ENDOSULFAN FINAL REVIEW REPORT AND REGULATORY DECISION

JUNE 2005

Volume II.   Occupational Health & Safety technical report

Endocrine Disruption technical report

TABLE OF CONTENTS

9. OH&S ASSESSMENT TECHNICAL REPORT

9.1 BACKGROUND

Following the 1998 APVMA interim report of the review of endosulfan, based on the available data, concerns were raised with regard to exposure for workers during certain end-use and re-entry activities. Consequently, the APVMA decided that these uses of endosulfan could only continue on a temporary basis until additional worker exposure data was obtained.

Due to the lack of actual worker exposure data and the high toxicity of the chemical, worker exposure data generated under actual Australian conditions was considered essential to fully assess OHS risks. Work practices identified as requiring additional exposure data 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)
- Workers using hand-directed spray applicators (including nursery/greenhouses)

The Australian Centre for Agricultural Health and Safety (Moree) and the Centre for Pesticide Application Safety (Gatton) conducted exposure studies for workers treating orchard, broadacre and nursery crops by ground and aerial applications and re-entry to broadacre crops. All studies used the same formulation of endosulfan containing 350 g ai/L, which was considered representative of each of the products under review. The studies were conducted according to a protocol approved *by NOHSC/APVMA prior* to the commencement of the studies.

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 has now been submitted for consideration (Singer, 1995).

9.2 Dermal Absorption

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.

9.2.1 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 $\mu g/animal$ 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.

9.2.2   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 350 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 0.019 – 0.033 : 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			
Epidermis	0.40	0.28	1.17	0.79
Receptor fluid	0.13	0.33	1.18	1.90

109

Total absorbed	0.53	0.61	2.35	2.69
	Rat epidermis: Percent of applied dose detected in sample matrix			
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	0.019	0.025	0.032	0.033

9.2.3 Dermal absorption factor for exposure to concentrates and spray mixtures

From the above consideration, it is apparent that endosulfan is less well absorbed across rat skin *in vivo* than *in vitro*. This probably arises from the use of epidermal membranes *in vitro*, which lack the dermal layer, and are hence thinner and more permeable than whole skin. Under identical

experimental conditions, human epidermis is at least 30-fold less permeable to endosulfan than rat epidermis. Due to saturability at high concentrations, absorption of endosulfan from spray mixture across isolated human epidermis is approximately 5-fold more extensive than from the undiluted concentrate. Similarly, there was about 2.5-fold and 3-fold more absorption from spray mixture 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.

Endosulfan tends to accumulate in the skin and is absorbed into the systemic circulation over a number of days. Based on the rat *in vivo* data, absorption is greatest during the first day, slightly lower on the second day and negligible subsequently. For the purposes of human risk assessment, the amount absorbed into the systemic circulation during the first 24 hours (i.e. the effective daily dose) is the appropriate dose metric for comparison against relevant NOELs, which are themselves based on daily doses. However, a single exposure will need to be considered to result in exposure on a number of subsequent days. Using the amount absorbed on day 1 will provide an appropriately conservative estimate of the systemic dose on subsequent days. For repeated exposure of the same skin area, such as for the decanting and mixing procedures where the hands will be exposed repeatedly, the subsequent exposures are unlikely to significantly increase the daily systemic dose above that for the first exposure so this value remains appropriate. However, if there is repeated dermal exposure at different sites, the daily systemic doses may be additive.

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 0.025 = 0.50%, while the factor for exposure to spray mixture will be 46% x 0.033 = 1.52%.

9.2.4 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 (i.e. 1.52%; see discussion above), rather than the extent of absorption from exposure to concentrated formulations. A dermal absorption factor of 1.52% will be used for re-entry exposure assessment.

9.3   OCCUPATIONAL EXPOSURE STUDIES

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:

Internal patches (patches fixed with velcro) on the singlet, under the overalls

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

Internal patches (patches fixed with velcro) on the body of the worker

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.

External patches (on the overalls)

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	
Front of neck	(Back patch) x 110
Chest/stomach	(Chest patch) x 150
Back	(Chest patch) x 3550
Upper arms	(Back patch) x 3550
Forearm	(Mean int shoulder and forearm) x 1210
Hand	(Arm patch) x 2910
Thigh	(Glove result) x 2
Lower leg	(Thigh patch) x 3820
Feet	(Low leg patch) x 2380
	(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 9.2.1 outlines the parameters of each study. Section 9.2.2 summarises the dermal exposure data generated for the various occupational scenarios studied.

9.3.1 Parameters used in exposure studies

Worker exposure to endosulfan in the course of application in 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 boomspray (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 facepiece 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.

Worker exposure to endosulfan in the course of 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 facepiece 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.

Worker exposure to endosulfan in the course of 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.

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/filte	Indicate the paddock to be sprayed by waving a 1 m² 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

		rs/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		
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 a.i. handled during cleaning operations is not known.

(4) Data on acreage sprayed indicates <u>low volume</u> spraying (~200 L/ha)

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.

Worker exposure to endosulfan in the course of 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 broadacre 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

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), i.e. 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 µg/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 µg/cm^2 and 100 x the LOQ. The overall recovery for the validation samples ranged between 84% and 95% (±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 ($\mu g/cm^2$)		
	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

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 an assessment of supplementary dermal data. The revised dermal absorption rates were 0.5% for mixer/loader exposure and 1.5% for applicators and re-entry workers. The amended worker exposure data are presented in Tables 7-9 of this report. The PHED exposure data have also been amended, and are presented in Table 10.

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	0.0168	-	0.0014	-		0.0182
	Y	0.0001	-	0.0014	-		0.0015
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	0.0065		0.0015			0.008
	Y	0.0010		0.0015			0.0025

*Based on an application rate of 2.1 L/ha, handling 36.75 kg ai/day, 70 kg bw person, dermal absorption factor (0.5% M/L; 1.52% A) and 100% inhalation absorption.

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, travelling 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 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	0.0001			
Air-assist spray- no cabin (H-1-2-U)	0.0048	0.0730	0.1920		0.0029		0.0033
Air-assist-with cabin (H-1-2-C)	0.0014	0.0213	0.0560		0.0008		0.0013
Air-shear- with cabin (H-2-2-C)	0.0005	0.0076	0.0200		0.0003		0.0007
Oscillating boom spray (H-5-2-C)	0.0013	0.0198	0.0520		0.0008		0.0012
Cleaning down (H-1-4)	0.0005	0.0076	0.0200			0.0003	

[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 (0.5% M/L; 1.52% 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	0.00001	-	-	-
Application (H-3-2)	0.0082	0.0041	0.0041		0.00006	-	-
Cleaning down (H-3-3)	0.0024	0.0012	0.0012		-	0.00002	-
							0.00009

[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 (0.5% M/L; 1.52% 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	0.0009			
Mixing/Loading small containers (open/remote) (A-1-2)	0.00011	0.089	0.162	0.0008			
Aerial applicators (A-1-3)	0.00003	0.024	0.044		0.0007		
Support workers (vehicles) (A-1-4)	0.00001	0.008	0.015				0.0002
Support workers (ATVs) (A-1-5)	0.00005	0.041	0.074				0.0011
Cleaning down (A-1-6)	0.00002	0.016	0.029			0.0004	

[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 (0.5% M/L; 1.52% A & C)

M/L=mixing/loading; A=application; C=cleaning down; S= support workers

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 ($\mu g/cm^2$, 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/cm²	Study dermal exposure estimates (mg/kg bw/day) (with PPE)			Transfer coefficient (cm²/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 (cm²/hr) calculated using measured dermal exposure values for cotton chipping and crop checking and measured DFR following aerial application of endosulfan, TC (cm²/hr) = dermal exposure (mg/day) ÷ time spent for activity (hrs/day) x DFR (µg/cm²); [3] refers to the day before endosulfan was sprayed; ND no data

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/cm²)	Study dermal exposure estimates [2] (mg/kg bw/day) (without PPE)			Transfer coefficient [3] (cm²/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 (cm²/hr) calculated using measured dermal exposure values for cotton chipping and crop checking and measured DFR following aerial application of endosulfan, TC (cm²/hr) = dermal exposure (without PPE (mg/day) ÷ time spent for activity (hrs/day) x DFR (µg/cm²)
[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/cm²), 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/cm²) 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/cm²)			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	0.0034	0.0004	0.0027
			1			0.31			
			3	0.54	0.16	0.11	0.0015	0.0002	0.0012
			5			0.09			
			7	0.15	0.09	0.03	0.0004	0.0001	0.0004
			10			0.02			
Peaches	3.36	2.1	14	0.09	0.07	0.04	0.0003	0.0001	0.0003
			17			0.05			
			21	0.06	0.04	0.02	0.0002	0.00003	0.0001
			24			0.04			
			28	0.05	0.03	<0.01	0.0001	0.00003	0.0001
Grapes	1.68	2.1		0.05	0.03		0.0001	0.00003	0.0002
				0.03	0.03		0.0001	0.00003	0.0002
				0.02	0.05		0.0001	0.00005	0.0001
				0.02	0.02		0.0001	0.00002	0.0002
				0.02	0.01		0.0001	0.00001	<0.00003

LOQ Limit of quantitation = 0.01 µg/cm²
*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 (1.52%)
(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	0.0009	0.0005	0.0073	ND	0.0004	0.0002	0.0027	ND	ND	ND	ND	ND
1	0.0017	0.0008	0.0127	ND	0.0007	0.0003	0.0048	ND	ND	ND	ND	ND
2	0.0009	0.0004	0.0065	0.0011	0.0003	0.0002	0.0024	0.0006	ND	ND	ND	ND
3	0.003	0.0001	0.0011	0.0002	0.0001	0.00003	0.0004	0.0002	ND	ND	ND	ND
4	0.0002	0.0001	0.0012	0.0002	0.0001	0.00003	0.0005	0.0002	ND	ND	ND	ND
5	0.0001	0.00004	0.0007	0.0001	0.00005	0.00002	0.0003	0.0001	ND	ND	ND	ND
7	0.0001	0.0001	0.0009	0.0001	0.00005	0.00002	0.0003	0.0001	ND	ND	ND	ND
13	0.0001	0.00003	0.0004	0.00003	0.00002	0.00002	0.0002	0.00005	ND	ND	ND	ND
Aerial (26 cm crop)	**(109)**				**(99)**				**(383)**			
0	0.0006	0.0005	0.0077	ND	0.0002	0.0002	0.0029	ND	0.0005	0.0001	0.0019	0.0015
1	0.0006	0.0006	0.0088	ND	0.0002	0.0002	0.0033	ND	0.0006	0.0002	0.0022	0.0008
2	0.0002	0.0002	0.0024	0.0002	0.0001	0.0001	0.0009	0.0001	0.0002	0.00005	0.0006	ND
3	0.0001	0.0001	0.0015	0.0001	0.00003	0.00003	0.0006	0.0001	0.0001	0.00003	0.0004	ND
4	0.0001	0.0001	0.0009	0.0001	0.00003	0.00003	0.0004	0.0001	0.0001	0.00002	0.0002	ND
5	0.00005	0.00005	0.0007	ND	0.00002	0.00002	0.0003	ND	0.00005	0.00002	0.0002	ND

[1] dermal absorbed dose (mg ai/kg bw/day) = mean dermal exposure x dermal absorption (1.52%).

[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 1.52% (dermal absorption).

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

133

9.4   OCCUPATIONAL RISK ASSESSMENT

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, i.e. 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 (i.e. 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, i.e. 0.5% for the concentrate and 1.5% 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 was established 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).

9.4.1 Margin of Exposure

The MOE calculated for the various crops/situations and application methods from dermal exposure values determined from the studies (Table 16). MOE calculated for broad acre crops using ground application equipment were determined from PHED data. These are presented in Table 18.

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)	19200	-		-	-
Spraying air-assist, no cabin (H-1-2-U)	-	1745		-	1371
Spraying air assist, with cabin (H-1-2-C)	-	2400		-	1745
Spraying air-shear, with cabin (H-2-2-C)	-	6400		-	3200
Oscillating boomspray (H-5-2-C)	-	2743		-	1920
Cleaning down (H-1-4)	-	-		9600	-
Nursery crops (0.5 kg ai/2 hours/day)					
Mixing/loading (H-3-1)	192000	-	-	-	-
Spraying (H-3-2)	-	38400	-	-	24000
Cleaning down (H-3-3)	-	-	-	96000	-
Broad acre crops (Aerial application) (1470 kg ai/2000 ha/day)					
Mixing/loading, bulk and mini bulk, closed base (A1-1)	2400	-	-	-	-
Mixing/loading, small containers, open/remote (A1-2)	2743	-	-	--	-
Aerial application (A1-3)		2743	-	-	-
Support workers, vehicles (A1-4)		-	9600	-	-
Support workers, ATVs (A1-5)		-	1745	-	-
Cleaning down (A1-6)		-	-	4800	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	114	9600	1371	2242	105
	Y	19200	9600	1371	2242	1280
PHED Surrogate Scenario 28: All liquids, open pour, ground boom, open cab	N	295		1280		240
	Y	1920		1280		768

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

Ground application to tree crops

Tree crop workers handling 40 kg ai/day had acceptable MOE (when head/face exposure was included) for mixer/loaders (19200), applicators using air-assist with no cabin (662), air assist with cabin (2400), air shear with cabin (6400), and oscillating boom spray (2400). MOE for cleaners were acceptable (6400). When mixer/loader activities were combined with application using air-assist with & without cabin, MOE were acceptable (1600 & 582) air shear with cabin (2473) and oscillating boom spray equipment (1600). All MOEs for scenarios (single and combined) were also acceptable when head/face exposure was excluded.

Ground application to nursery crops

Nursery workers handling 0.5 kg ai/day had acceptable MOE (when head/face exposure was included) for mixer/loaders (192000), applicators (32000) and cleaners (96000). When all activities were combined, the MOE was also acceptable (21333). Similar results were seen when head/face exposure was excluded.

Aerial application to broadacre crops

Broadacre workers handling 1470 kg ai/day for aerial application had acceptable MOE (when head/face exposure was included) for mixer/loaders (2133 & 2400 for closed & open mixing), aerial applicators (2743), vehicle support workers (9600), ATV support workers (1745) & cleaners (4800). MOE for combined activities for broadacre crops (aerial) was not estimated as these activities are usually undertaken by different workers. Similar results were seen when head/face exposure was excluded.

Ground application to broadacre crops (from PHED data)

According to PHED estimates, workers handling 36.75 kg ai/day had acceptable MOE for open mixing/loading while not using gloves (105), and while using gloves (1280). MOE for applicators using open cab with and without using gloves were acceptable (9600) while treating broadacre crops using ground equipment. Acceptable MOE were also determined for workers combining both activities while not using gloves (240) and while using gloves (768).

9.4.2   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 19.

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) 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 absorbed 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 (1.52%). The MOE were then determined using the dermal absorbed dose and NOEL of 1.92 mg/kg bw/day.

Risks to re-entry workers (cotton crop)

MOE for re-entry activities (cotton chipping, crop checking and irrigating) were determined for workers from DFR (using calculated and generic transfer coefficients) and from measured worker exposure data (dosimeters) (Table 17). 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 .

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	2133	3840	263	ND	4800	9600	711	ND	ND	ND	ND	ND
1	1129	2400	151	ND	2743	6400	400	ND	ND	ND	ND	ND
2	2133	4800	295	1745	6400	9600	800	3200	ND	ND	ND	ND
3	640	19200	1745	9600	19200	64000	4800	9600	ND	ND	ND	ND
4	9600	19200	1600	9600	19200	64000	3840	9600	ND	ND	ND	ND
5	19200	48000	2743	19200	38400	96000	6400	19200	ND	ND	ND	ND
7	19200	19200	2133	19200	38400	96000	6400	19200	ND	ND	ND	ND
13	19200	64000	4800	64000	96000	96000	9600	38400	ND	ND	ND	ND
Aerial (26 cm crop)	**(109)**				**(99)**				**(383)**			
0	3200	3840	249	ND	9600	9600	662	ND	3840	19200	1011	1280
1	3200	3200	218	ND	9600	9600	582	ND	3200	9600	873	2400
2	9600	9600	800	9600	19200	19200	2133	19200	9600	38400	3200	ND
3	19200	19200	1280	19200	64000	64000	3200	19200	19200	64000	4800	ND
4	19200	19200	2133	19200	64000	64000	4800	19200	19200	96000	9600	ND
5	38400	38400	2743	ND	96000	96000	6400	ND	38400	96000	9600	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

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	564	4800	711	343	392	325	565	914
1	1280	9600	1600	800	873	914	1280	2133
3	4800	19200	4800	2743	3200	2743	4800	6400
5	6400	19200	6400	4800	4800	4800	6400	9600
7	9600	64000	19200	6400	9600	6400	9600	19200
10	19200	64000	19200	9600	9600	9600	19200	19200
14	19200	64000	9600	9600	9600	9600	19200	19200
17	19200	64000	9600	19200	19200	19200	19200	19200
21	19200	38400	19200	21333	19200	19200	19200	64000
24	19200	96000	9600	21333	19200	19200	19200	64000
28	19200	192000	<64000	21333	19200	19200	19200	64000

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

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.

9.5   Validity of new OHS studies

The Australian Centre for Agricultural Health and Safety (Moree) and the Centre for Pesticide Application Safety (Gatton) carried out and submitted worker exposure data for endosulfan for the following work practices:

- Mixer/loaders in ground and aerial applications
- Orchard ground spray applicators
- Broadacre aerial spray applicators including re-entry
- Broadacre ground spray (re-entry only)
- Workers using hand-directed spray applicators (nursery)

The study protocols (OHS Appendix 1) were adapted from the United States Environmental Protection Agency (US EPA) Occupational and Residential Exposure Test Guidelines and were approved by the APVMA, NOHSC and the New England Health Research Ethics Committee and the University of Sydney Research Ethics Committee. The EC formulation of endosulfan of 350 g ai/L was used in all the studies.

Generation of data on inhalation exposure was not included in the study protocols, since workers are required to wear respirators to protect against acute inhalation toxicity of endosulfan. In addition, data assessed in the interim report (APVMA 1998) indicated that dermal exposure represents >98% of exposure to endosulfan EC formulations during ground use (hand-held and mechanical spraying).

Overall, the studies were conducted according to the specifications of the protocol approved by NOHSC/APVMA, and in accordance with current label instructions, and use patterns, where deficient, in labels. The quality analysis for these studies including statistical analysis is presented in Appendices 2 and 3.

9.6 OHS REFERENCES

Clarke L & Churches T (1992) Pesticide Exposure in Cotton Chippers in the Gwydir Valley 1991-1992, Agricultural Health Unit, Moree District Hospital and NSW Health Department, December 1992.

Ebert E & Leist KH. (1989a) Endosulfan; Emulsifiable Concentrate; 353 g/L (Code HOE 002671 00 EC33 B317). Testing for acute oral toxicity in the male and female NMRI mice. Pharma Research Toxicology and Pathology, Frankfurt am Main, Research Project ID Study Number 89.0994, completed 19 October 1989. Hoechst Report no 89.1560, 18 December 1989. Hoechst document no A42359.

Ebert E & Leist KH. (1989b) Endosulfan; Emulsifiable Concentrate; 352 g/L (Code HOE 002671 00 EC33 B317). Testing for acute oral toxicity in the male and female Wistar rat. Pharma Research Toxicology and Pathology, Frankfurt am Main, Research Project ID Study Number 89.0844, completed 11 October 1989. Hoechst Report no 89.1565, 22 December 1989. Hoechst document no A42355.

Ebert E & Leist KH. (1989c) Endosulfan; Emulsifiable Concentrate; 352 g/L (Code HOE 002671 00 EC33 B317). Testing for acute dermal toxicity in the male and female Wistar rat. Pharma Research Toxicology and Pathology, Frankfurt am Main, Research Project ID Study Number 89.0845, completed 11 September 1989. Hoechst Report no 89.1366, 1 December 1989. Hoechst document no A42278.

Ebert E & Leist KH. (1989d) Endosulfan; Emulsifiable Concentrate; 352 g/L (Code HOE 002671 00 EC33 B317). Testing for primary eye irritation in the rabbit. Pharma Research Toxicology and Pathology, Frankfurt am Main, Research Project ID Study Number 89.0847, completed 26 September 1989. Hoechst Report no 89.1430, 5 December 1989. Hoechst document no A42223.

Ebert E & Leist KH. (1989e) Endosulfan; Emulsifiable Concentrate; 352 g/L (Code HOE 002671 00 EC33 B317). Testing for primary dermal irritation in the rabbit. Pharma Research Toxicology and Pathology, Frankfurt am Main, Research Project ID Study Number 89.0846, completed 12 September 1989. Hoechst Report no 89.1300, 14 November 1989. Hoechst document no A42256.

Ebert E & Leist KH. (1990) Endosulfan; Emulsifiable Concentrate; 352 g/L (Code HOE 002671 00 EC33 B317). Testing for acute oral toxicity in the male and female rabbit. Pharma Research Toxicology and Pathology, Frankfurt am Main, Research Project ID Study Number 90.0013, completed 1 February 1990. Hoechst Report no 90.0104, 17 April 1990. Hoechst document no A43165.

Handbook of First Aid Instructions and Safety Directions, (1998) Commonwealth Department of Health and Family Services and National Occupational Health and Safety Commission, Australian Government Publishing Service, Canberra.

Hollander & Weigand. (1976) Thiodan 25 ULV Pfl.-Ausl. 1347. Manuf. 1974 Batch No. 4491. Inhalation toxicity to the female SPF-Wistar-Rat : 4 h -LC 50. Pharma Research Toxicology and Pathology, Frankfurt am Main, Report no. 356/76, 20 August 1976. Hoechst document no A11643. Translation of document no. A08294

Hollander H & Weigand W. (1983) Acute aerosol toxicity in male and female SPF Wistar Rats 4 hours - LC50. Hoechst. Report No. 83.0397, 7 December 1983.

National Occupational Health and Safety Commission (1994) Control of Workplace Hazardous Substances [NOHSC: 1005(1994), 2007(1994)], Australian Government Publishing Service, Canberra.

National Registration Authority (1998) The NRA Review of Endosulfan, Existing Chemical Review Program, Canberra, Australia.

Singer S (1995) Dissipation of foliar residues of endosulfan following application of Phaser EC and Phaser WP to melons, peaches and grapes, Agrevo USA Company, USA.

Worker exposure to endosulfan (EC) in the course of application in tree crops (Mixing/loading)– Lyn Fragar, Report No. H-1-1 (February, 2002).

Worker exposure to endosulfan (EC) in the course of application in tree crops (Spraying air-assist spray-tractor without cabin)– Lyn Fragar, Report No. H-1-2U (February, 2002).

Worker exposure to endosulfan (EC) in the course of application in tree crops (Spraying air-assist spray-tractor with cabin)– Lyn Fragar, Report No. H-1-2C (February, 2002).

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).

Worker exposure to endosulfan (EC) in the course of application in tree crops (Oscillating boomspray)– Lyn Fragar, Report No. H-5-2-C (February, 2002).

Worker exposure to endosulfan (EC) in the course of application in tree crops (Cleaning down)– Lyn Fragar, Report No. H-1-4 (February, 2002).

Worker exposure to endosulfan (EC) in the course of application to nursery crops (Mixing/loading)– Lyn Fragar, Report No. H-3-1 (February, 2002).

Worker exposure to endosulfan (EC) in the course of application to nursery crops (Spraying)– Lyn Fragar, Report No. H-3-2 (February, 2002).

Worker exposure to endosulfan (EC) in the course of application to nursery crops (Cleaning down)– Lyn Fragar, Report No. H-3-3 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Mixing/Loading Bulk and Mini Bulk – closed base)– Lyn Fragar, Report No. A1-1 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Mixing/Loading small containers-open/remote)– Lyn Fragar, Report No. A1-2 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Aerial applicators)– Lyn Fragar, Report No. A1-3 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Support workers - vehicles)– Lyn Fragar, Report No. A1-4 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Support workers - ATVs)– Lyn Fragar, Report No. A1-5 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Cleaning down)– Lyn Fragar, Report No. A1-6 (February, 2002).

Worker exposure to endosulfan (EC) in the course of re-entry in broadacre cropping industries (Cotton chipping)– Lyn Fragar, Report No. RC 1-1 (February, 2002).

Worker exposure to endosulfan (EC) in the course of re-entry in broadacre cropping industries (Crop checking)– Lyn Fragar, Report No. RC 1-2 (February, 2002).

Worker exposure to endosulfan (EC) in the course of re-entry in broadacre cropping industries (Foliar Residue)– Lyn Fragar, Report No. RC 1-5 (February, 2002).

OHS APPENDIX 1

STUDIES USED IN THE OHS EXPOSURE ASSESSMENT OF ENDOSULFAN

The following end use and re-entry studies provided adequate data in order to conduct an OHS risk assessment for endosulfan and to provide recommendations for the safe use of endosulfan for the various activities assessed.

Singer S (1995) Dissipation of foliar residues of endosulfan following application of Phaser EC and Phaser WP to melons, peaches and grapes, Agrevo USA Company, USA.

Worker exposure to endosulfan (EC) in the course of application in tree crops (Mixing/loading)– Lyn Fragar, Report No. H-1-1 (February, 2002).

Worker exposure to endosulfan (EC) in the course of application in tree crops (Spraying air-assist spray-tractor without cabin)– Lyn Fragar, Report No. H-1-2U (February, 2002).

Worker exposure to endosulfan (EC) in the course of application in tree crops (Spraying air-assist spray-tractor with cabin)– Lyn Fragar, Report No. H-1-2C (February, 2002).

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).

Worker exposure to endosulfan (EC) in the course of application in tree crops (Oscillating boomspray)– Lyn Fragar, Report No. H-5-2-C (February, 2002).

Worker exposure to endosulfan (EC) in the course of application in tree crops (Cleaning down)– Lyn Fragar, Report No. H-1-4 (February, 2002).

Worker exposure to endosulfan (EC) in the course of application to nursery crops (Mixing/loading)– Lyn Fragar, Report No. H-3-1 (February, 2002).

Worker exposure to endosulfan (EC) in the course of application to nursery crops (Spraying)– Lyn Fragar, Report No. H-3-2 (February, 2002).

Worker exposure to endosulfan (EC) in the course of application to nursery crops (Cleaning down)– Lyn Fragar, Report No. H-3-3 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Mixing/Loading Bulk and Mini Bulk – closed base)– Lyn Fragar, Report No. A1-1 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Mixing/Loading small containers-open/remote)– Lyn Fragar, Report No. A1-2 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Aerial applicators)– Lyn Fragar, Report No. A1-3 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Support workers - vehicles)– Lyn Fragar, Report No. A1-4 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Support workers - ATVs)– Lyn Fragar, Report No. A1-5 (February, 2002).

Worker exposure to endosulfan (EC) in the course of aerial application in broadacre cropping industries (Cleaning down)– Lyn Fragar, Report No. A1-6 (February, 2002).

Worker exposure to endosulfan (EC) in the course of re-entry in broadacre cropping industries (Cotton chipping)– Lyn Fragar, Report No. RC 1-1 (February, 2002).

Worker exposure to endosulfan (EC) in the course of re-entry in broadacre cropping industries (Crop checking)– Lyn Fragar, Report No. RC 1-2 (February, 2002).

Worker exposure to endosulfan (EC) in the course of re-entry in broadacre cropping industries (Irrigating)– Lyn Fragar, Report No. RC 1-3 (February, 2002).

Worker exposure to endosulfan (EC) in the course of re-entry in broadacre cropping industries (Siphon Residue)– Lyn Fragar, Report No. RC 1-4 (February, 2002).

APPENDIX 2

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 OCS (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 <u>internal</u> 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. <u>externally</u>) 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 lyers" were provided by the study authors. However, when the data were plotted on a log-normal distribution, the so-called "out lyers" 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 3

COMPARISON OF NORMAL AND LOGARITHMIC DISTRIBUTION OF MIXER/LOADER/APPLICATOR/CLEANER/SUPPORT WORKER DATA FOR TREE, NURSERY AND BROADACRE CROPS.

Support workers for aerial application (normal)

Support workers for aerial application (Logarithmic)

Support workers (ATV) for aerial application (normal)

Support workers (ATV) for aerial application (Logarithmic)

Cleaners for aerial application (normal)

Cleaners for aerial application (Logarithmic)

150
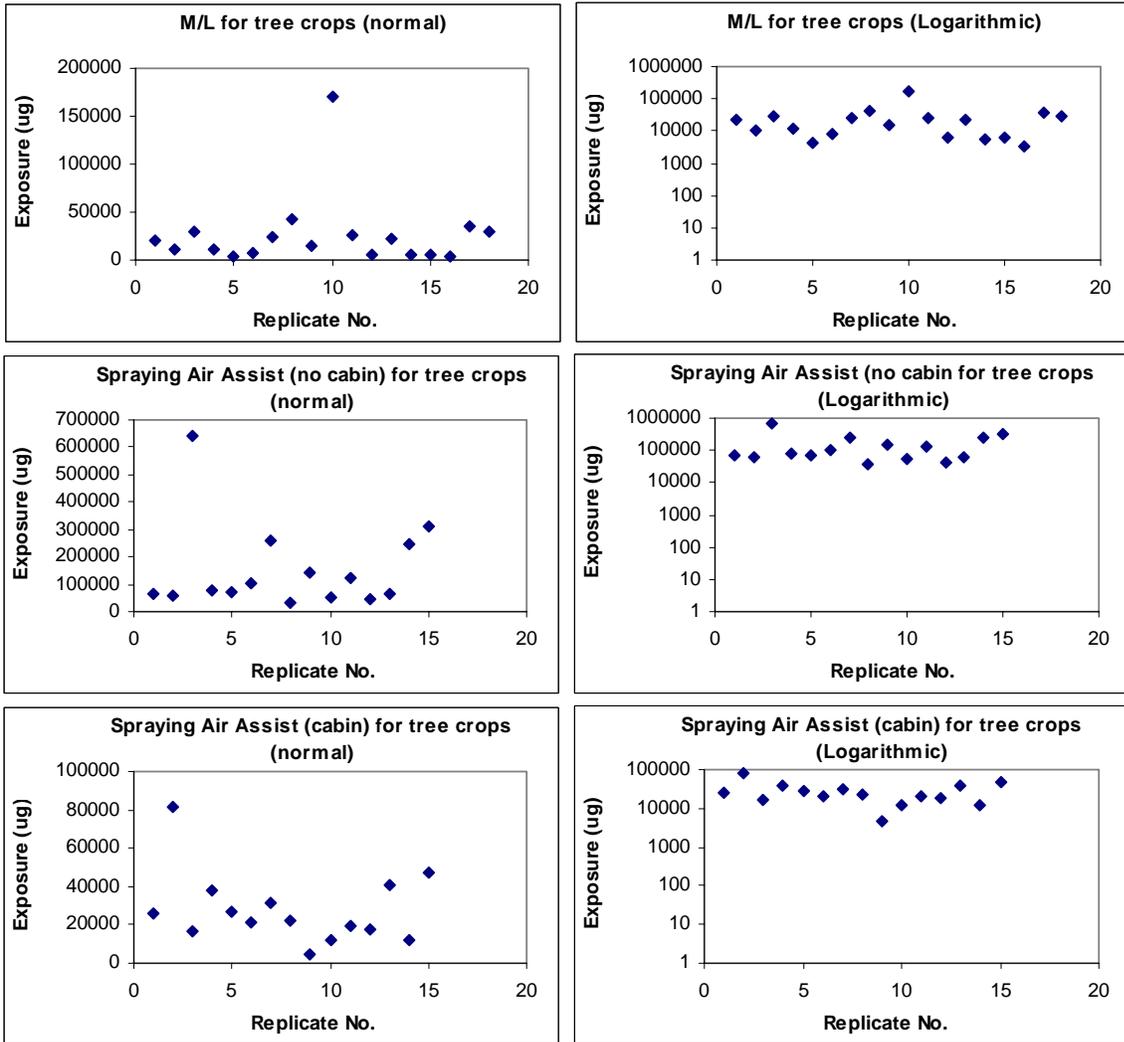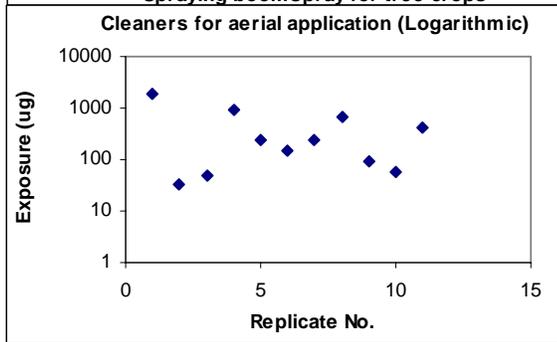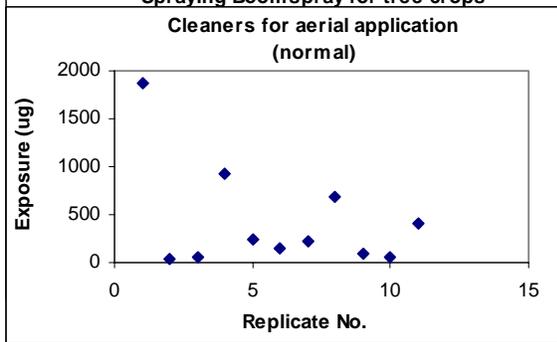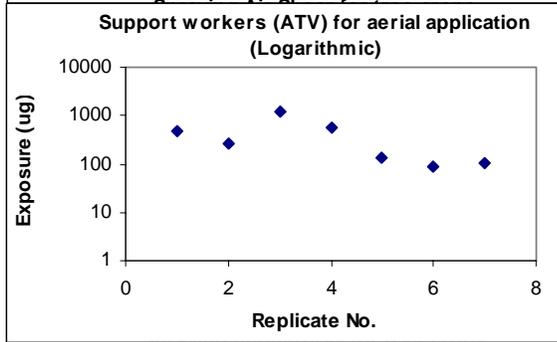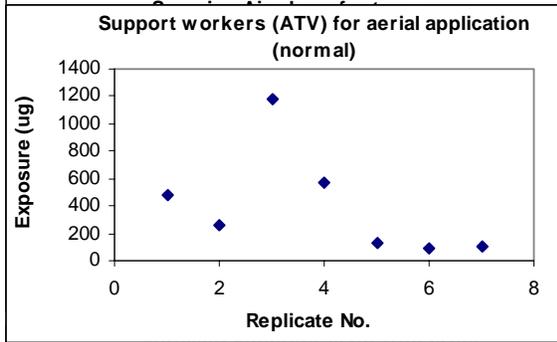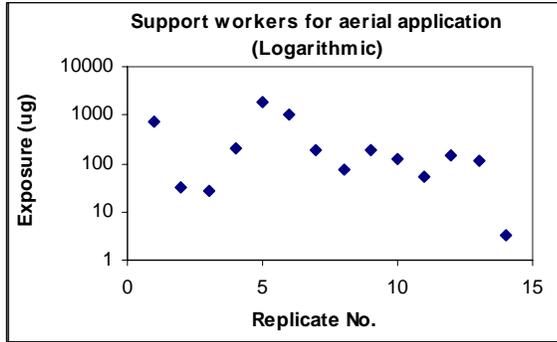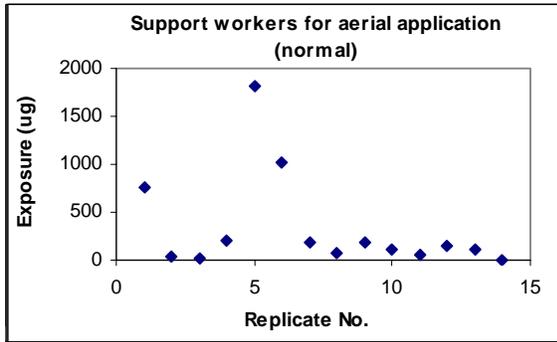
APPENDIX 4

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 (kg ai)	handled 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 (kg ai)	handled 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 (µg)	exposure 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 5 - NOHSC Risk Phrases

Endosulfan is classified by NOHSC in the List of Designated Hazardous Substances, with the following risk phrases:

R26	Very toxic by inhalation
R24/25	Toxic in contact with skin and if swallowed
R36	Irritating to eyes
R21/22	Harmful in contact with skin and if swallowed
R23	Toxic by inhalation
R20	Harmful by inhalation

The following cut-off concentrations apply for endosulfan:

Conc $\geq$ 25%	R26; R24/25; R36
$\geq$20% Conc<25%	R26; R21/22; R36
$\geq$7% Conc<20%	R26; R21/22
$\geq$3% Conc<7%	R23; R21/22
$\geq$1% Conc<3%	R23
$\geq$0.1% Conc<1%	R20

10.   ENDOCRINE DISRUPTION TECHNICAL REPORT

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

10.1   INTRODUCTION

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

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

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

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

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

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

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

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

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 end of April 2003 has been evaluated.

10.2   US EPA AND APVMA REPORTS

10.2.1 APVMA review of endosulfan

Regulatory history of endosulfan in Australia

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

Toxicology and Public Health Issues

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

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

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

No-observed-effect-level (NOEL) seen in a range of endosulfan studies
• 0.58 mg/kg/day in female mice in a 78-week dietary study, the highest dose tested;
• 0.64 mg/kg/day in rats in a 13-week dietary study, based on haematological changes and granular pigment formation in renal proximal tubules at 1.92 mg/kg/day;
• 0.57 mg/kg/day (females) and 0.65 mg/kg/day (males) in dogs in a 1-year dietary study, based on clinical signs and reduced body weights at 2.3 mg/kg/day;

- 0.66 mg/kg/day in female rats in a developmental study, based on decreased body weights at 2 mg/kg/day.
- 0.6 mg/kg/day in a 2-year rat dietary study, based upon reduced body weights and kidney pathology at 2.9 mg/kg/day.

10.2.2 The US EPA Reregistration Eligibility Decision

USA Regulatory history

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

Summary conclusions of endosulfan Reregistration Eligibility Decision

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

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

The US Chronic Reference Dose is 0.006 mg/kg bw/day derived by applying a 100-fold safety factor to the NOAEL of 0.6 mg/kg bw/day; it is based on reduced body weight gain, enlarged kidneys, increased incidences of marked progressive glomerulonephrosis; and blood vessel

aneurysms in male rats seen at the LOAEL of 2.9 mg/kg/day in a combined chronic toxicity/carcinogenicity study in rats.

FQPA safety factor: The FQPA Safety Factor of 10x for protection of children was retained for endosulfan. The RED comments that a weight-of-the-evidence approach indicated that there were no reliable data available to address concerns or uncertainties raised by the following matters: 1) evidence for increased susceptibility of young rats, (2) additional evidence for endocrine disruption, 3) uncertainty regarding neuroendocrine effects in the young, and 4) the need for a developmental neurotoxicity study (DNT). Hence an extra 10-fold safety factor was applied to each of the acute and chronic RfDs to derive the respective acute and chronic PADs of 0.0015 mg/kg bw and 0.0006 mg/kg bw/d.

Occupational health and safety issues: The EPA review concluded that there are potential mixer, loader, applicator as well as post-application exposures to occupational handlers. Based on current use patterns, there are some short-term dermal and inhalation risks of concern for workers who mix, load and apply endosulfan to agricultural sites, as well as to those workers who re-enter a treated area following application of endosulfan. To mitigate these risks, the US EPA mandated changes to packaging, deleted aerial application of WP products for some crops, and stipulated closed mixing/loading systems, closed cabs for air-blast equipment and restricted re-entry periods.

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

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

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

> Avian acute oral toxicity of bobwhite quail and mallard ducks
> Avian subchronic oral toxicity of bobwhite quail and mallard ducks
> Avian reproduction study
> Freshwater fish acute toxicity study of bluegill sunfish
> Early life stage fish
> Life cycle invertebrate
> Freshwater fish full life cycle using rainbow trout
> Estuarine/marine fish acute toxicity study
> Estuarine/marine invertebrate acute toxicity study of mysid shrimp
> Whole sediment acute toxicity testing using a freshwater invertebrate
> Whole sediment acute toxicity testing using a estuarine/marine invertebrate
> Whole sediment chronic toxicity testing using a freshwater invertebrate
> Whole sediment chronic toxicity testing using an estuarine/marine invertebrate
> Vegetative buffer effectiveness study
> Groundwater monitoring study

Surface drinking water monitoring study
Subchronic Neurotoxicity - Rat
Developmental Neurotoxicity Toxicity Study - Rat
Storage stability (oils seed, non-oily grain and processed commodities)
Crop field trials for the following raw agricultural commodities: barley hay, and pearled barley; oat forage, hay, and rolled oats; rye forage; wheat forage, and hay
Crop field trials for tobacco and a pyrolysis
Magnitude of residue in processed food/feed commodities
Dermal outdoor exposure for applying dip treatments to trees and roots or whole plants
Product use information for applying dip treatments to trees and roots or whole plants

10.3   IS ENDOSULFAN AN ENDOCRINE DISRUPTOR?

Definition and mechanisms

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

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

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

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

Australian and US EPA policy relating to Endocrine Disruptor Effects

The Australian Government first produced a paper on EDCs in April 1998 in response to public concerns. This document was redrafted in 2002; it acknowledges that Australian policy on EDCs remains under ongoing review and lends support to the IPCS EDC framework and the development and/or extension of appropriate OECD Test Guidelines. Australian agencies consider that endocrine disruption is but one part of a spectrum of effects that chemicals can cause if animals and humans are exposed to levels that overwhelm normal inactivation processes such as metabolism and excretion. That is, endocrine disruption is not considered to be an adverse end-point per se, but rather is a mode or mechanism of action potentially leading to other toxicological or eco-toxicological outcomes eg. reproductive, developmental, carcinogenic or ecological effects;

these effects are routinely considered in reaching regulatory decisions (at least for pesticides, food additive chemicals and high production volume industrial chemicals for which the required toxicology database is extensive). This position is quite similar to the US EPA position.

The US EPA view of endocrine disruption has resulted from changes in its underlying legislation. Under the 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.

The Australian vs. USA position on endosulfan as an endocrine disruptor

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

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

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

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

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

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

10.3.1 The toxicological database for endosulfan

A variety of chronic/carcinogenicity, reproductive and developmental studies on endosulfan, either published or submitted by the sponsors, have been evaluated for regulatory purposes. These

studies are suitable for evaluating the endocrine disrupting ability of endosulfan because they encompass a broad dose range often including the MTD, they assess a range of endpoints including indicators of endocrine disruption and they generally demonstrate a NOEL for most treatment effects. Several generalities are evident from the individual studies evaluated below. The chronic studies in mice, rats and dogs indicate that oral doses of endosulfan above ca. 1 mg/kg/d lead to hepatotoxicity and renal toxicity as the most common findings.

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

Chronic toxicity studies

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

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

Male and female Osborne-Mendel rats were dosed with endosulfan in the diet, with time-weighted average doses of 0, 223, and 445 ppm (0, 10, 20 mg/kg/d) for females, and 0, 408 and 952 ppm (0, 20, 40 mg/kg/d) for males for 78 weeks, with a return to control diets for a further 4 weeks. A dose related reduction in body weights was found at all doses in male rats as well as a highly significant morbidity rate such that by week 54, 52% of the high dose males had died. Histopathological examination revealed a high incidence of toxic nephropathy (>90%) in treated but not control males and females. Renal calcium deposits were also observed in treated males. The toxic nephropathy observed in animals was characterised as degenerative changes in the proximal convoluted tubules at the junction of the cortex and medulla, and associated cloudy swelling, fatty degeneration, and necrosis of the tubular epithelium. Parathyroid hyperplasia occurred in treated males, as did medial calcification of the aorta and medial calcification of the mesenteric artery, and calcium deposits in the stomach. A dose related increase in testicular atrophy occurred in treated male rats, characterised by degeneration and necrosis of the germinal cells lining the seminiferous tubules, multinucleated cells (fusion bodies), and calcium deposition resulting in aspermatogenesis. No treatment related effects were noted on the reproductive organs in female rats (Powers et al, 1978).

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

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

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

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

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

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

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

Reproductive Toxicity

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

Developmental Toxicity

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

Female Wistar rats were orally dosed with endosulfan from days 7-16 of gestation, at of 0, 0.66, 2, and 6 mg/kg body weight/day. No clinical signs of toxicity were reported in females at 0.66 or 2 mg/kg/day but four dams died with typical convulsive symptoms at 6 mg/kg/day. Body weight and bodyweight gain were reduced at 6

mg/kg/day. No statistically significant changes in reproductive or pup parameters were observed at any dose level in this study, and the foetal sex ratio was relatively balanced. No statistically significant increase in the incidence of abnormalities was observed in foetuses during examination. Skeletal examination revealed a statistically significant increase in fragmented thoracic vertebral centra at 6 mg/kg, an effect considered to reflect the frank maternotoxicity of endosulfan seen at the high dose level (Albrecht & Baeder, 1993).

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

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

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

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

Female Wistar rats were dosed with endosulfan orally at 0, 0.5 or 1.5 mg/kg bw/d for 21 d prior to mating, during the mating, pregnancy and lactation. Maternal and reproductive outcome data and male sexual development landmarks (testis descent

and preputial separation) were assessed. Reproductive endpoints of the male offspring examined at adulthood included: sex organ weights, daily sperm production, spermatid number, sperm transit, sperm morphology and testosterone level. No signs of maternal toxicity were detected at the dose levels tested. Sexual development landmarks were also unaffected. There were no statistically significant adverse effects of treatment on the reproductive endpoints investigated at adulthood except for a significant increase in the relative epididymis weight, not dose-related as it was seen only in the 0.5 mg/kg group (Dalsenter et al, 2003).

Testicular toxicity

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

In a later study by the same authors, the effect of sub-chronic endosulfan treatments on plasma and testicular testosterone, plasma gonadotrophins (follicle stimulating hormone (FSH), and luteinising hormone (LH)), and two of the four main enzymes of the testes involved in biosynthesis of testosterone from pregnenolone (3-β hydroxysteroid dehydrogenase (3-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).

Technical grade endosulfan was administered via oral gavage to groups of male Druckrey rats at doses of 0, 2.5, 5, and 10 mg/kg/day, on 5 days/week for 70 days. No changes in body weights or testis weight were seen in treated animals compared with controls. Statistically significant, dose related increases in testicular lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), gamma glutamyl transpeptidase (GGT), and glucose-6-phosphate dehydrogenase (G6PDH) activity were seen at all endosulfan dose levels. Statistically significant decreases in cauda epididymis sperm counts were seen at all test doses, with reductions of 22%, 43%, and 47%, at 2.5, 5, and 10 mg/kg/day, respectively. In the absence of historical control data, it is unclear whether the decrease in sperm count at 2.5 mg/kg/day (22%) was within the expected biological range for the test animals. Statistically significant reductions in spermatid count (about 16%) and sperm production rate (about 22%) were also reported at 5 and 10 mg/kg/day but the biological significance of these changes is unclear as there was no dose relationship. Thus, the administration of endosulfan at doses of 2.5 mg/kg/day and above for several months resulted in testicular toxicity as evidenced by increased testicular enzyme activity and marked reduction in sperm counts at 5 mg/kg/day and above (Sinha et al, 1995).

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

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

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

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

Signs of hepatotoxicity were dose related (liver enlargement, reduced serum LDH activity, increased serum cholesterol and protein levels) and elevated hepatic 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 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).

Thyroid toxicity

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

the TDI (USA). Adult male rats were exposed to the mixture by gavage at 0, 1, 10, 100, and 1000 times the estimated safe levels daily for 70 days. Endpoints related to circulating thyroid hormone (serum thyroxine [T(4)], triiodothyronine [T(3)], thyroid stimulating hormone [TSH], and serum T(3) uptake [T(3)-up]), thyroid gland histomorphology (thyroid follicle cross sectional area, epithelial height, follicle roundness or aspect ratio, colloid/epithelial ratio) and hepatic metabolism of thyroid hormone (UDP-glucuronyl transferase [UGT] and outer-ring deiodinase [ORD]) were assessed.

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

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

Adrenal toxicity

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

Pituitary toxicity

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

Oestrogenic effects

A study primarily designed to examine the interaction between endosulfan and dieldrin in the activation of ER in or extracted from mammalian cells showed that endosulfan induced cell proliferation in the MCF-7 human breast cancer cell line between 2 and 4 times control levels at exposure levels of 10 and 50 µM, but had no proliferative effect at 2 µM. Endosulfan and dieldrin showed no synergism in displacing ^{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 estrogenic synergism when incubated in combination with dieldrin (Arcaro et al. 1998).

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

In apparent contradiction of these positive findings, endosulfan (isomeric composition not reported) did not substantially affect the growth of either ER-positive (MCF-7) or ER-negative (SK-BR-3) cultured human breast cancer cell lines at concentrations of 35 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 activities of these compounds was essentially additive. In addition, the investigators reinvestigated all of the binary mixtures of organochlorine pesticides reported by Arnold et al (1996). in two yeast based assays, and found that the estrogenic activities of all of the binary mixtures of organochlorine pesticides were additive, not synergistic (Ramamoorthy et al, 1997).

Continuous exposure of adult male sheepshead minnow (*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).

Neurobehavioural effects

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

Immunotoxicity

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

Another study by the same authors also investigated immune competence in male Wistar rats treated with endosulfan in the diet for 22 weeks with interim sacrifices. At 19 weeks of exposure the rats were immunised with tetanus toxoid. There were no clinical signs or effects on bodyweights but there was a decrease in thymus weight at the high dose of 1.0 mg/kg/d. Measures of immune response showed a significant time and dose-related decrease at 0.5 and 1.0 mg/kg/d (Banarjee & Hussain, 1986).

10.4 DISCUSSION

Chronic, developmental and reproductive toxicity

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

One criticism of the developmental studies is that the mandated observations do not address subtle endocrine-related changes that might only be evident in maturity. It is biologically plausible that the earliest life stages are the most sensitive to endocrine disruption, whether because the foetus is uniquely sensitivity or merely quantitatively more sensitive. The developmental effects of endocrine 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/d administered to Wistar rats pre-mating and throughout mating, pregnancy and lactation, did not induce maternotoxicity and had no effect on sex organ weights, daily sperm production, spermatid number, sperm transit, sperm morphology and testosterone level in male offspring (Dalsenter et al, 2003). Another study dosed pregnant Druckrey rats with endosulfan at 0, 1 or 2 mg/kg bw/d from day 12 of gestation through parturition and reported dose related increases in testicular LDH and SDH as well as reduced spermatid and sperm counts and decreases in testis, epididymis and seminal vesicle weights (Sinha et al, 2001). These contrasting results indicate that there may be differences in susceptibility of the male reproductive system to endosulfan depending on the rat species and treatment period used.

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

Testicular toxicity

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

Thyroid toxicity

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

Adrenal toxicity

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

Pituitary toxicity

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

Oestrogenicity

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

Immune toxicity

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

Synergy

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

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

Similar difficulties arise when extrapolating data obtained from the reported *in vitro* assays to effects observed *in vivo*, and for the extrapolation of evidence of endocrine

activity in what are simple screening assays to the ability to induce adverse effects in more traditional testing protocols.

Exposure

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

Bioaccumulation

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

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

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

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

10.5 CONCLUSION

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

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

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

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

10.6 TOXICOLOGY REFERENCES

Albrecht & Baeder (1993). Hoe 002671 - substance technical (Code: Hoe 002671 00 ZD98 0005). Testing for embryotoxicity in the Wistar rat after oral administration. Pharma Development Central Toxicology, Laboratory project RT: RR0663, TOXN: 92.0695, 18 November 1993. Hoechst report 93.0716. Hoechst document A51695.

Arcaro KF, Vakharia DD, Yang Y, Gierthy JF (1998) Lack of synergy by mixtures of weakly estrogenic hydroxylated polychlorinated biphenyls and pesticides. Environ Health Perspect 106 Suppl 4:1041-6.

Arnold SF, Klotz DM, Collins BM, Vonier PM, Guilette LJ and McLachlan JA (1996) Synergistic activation of oestrogen receptor with combinations of environmental chemicals. Science, 272, 1489-1492.

ATSDR (2000) Toxicological profile for endosulfan. Agency for Toxic Substances and Disease Registry.

Banarjee & Hussain (1986) Effect of subchronic endosulfan exposure on humoral and cell-mediated immune responses in albino rats. Arch. Toxicol. 59: 279-284.

Banarjee & Hussain (1987) Effects of endosulfan on humoural and cell-mediated immune responses in rats. Bull Environ. Contam. Toxicol. 38: 438-441

Bisson M, Hontela A (2002) Cytotoxic and endocrine-disrupting potential of atrazine, diazinon, endosulfan, and mancozeb in adrenocortical steroidogenic cells of rainbow trout exposed in vitro. Toxicol Appl Pharmacol 180(2):110-7.

Blair RM, Fang H, Branham WS, Hass BS, Dial SL, Moland CL, Tong W, Shi L, Perkins R, Sheehan DM (2000) The oestrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands. Toxicol Sci 54(1):138-53.

Brunk R (1990). Addendum to report 89.0188. Hoechst report 89.0188. Hoechst document no A44605.

Brunk R (1989). Endosulfan-substance technical (Code: Hoe 002671 OI ZD96 0002). Testing for toxicity by repeated oral administration (1-year feeding study) to Beagle dogs. Pharma Research Toxicology and Pharmacology Study no 87.0643, 20 January 1989. Hoechst report no 89.0188, 16 March 1989. Hoechst document no A40441.

Choudhary N & Joshi SC (2003) Reproductive toxicity of endosulfan in male albino rats Bull Environ Contam Toxicol 70(2):285-9

Dalsenter PR, de Araujo SL, de Assis HC, Andrade AJ, Dallegrave E (2003). Pre and postnatal exposure to endosulfan in Wistar rats. Hum Exp Toxicol. 22:171-5.

Dalsenter PR, Dallegrave E, Mello JR, Langeloh A, Oliveira RT, Faqi AS. (1999) Reproductive effects of endosulfan on male offspring of rats exposed during pregnancy and lactation. Hum Exp Toxicol. 18:583-9.

Donaubauer, HH (1988). Endosulfan-substance technical (Code: Hoe 002671 OI ZD97 0003). Carcinogenicity study in mice: 24 months feeding study. Pharma Research Toxicology and Pathology, Germany: Study no 745; TOXN no 83.0113; Completed 6 April, 1988. Hoechst report no 88.0278, 6 April, 1988. Hoechst document no A38008.

Donaubauer, HH (1989). Amendment to the report no 88.0278 Endosulfan-substance technical (Code: Hoe 002671 OI ZD97 0003). Carcinogenicity study in mice: 24 months feeding study. Pharma Research Toxicology and Pathology, Germany: Study no 745; TOXN no 83.0113; Completed 13 September, 1989. Hoechst report no 89.1288, 13 September 1989. Hoechst document no A41617.

EDSTAC 1998. Endocrine Disrupter Screening and Testing Advisory Committee Final Report http://www.epa.gov/scipoly/oscpendo/history/finalrpt.htm .

Edwards JA, Reid YJ, Offer JM, Almond RH, Gibson WA (1984). Effect of endosulfan technical (Code: Hoe 02671 O I AT209) on reproductive function of multiple generations in the rat. Huntingdon Research Centre, UK, Report no HST/204/83768, 19 July 1984. Hoechst document A29428.

Gupta PK, Chandra SV, Saxena DK (1978). Teratogenic and embryotoxic effects of endosulfan in rats. Acta pharmacol. et toxicol., 1978, 42, 150-152.

Harris HA, Bapat AR, Gonder DS, Frail DE The ligand binding profiles of estrogen receptors alpha and beta are species dependent. Steroids 67(5):379-84.

Hazelton Laboratories. (1959a) 2 Year dietary study in rats. Hazleton Laboratories, Ref: A-199-119, 22 May 1959. [SB:HO, 1973, m/f].

Hazelton Laboratories. (1959b) One-year oral study in dog. Hazleton Laboratories, 12 May 1959. [SB:HO, 1973, m/f].

Hemmer MJ, Hemmer BL, Bowman CJ, Kroll KJ, Folmar LC, Marcovich D, Hoglund MD, Denslow ND (2001) Effects of p-nonylphenol, methoxychlor, and endosulfan on vitellogenin induction and expression in sheepshead minnow (Cyprinodon variegatus) Environ Toxicol Chem 20:336-43.

Hsu J-T, Ying C, Lan H-C (1998) The effects of pesticides chlordane, dieldrin and endosulfan on the growth of human breast cancer cell lines MCF-7 and SK-BR-3. J Chinese Agri Chem Soc 36(6):535-546. Cited in ATSDR, 2000.

Khan PK & Sinha SP (1996). Ameliorating effect of vitamin C on murine sperm toxicity induced by three pesticides (endosulfan, phosphamidon and mancozeb). Mutagenesis 11 (1), 33-36.

MacKenzie (1980) Teratology Study with FMC 5462 in Rats. Raltech Scientific Services, Study No. 79041, 1980.

MacKenzie (1981) Teratology Study with FMC 5462 in Rabbits. Raltech Scientific Services, Study No. 80070, 27 July 1981.

Matthews JB, Fertuck KC, Celius T, Huang YW, Fong CJ, Zacharewski TR (2002) Ability of structurally diverse natural products and synthetic chemicals to induce gene expression mediated by estrogen receptors from various species.J Steroid Biochem Mol Biol 82(2-3):181-94.

Murono EP, Derk RC, de Leon JH (2001). Differential effects of octylphenol, 17beta-estradiol, endosulfan, or bisphenol A on the steroidogenic competence of cultured adult rat Leydig cells.Reprod Toxicol 15(5):551-60

National Research Council 1999. Hormonally Active Agents in the Environment . National Academy Press, Washington .

OECD (1998) Report of the First Meeting of the OECD Endocrine Disrupter Testing and Assessment (EDTA) Working Group, 10-11 March 1998,

ENV/MC/CHEM/RA(98)5. Paris: Organization for Economic Cooperation and Development, 1998.

Offer (1985). Addendum to HST 204 Effect of endosulfan-technical (Code: HOE 02671 0I AT209) on the reproductive function of multiple generations in the rat. Histopathological review of the kidneys in adult rats of the F1B generation and in weanling rats of the F2B generation. Huntingdon Research Centre (HRC), UK, 22 March, 1985. Hoechst document A30757.

Pandey N, Gundevia F, Prem AS, Ray PK (1990) Studies on the genotoxicity of endosulfan, an organochlorine insecticide, in mammalian germ cells. Mutat Res 242:1-7.

Paul V, Easwaramoorthy B, and Kazi M (1994) The neurobehavioural toxicity of endosulfan: a serotonergic involvement in learning impairment. European Journal of Pharmacology-Environmental Toxicology and Pharmacology Section, 270, 1-7.

Paul V, Easwaramoorthy B, Arumugam RJ and Kazi M (1995) A sex-related difference in the neurobehavioural and hepatic effects following chronic endosulfan treatment in rats. European Journal of Pharmacology-Environmental Toxicology and Pharmacology Section, 293, 355-360.

Paul V, Sheela S, Balasubramaniam E and Kazi M (1993) Behavioural and biochemical changes produced by repeated oral administration of the insecticide endosulfan in immature rats. Indian Journal of Physiology and Pharmacology, 37, 204-208.

Powers MB et al (1978) National Cancer Institute Bioassay of endosulfan for possible carcinogenicity:78-week dietary study in Osborne-Mendel rats and B6C3F1 mice. NCI Study No.NCI-CG-TR62, Technical Report Series No. 62. Carcinogenesis testing program, Division of Cancer Cause and prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

Ramamoorthy K, Wang F, Chen I-C, Safe S (1997). Potency of combined estrogenic pesticides. Technical Comments, Science 275, p 405, 17 January 1997.

Rousseau J, Cossette L, Grenier S, Martinoli MG (2002) Modulation of prolactin expression by xenoestrogens. Gen Comp Endocrinol 126(2):175-82

Ruckman SA, Waterson LA, Crook D, Gopinath C, Majeed SK, Anderson A, Chanter DO (1989). Endosulfan, active ingredient technical (Code: Hoe 002671 OI ZD97 0003). Combined chronic toxicity/carcinogenicity study (104-week feeding study in rats). Huntingdon Research Centre, UK, report HST/289/881067, 1 April 1989. Hoechst document A40440.

Singh SK and Pandey RS (1990) Effect of sub-chronic endosulfan exposures on plasma gonadotrophins, testosterone, testicular testosterone and enzymes of androgen biosynthesis in rat. Indian Journal of Experimental Biology, 28, 953-956.

Singh SK, Pandey RS (1990) Effect of sub-chronic endosulfan exposures on plasma gonadotrophins, testosterone, testicular testosterone and enzymes of androgen biosynthesis in rat. Indian J Exp Biol 28(10):953-6.

Singh SK, Pandey RS (1989) Gonadal toxicity of short term chronic endosulfan exposure to male rats. Indian J Exp Biol 27(4):341-6.

Sinha N, Narayan R, Shanker R, Saxena DK (1995) Endosulfan-induced biochemical changes in the testis of rats. Vet Hum Toxicol 37(6):547-9.

Sinha N, Adhikari N, K Saxena D. (2001) Effect of endosulfan during fetal gonadal differentiation on spermatogenesis in rats. Environ. Toxicol. Pharmocol. 10:29-32.

Sinha N, Lal B, Singh TP (1991) Effect of endosulfan on thyroid physiology in the freshwater catfish, Clarias batrachus. Toxicology 67:187-97.

Soto AM, Chung KL and Sonnenschein C (1994) The pesticides endosulfan, toxaphene and dieldrin have oestrogenic effects on human oestrogen-sensitive cells. Environmental Health Perspectives, 102, 380-383.

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO (1995) The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 103 Suppl 7:113-22.

Wade MG, Desaulniers D, Leingartner K, Foster WG (1997) Interactions between endosulfan and dieldrin on estrogen-mediated processes *in vitro* and *in vivo*. Reprod Toxicol 11(6):791-8

Wade MG, Foster WG, Younglai EV, McMahon A, Leingartner K, Yagminas A, Blakey D, Fournier M, Desaulniers D, Hughes CL (2002b) Effects of subchronic exposure to a complex mixture of persistent contaminants in male rats: systemic, immune, and reproductive effects Toxicol Sci 67(1):131-43

Wade MG, Parent S, Finnson KW, Foster W, Younglai E, McMahon A, Cyr DG, Hughes C. (2002a) Thyroid toxicity due to subchronic exposure to a complex mixture of 16 organochlorines, lead, and cadmium. Toxicol Sci 67(2):207-18