender and/or because the incidence was reported to be within the historical control range (though no historical control data was provided).

Post mortem results

Sperm analysis

There were no treatment related effects on sperm motility, total sperm count or sperm morphology in high dose males compared to controls.

Gross pathology

No treatment related necropsy findings were seen in animals found dead, those sacrificed on PND 21 or at study termination (PND 75).

Terminal body weight and brain weight

PDN 21, a statistically significant decrease in terminal body weights compared to controls, and was considered a treatment related effect. PND 75 terminal bodyweight for perfused and non-perfused males and females was not affected by treatment at any dose level. A summary of terminal body weight and brain weight is presented in Table 10.

At PND 21, absolute fixed brain weights were significantly decreased in high-dose males only. This decrease was attributed to the decreased term body weight, as relative brain weight was not affected by treatment at any dose level in either sex. Furthermore, at PND 75, absolute and relative fixed brain weights were not affected by treatment at any dose level in either sex.

Brain measurement morphometry

Gross necropsy brain measurement: There was no difference in cerebrum and cerebellum lengths compared to controls in males or females at any dose level at either PND 21 or at experiment termination (PND 75).

Micropathology brain measurements: On PND 21 termination, there was a statistically significant decrease in the hippocampus thickness in high-dose females compared to controls. However, this finding was not considered to be treatment related as it is reported that the value is within the historical control range of the laboratory, and no similar finding observed in terminal high-dose females (though historical control data was not provided). On PND 75 termination there were no treatment related effects on brain measurements in high-dose males or females compared to controls.

Micropathology brain measurements: There were no treatment related findings in brain tissue from high-dose males and females at either PND 21 or PND 75.

Additional non-brain tissues

In addition, there were no treatment related microscopic lesions present in non-brain tissue from high-dose terminal males and females. Non-brain tissues examined were spinal cord, cauda equinea, spinal nerve roots, dorsal root ganglia, gasserian ganglion, eyes, optic nerves, gastrocnemius muscle, sciatic nerve, tibial nerve and sural nerves.

Table 10. Summary of terminal body weight on PND 21 and PND 75.

Observation	Dose (ppm in diet)			
	control	50 ppm	150 ppm	400 ppm
Males				
PND 21 (Perfused)				
Terminal body weight	49.2 ± 3.3	45.6 ± 5.4	46.6 ± 2.4	43.4* ± 4.5

(g)	(10)	(10)	(10)	(10)
Brain, fixed (g)	1.403 ± 0.06 (10)	1.398 ± 0.04 (10)	1.413 ± 0.056 (10)	1.331* ± 0.063 (10)
Brain, fixed/body weight (%)	2.858 ± 0.151 (10)	3.105 ± 0.375 (10)	3.039 ± 0.155 (10)	3.088 ± 0.214 (10)
PND 75 (Termination -perfused)				
Terminal body weight (g)	311.4 ± 33.9 (10)	307.0 ± 16.7 (10)	304.5 ± 27.3 (10)	302.4 ± 28.1 (10)
Brain, fixed (g)	1.833 ± 0.070 (10)	1.791 ± 0.056 (10)	1.783 ± 0.083 (10)	1.812 ± 0.122 (10)
Brain, fixed/body weight (%)	0.594 ± 0.057 (10)	0.585 ± 0.037 (10)	0.589 ± 0.050 (10)	0.602 ± 0.052 (10)
PND 75 (Termination- non perfused)				
Terminal body weight (g)	317.1 ± 25.0 (10)	304.5 ± 19.2 (10)	311.6 ± 19.2 (10)	310.9 ± 28.9 (10)
Brain, fixed (g)	1.910 ± 0.082 (10)	1.860 ± 0.077 (10)	1.909 ± 0.090 (10)	1.981 ± 0.084 (10)
Brain, fixed/body weight (%)	0.606 ± 0.054 (10)	0.613 ± 0.043 (10)	0.614 ± 0.039 (10)	0.612 ± 0.047 (10)
Females				
PND 21 (Perfused)				
Terminal body weight (g)	4.62 ± 3.1 (10)	43.7 ± 2.7 (10)	47.1* ± 5.6 (10)	41.5* ± 3.1 (10)
Brain, fixed (g)	1.344 ± 0.048 (10)	1.361 ± 0.021 (10)	1.306 ± 0.072 (10)	1.317 ± 0.031 (10)
Brain, fixed/body weight (%)	2.920 ± 0.118 (10)	3.128 ± 0.201 (10)	3.173 ± 0.365 (10)	3.194 ± 0.282 (10)
PND 75 (Termination -perfused)				
Terminal body weight (g)	197.5 ± 18.4 (10)	190.8 ± 18.1 (10)	187.4 ± 15.8 (10)	182.2 ± 16.6 (10)
Brain, fixed (g)	1.691 ± 0.074 (10)	1.722 ± 0.061 (10)	1.665 ± 0.049 (10)	1.669 ± 0.070 (10)

Brain, fixed/body weight (%)	0.861 ± 0.067 (10)	0.908 ± 0.073 (10)	0.894 ± 0.080 (10)	0.913 ± 0.089 (10)
PND 75 (Termination- non perfused)				
Terminal body weight (g)	193.5 ± 16.0 (10)	190.4 ± 8.8 (10)	193.0 ± 15.8 (10)	181.2 ± 17.39 (10)
Brain, fixed (g)	1.814 ± 0.057 (10)	1.810 ± 0.066 (10)	1.779 ± 0.112 (10)	1.728 ± 0.105 (10)
Brain, fixed/body weight (%)	0.943 ± 0.073 (10)	0.953 ± 0.064 (10)	0.927 ± 0.093 (10)	0.959 ± 0.079 (10)

Values are mean ± standard error (n). * Statistically different from control, p≤ 0.05

Discussion and conclusion:

Technical-grade endosulfan was administered via the diet from GD 6 through to LD 21 to mated female Wistar rats at doses of 0, 50, 150 or 400 ppm (equivalent to 0, 3.74, 10.8 and 29.8 mg/kg bw/day) in a developmental neurotoxicity study. The offspring were evaluated using detailed clinical observations, body weight, food consumption, developmental landmarks for sexual maturation, automated measures of activity, auditory startle habituation, learning and memory, and an ophthalmic examination. Tissues were collected for morphometry (brain) and microscopic examination on PND 21 (brain) and at study termination (brain, other neural tissues and skeletal muscle). Sperm analysis was also performed on selected control and high-dose males.

General observations: the average daily intake of endosulfan by dams during gestation and lactation was 0, 3.74, 10.8 and 29.8 mg/kg bw/day. There was no effect on reproduction parameters at any dietary level.

Treatment related effects in dams

At 50 ppm there was a statistically significant and dose related decrease in body weight gain (≥11%) and food consumption (≥12%) during gestation. Although these findings were ascribed by the study authors to result from palatability and not toxicity this cannot be reliably determined. Statistically significant decreases in body weight gain were also seen on LD days 0, 4 and 7 in the dams receiving 150 and 400 ppm endosulfan in the diet (6-9% and 8-13%, respectively). Consequently a maternal NOEL could not be established for this study as statistically significant decreases in body weight gain and food consumption were seen at the lowest dose tested. Thus, a maternal LOEL of 50 ppm (3.75 mg/kg bw/day) could only be established in this study.

Treatment related effects in offspring

At 50 ppm, a statistically significant decrease in body weight gain was seen in both sexes on PND 11 (9%), in males only on PND 17 (7%) and in females only on PND 21 (7%). However, the study authors did not consider these findings to be treatment related since the changes were modest and

inconsistent, and were within the historical control range for this laboratory. In contrast, a statistically significant and dose related decrease in body weight gain were seen at 150 ppm and above, the magnitude of which was statistically significant and of toxicological significance: ≥13% in both sexes on PND 11; and ≥10% in both sexes on PND 17. Additionally, the onset of preputial separation was significantly delayed in males at 150 ppm and above (day 47.1 and 46.8 at 150 and 400 ppm respectively, compared to day 44.9 in controls). No treatment related effect was observed on any behavioural parameter assessed. Additionally, no treatment related necroscopic findings were seen in the brain and non-brain tissues, and no effect was observed on the assessed sperm parameters. Consequently, a NOEL of 50 ppm (3.75 mg/kg bw/day) for offspring was established in this study, due to the observed decreases in body weight gain in both sexes and a delay in preputial separation in males at 150 ppm.

In conclusion, dietary administration of endosulfan to pregnant rats at the highest tolerated dose of 400 ppm from GD 6 to LD 21 (a dose which produced marked maternal toxicity) does not produce evidence of neurotoxicity in the offspring.