Section 5

EVALUATION OF THE MAMMALIAN TOXICOLOGY AND METABOLISM/TOXICOKINETICS

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1. INTRODUCTION

1.1 Regulatory History of Health Considerations in Australia

Endosulfan is a synthetic cyclodiene compound used in agriculture to control a range of insects and mites on a broad spectrum of crops. Endosulfan has been available in Australia for over 30 years and over this period has been used in the home garden (currently not permitted), commercial food crops and other crops such as cotton. There are some 15 end use products (EUPs) of endosulfan with over 400 approved (registered) uses Australia wide. Endosulfan has established maximum residue limits (MRLs) in a wide range of food crops (see below).

The toxicology of endosulfan was first considered by the National Health & Medical Research Council (NHMRC) of Australia for the Department of Health and Family Services (here after called the Department) in 1968. The NHMRC set an acceptable daily intake (ADI) of 0.007 mg/kg/day based on the No-Observed-Effect-Level (NOEL) of 0.75 mg/kg/day established in a 12 month dietary study in dog.

The toxicology of endosulfan has been evaluated and reviewed by the Department on a number of occasions since 1965. This has occurred for a number of reasons including the identification, by the IBT Taskforce Program, of endosulfan as having IBT generated data in its toxicological data package which required either verification or replacement. In addition, as part of the Technical Grade Active Constituent (TGAC) Clearance Scheme (1985-1992), the Department reviewed the toxicology profile of some 400 agricultural chemicals, including endosulfan.

Endosulfan has also been considered as part of the Department's review of organochlorine pesticides. As a result, the toxicology data for endosulfan was re-evaluated by the Department over the period 1987 to 1989. In addition, three applications for clearance of a source of manufacture for endosulfan have been evaluated since 1990.

Public Health Standards

In Australia, public health standards for agricultural and veterinary chemicals, such as the poison schedule, first aid and safety directions and an acceptable daily intake (ADI), are set by the Department. Poisons schedules are set by the National Drugs and Poisons Schedule Committee (NDPSC) of the Australian Health Minister's Advisory Council (formerly the Drugs and Poisons Schedule Committee (DPSC) of the National Health and Medical Research Council (NHMRC). In the case of maximum residue limits (MRLs), these were formerly established by the Pesticide and Agricultural Chemicals Standing Committee (PACSC) of the NHMRC, however, in 1992, the Department became directly responsible for establishing MRLs, a function then formally transferred to the National Registration Authority (NRA) in June 1994.

The regulatory history of public health considerations of endosulfan in Australia is summarised below.

DATE	REGULATORY ACTIVITY
1968	ADI set at 0.007 mg/kg/day; Schedule 6 (S6); MRLs established.
1971	Change from S6 to S7 (acute toxicity concerns).
1973	Reschedule S7 to S6 (acute toxicity concerns addressed by sponsor).
1980-1983	MRLs: goat meat, sorghum, lupins, maize, cereals, nuts, soybeans etc.
1981-1982	Endosulfan toxicology data package contains some IBT studies. Endosulfan under watching brief until replacement studies generated.
1982	Claims of carcinogenicity potential of endosulfan by RM Ruber (The Science of the Total Environment, 20, 23-47, 1981) discounted after review of available data, consideration of the US EPA decision and sponsor comments.
1985	TGAC clearance evaluation.
1987	Replacement IBT studies reviewed. NOEL 0.75 mg/kg/day & ADI of 0.007 mg/kg confirmed, S6 Organochlorine (OCs) pesticides under review (including endosulfan), foreshadow the restriction of OCs for indoor and outdoor living area use and home garden use. NHMRC call for full industry consultation.
1988	TGAC clearance, review of all available toxicology data, NOEL & ADI confirmed. Home garden use concerns, schedule changes foreshadowed restricting concentrations available for such use.
1989	Sponsors requested for formulation details and toxicology on EUPs. Review of safety directions. Published reports of possible immunotoxicity of endosulfan evaluated.
1990	Additional toxicology data reviewed, TGAC clearance evaluation. Poisons information reviewed. Endosulfan withdrawn from the home market and a change from S6 to the more restrictive S7.
1990-1993	Three separate applications for approval of a source of manufacture approved.
1993	Current ADI is 0.007 mg/kg/day, poison schedule 7.
1995	NDPCS confirm S7;

Nominated onto the NRA's ECRP Priority Review Candidate List

1996/7 Data call in and public submissions received and evaluation of all available toxicology data undertaken

Some 400 approved uses are listed for endosulfan and MRLs are established for a wide range of food groups. The Australian MRL Standard (1994), a Standard for maximum residue limits of pesticides, agricultural chemicals, feed additives and veterinary medicines in food and feedstuffs, listings for endosulfan are summarised in Table below.

The Australian MRL standard listings for endosulfan

CODEX CODE	FOOD	MRL(mg/kg)
VR 0577	Carrot	0.2
MO 0812	Cattle, edible offal of	0.2
MM 0812	Cattle meat (in the fat)	0.2
GC 0080	Cereal grains	0.2
VD 0526	Common bean (dry)[navy bean]	1
OC 0691	Cotton seed oil, crude	0.5
PE 0112	Eggs	*0.05
	Fruits	2
VO 0050	Fruiting vegetables, other than curcurbits	2
MO 0814	Goat, edible offal of	0.2
MM 0814	Goat meat (in the fat)	0.2
VD 0545	Lupin (dry)	1
ML 0106	Milks (in the fat)	0.5
VD 0536	Mung bean (dry)	1
SO 0088	Oilseed	1
VA 0385	Onion, bulb	0.2
SO 0697	Peanut	1
VR 0589	Potato	0.2
PO 0111	Poultry, edible offal of	0.2
PM 0110	Poultry meat (in the fat)	0.2
GC 0649	Rice	0.1
MO 0822	Sheep, edible offal of	0.2
MM 0822	Sheep meat (in the fat)	0.2
VD 0541	Soya bean (dry)	1
VO 0447	Sweet corn (corn-on-the-cob)	0.2
VR 0508	Sweet potato	0.2
DT 1114	Tea, green, black	30
TN 0085	Tree nuts	0.2
VO 0448	Tomato	2
	Vegetables [except carrot, common bean, fruiting vegetables, curcurbits, lupins (dry), mung bean (dry),	

onion, potato, soya bean (dry), sweet corn (corn-on-the-cob), sweet potato]	2

* at or about the limit of detection

Existing Chemicals Review Program

Endosulfan is one of some 80 agricultural and veterinary chemicals identified as candidates for priority review under the ECRP. Following data call-in processes, a number of additional data submissions on the toxicology of endosulfan have been received from industry and the public. These data, together with all previously submitted data have been evaluated and are detailed in this report.

1.2 International Toxicology Reviews

WHO/FAO Evaluations

Endosulfan has been evaluated for acceptable daily intake by the Joint Meeting of Pesticide Residues (JMPR) in 1963, 1965, 1967 and 1968, and reviewed in 1971, 1974, 1975, 1982 and 1985 and most recently in 1989. In 1968, the JMPR established an ADI for endosulfan of 0.0075 mg/kg/day based partly on long term feeding studies in rats and dogs. In 1982, the JMPR recommended that the ADI be given temporary status pending independent validation and/or replacement studies for the non-validated data of IBT studies.

In 1989, a complete re-evaluation of the endosulfan toxicological database was carried out and the following levels causing no toxicological effect identified:

- mouse 6 ppm in the diet, equal to 0.84 mg/kg/day;
- rat 15 ppm in the diet, equal to 0.6 mg/kg/day;
- dog 10 ppm in the diet, equal to 0.57 mg/kg/day.

The current JMPR estimate of ADI for humans is 0-0.006 mg/kg bw.

In addition, the International Programme on Chemical Safety (IPCS) produced an Environmental Health Criteria Document on Endosulfan in 1984 (EHC 40).

Available Country Reports

The US Environmental Protection Agency (EPA) published a Pesticide Registration Standard on endosulfan in March 1982.

Sweden has completed a review of the toxicology of endosulfan authored by Ronny Fransson, Karolinska Institute of Environmental Medicine, KEMI, 1993. The Swedish report has been used to supplement the main report wherever additional studies have been reported by

Sweden but have not been submitted in Australia. These data are included for completeness and are clearly indicated in the main text.

Under the OECD Pesticide Review Exchange Program, Canada have provided, on NRA request, their "*Pesticide Rulings Proposal Toxicology- Endosulfan*" March 1993. This document presents a summary of the toxicology for endosulfan

1.3 Chemistry

Chemistry details for endosulfan are contained in Section 3 of this report.

Composition of TGAC

The Department has evaluated data from a number of sponsors for approval of the sources of manufacture for technical endosulfan, namely Hoechst Australia Ltd, Koor Inter-Trade (Asia) Pty Ltd, and Mineral and Chemical (M&C) Traders Pty Ltd and George Wills & Co. Ltd (for Excel Industries Ltd). Current compositional standards were met. The NRA will adopt the FAO compositonal standard for endosulfan; in which case, future TGAC suppliers will be required to meet that standard.

1.4 End Use Products

In Australia, there are 15 products containing endosulfan, formulated either as emulsifiable concentrates (350 g/L) or ULV insecticides (240 g/L and 295 g/L). The department has evaluated those formulations and noted their compositions. Product formulations are commercial-in-confidence information and as such are not published in this review.

2. METABOLISM AND TOXICOKINETICS

2.1 Mouse

2.1.1 Oral & Dietary Dosing Study

Christ & Kellner (1968). Investigations with endosulfan-14C in mice. Hoechst Radiochemical Frankfurt, Ch/He-8412, 31 December, 1968. Hoechst document A53842, translation of document A14217 (AgrEvo 11303).

To investigate the excretion and distribution of ^{14}C-endosulfan in mice, the test material (specific radioactivity 50 mCi/g; 65% -endosulfan: 35% -endosulfan; Hoechst) was administered to mice (strain, sex and source unstated) via oral gavage or in a feeding study. In the gavage study, animals received a single dose of 4 mg/kg body weight (80µg endosulfan dissolved in 0.5 mL edible oil), and then Altromin diet for the remainder of the excretion

period (24 days). In the feeding study, some mice received Altromin diet spiked with the radiolabelled test material for one day, receiving a mean dose of 4.7 mg/kg body weightThe doses ranged from 3.2 to 5.6 mg/kg/day. The remaining mice were administered a diet containing the radiolabel at 18.6 ppm for 21 days, and afterwards with a normal diet without endosulfan (for 35 days post dosing). In the feeding study the mean intake of test material was calculated to be 2.4 mg/kg body weight, with doses ranging from 1.7 to 3.0 mg/kg/day.

Following the single gavage dose, groups of five mice were sampled on days 1, 5, or 24, with urine and faeces collected daily and the mice killed for tissue residue determinations at the sample intervals. For the feeding studies (1 day and 21 day), determinations of radiolabel in the excreta were conducted daily, and groups of five animals were killed for tissue residue determination on days 1, 5, 21, 35, and 46 following the 1 day feeding study, and on days 1, 5, 23, and 35 days after the 21 day feeding period. Tissue residue determinations were made on the following: stomach (± contents), small intestine (± contents), large intestine (± contents), liver, kidneys, spleen, lungs, heart, brain, fat, muscle, carcass.

Following the single gavage dose of radiolabelled endosulfan of 4 mg/kg, excretion was mainly via the faeces, and to a lesser extent in the urine. The percentage of the administered dose recovered from the faeces and urine were 36% and 7.9% (day 1), 77% and 13.8% (day 5), and 86% and 8.5% (day 24), respectively. For animals that received the test material in the diet for 1 day (mean 4.7 mg/kg/day), excretion was 98% of the administered dose or greater between 2 days and 46 days after dosing, with the majority of the radiolabel recovered from the faeces (up to 94%) and the urine (7-9%). For animals that received the test material in the diet for 21 days (mean 2.4 mg/kg/day), excretion was also high, with total recovered radiolabel of 87-93% of the administered dose after 22-56 days of the study, with the majority of the radiolabel recovered from the faeces.

Following the single oral dose of test material at 4 mg/kg, residues were detected in all of the organs assayed after one day, with the highest residues seen in the liver (12.3 ppm), fat (4.9 ppm), kidneys (2.4 ppm), stomach (8.6 ppm) and lungs (2.5 ppm). The residue levels dropped quickly, and by day 24, the highest residues were detected in the spleen (.25 ppm), liver (0.16 ppm), and lungs (0.18 ppm). The liver and other tissues were below 0.1 ppm, including kidneys (0.09 ppm), and fat (0.06 ppm).

In animals receiving a single dietary dose endosulfan (mean 4.7 mg/kg), residues on day 1 after the end of the feeding period were detected in all tissues, including the liver (1.84 ppm), kidneys (0.49 ppm), and fat (0.32 ppm). By day 21 after treatment, liver residues were 0.11 ppm, and all other tissues were very low, including kidneys (0.03 ppm) and fat (0.02 ppm), while by day 45, liver (0.04 ppm), kidney (0.008 ppm), and fat (0.01 ppm) residues were all low. The spleen residues levelled after about day five, with 0.06 ppm detected on day 45.

After 21 days of dietary administration (mean 2.4 mg/kg/day), tissue residues were again detected in all tissues, including the liver (7 ppm), kidneys (1.7 ppm), and fat (0.61 ppm) on day 1 after treatment. Residues were still detected in tissues after 35 days post dosing, including the liver (0.86 ppm), kidneys (0.22 ppm), and fat (0.06 ppm), while the residues in the spleen remained consistently above 1 ppm for most of the study, and were still at 0.34 ppm by the completion of the study.

Summary

When radiolabelled endosulfan was administered to mice as a single gavage dose (4 mg/kg), single dietary dose (4.7 mg/kg), or 21 day dietary administration (2.4 mg/kg/day), recovery of the radiolabel was predominantly via the faeces, with a smaller amount excreted in the urine. Within three weeks after cessation of treatment, total recovery of the radiolabel was 87% - 100% of the administered dose, and did not differ greatly between the various dosing regimes. Whilst biliary excretion was not studied, oral absorption would appear to be moderate to high. Three weeks after the final test material administration, tissue residues were greater in those animals on the 21 day feeding study, with the highest residues remaining in the livers (about 2 ppm) and spleen (about 1.4 ppm) at this time. Residues in the kidneys and fat were generally low, even after repeat administration, and endosulfan residues did not accumulate in the tissues following oral or dietary administration of the radiolabelled test material.

2.1.2 Oral Dosing Study

Deema et al (1966) Metabolism, Storage and Excretion of ^{14}C Endosulfan in the Mouse. Journal of Economic Entomology, 59, 546-550, 1966.

(a) Single Dose Study

Endosulfan technical and its two component isomers were each fed to male Balb/c mice at a dose level of 0.3 mg/mouse. Purified endosulfan was fed to 9 male mice, the alpha-isomer was fed to 2 males and the beta isomer fed to 2 males; a control animal was included in each test. Animals were housed in metabolism cages and urine and faeces collected over a 24 h observation period.

Endosulfan (and its two isomers) was not completely absorbed from the gastrointestinal tract but was excreted, along with the metabolites endosulfan sulfate and diol, in the faeces. Only the diol metabolite was excreted via the urine while the sulfate metabolite was the only form of endosulfan found in tissues. Relatively large amounts were found in liver, small intestine and visceral fat with trace amounts in muscle and kidney. The actual tissue levels and elimination rates were not stated.

(b) Repeat Dose Study

Endosulfan technical was fed to Balb/c mice (8/sex) in their diets at a dose level of 10 ppm for up to 49 days. The control group (2/sex) was fed food only treated with the vehicle, acetone. In the treated groups, two animals (1/sex) were killed at 7, 14, 21, 28, 35, 42 or 49 days of treatment. Control animals were killed at 14, 28, 42 and 49 days. Urine and faeces were collected and tissues assayed for endosulfan and its metabolites.

The sulfate metabolite was detected in the liver and visceral fat of all mice tested, while the actual tissue levels were not stated it was claimed that levels in these tissues were lower than those occurring 24 h after a single oral administration [(a) above]. Both isomers and the sulfate and diol metabolites of endosulfan were detected in the faeces at all sample times. The only endosulfan product detected in the urine of these animals was the diol metabolite.

(c) ^{14}C Radiolabelled Distribution Study

^{14}C-labelled endosulfan was fed to Balb/c mice (2/group) at dose levels of 0, 0.2, 0.25 and 0.3 mg/mouse as a single dose. Animals were kept in metabolism cages and urine, faeces and expired air was collected over a 24 h period. The radioactivity of organs was also determined following a 24 h period.

From 0.1 to 0.2% of the administered radioactivity was detected in respired air in the form of CO_2 indicating that only slight metabolism of the cyclodiene ring occurs.

Approximately 65% of the radiolabel was recovered; based on the radioactivity/g of tissue and excreta, the faeces accounted for the highest levels followed (in rank order) by visceral fat > urine > small intestine > kidney > brain > respired CO_2 > blood. No breakdown of the actual amounts of endosulfan was provided.

2.1.3 Residue Study following 24 Month Dietary Administration

Leist (1989). Endosulfan-substance technical (Code: Hoe 002671 OI ZD97 0003). Carcinogenicity study in mice, 24 months feeding study-Residue determination. Pharma Research Toxicology and Pharmacology, Study no 745, TOXN no 83.0113, 13 July 1989. Hoechst report no 89.1104, 27 July 1989. Hoechst document no A41284 (AgrEvo 11303).

After completion of a 24-month feeding study in mice (Donaubauer, 1988; Hoechst document A38008), the levels of endosulfan and its main metabolites endosulfan-hydroxyether, -sulfate, -lactone, and -diol were investigated in the liver and kidneys of the animals. No parent compound was detected in either liver or kidney samples. In animals administered endosulfan at 18 ppm in the diet, the levels of the hydroxyether, lactone and diol metabolites were at or below the level of detection (0.02 ppm), while the endosulfan-sulfate concentrations were 0.1-0.2 ppm (kidneys) and 0.7-1.1 ppm (liver). At a dietary concentration of 2 ppm the endosulfan-sulfate concentrations were 0.2-0.4 ppm in kidneys and 0.06-0.07 ppm in the livers, and at a dietary concentration of 6 ppm, the kidney residues were 0.04 ppm, and liver residues were 0.12-0.45 ppm.

2.2 Rat

2.2.1 Oral and Intravenous Study

Kellner & Eckert (1983). Hoe 02671-^{14}C. Pharmacokinetics and residue determinations after oral and intravenous administration to rats. Hoechst study TEP 74/1; Bereich C/Analytisches Project OE 87/45; Hoechst report 01-L42-0382-83, 15 February 1983; document A49475, translation of document A27971 (AgrEvo 11303).

Stumf & Lehr (1993). Amendment to document A49584, Hoechst document A49584, 2 February 1993 (AgrEvo 11303).

Radiolabelled endosulfan (^{14}C-labelled in the 1, 2, 3, 4, 7 positions of the molecule; radiochemical purity 98%; Hoechst) was administered to male and female SPF-Wistar rats (Winkelmenn) via oral or intravenous routes. For oral administration, ^{14}C-endosulfan was dissolved in cooking oil at a concentration of 0.42 mg/mL, and administered by oral gavage at a dose level of 2 mg/kg body weight. For intravenous administration, the test material was dissolved in 1,2-propanediol to a concentration of 0.2 mg/mL, and injected into the caudal vein. The planned dose was 2 mg/kg, but was reduced to 0.5 mg/kg. Animals were kept in metabolism cages with separate collection of urine and faeces. Blood was sampled from the retrobulbar venous plexus, and tissue residual concentrations were determined on samples obtained seven days after test material administration.

Following oral administration, the highest blood concentrations were found between 3 and 8 h after treatment in males, with a mean of the highest observed blood concentrations of 0.25 $\pm$ 0.06 µg/mL, and in females the highest blood levels of 0.18 $\pm$ 0.05 µg/mL was reached after about 18 h. The elimination from blood was biphasic in the males, with biological half-lives of 8.07 $\pm$ 1.12 h and 110 $\pm$ 21 h. In females, the elimination was monophasic with half lives of 75.4 $\pm$ 13.5 h. After oral administration, the majority of the radiolabel was excreted in the faeces, with 82.2 $\pm$ 6.5% for males, and 71.8 $\pm$ 16.2% in the females. Urinary excretion in males was lower than for females, with 11.87 $\pm$1.84% in males, and 22.28 $\pm$ 2.74% in females. Urinary and faecal excretion was biphasic in both males and females. Urinary half lives were 6.17 $\pm$ 1.43 h or 67.5 $\pm$ 14.4 h in males, and 5.59 $\pm$ 1.1 h or 32.8 $\pm$ 3.4 h in females. Faecal half lives were 7.67 $\pm$ 1.07 h or 34.3 $\pm$ 4 h in males and 11.41 $\pm$ 3.71h or 29.5 $\pm$ 3.3 h in females. After seven days following administration, 94% of the administered dose had been eliminated, most within the first 1-2 days, but small amounts of radiolabel were still present in urine and faeces.

Following intravenous administration, elimination consisted of three phases in males, with half lives of 0.77 $\pm$ 0.2 h, 12.5 $\pm$ 2.9 h, and 157 $\pm$ 57 h. By day 7 of the study, blood concentrations accounting for approximately 9% of the maximum values were measured. In females, the decrease in blood concentration was biphasic, with half lives of 1.2 h and 47 $\pm$ 12.5 h. After day 6 following dosing, the blood concentrations were below the level of detection. Elimination was incomplete after 7 days following intravenous administration, with 79.1 $\pm$ 13.2 % of the administered radiolabel excreted by males and 83 $\pm$ 7.3% by females, and small amounts of the radiolabel were still detected in the excreta on day 7. Urinary excretion was 13.33 $\pm$ 2.34% in males, and 24.06 $\pm$ 3.72 % in females, while faecal

excretion was 65.72 ± 11.87% in males, and 58.98 ± 5.11 % in females. The range of half lives following intravenous administration was similar to that seen following oral administration. For urinary excretion, these ranges were 7.48 ± 0.94 h or 59.3 ± 19.3 h for males and 7.58 ± 1.48 h or 41.6 ± 5.8 h in females. For faecal elimination, the ranges of half lives was 8.57 ± 2.37 h or 34.5 ± 8 h in males and 13.59 ± 4.89 h or 40.2 ± 10.3 h in females.

Summary of 14C-endosulfan pharmacokinetics in rats following oral (po) and intravenous (iv) dosing.

	po (2 mg/kg)	iv (0.5 mg/kg)
T_{max}	males 3-8 h females 18 h	males and females 5 minutes
C_{max}	males 0.25 ± 0.06 µg/mL females 0.18 + 0.05µg/mL	males and females 0.18 + 0.04µg/mL
Elimination $t_{1/2}$	Males (biphasic) 8 h , 110 h Females (monophasic): 75 h	Males (triphasic) 0.77 h, 12.5 h, 157 h Females (biphasic) 1.2 h, 47 h
Excretion - Faecal	Males 82% Females 72%	Males 66% Females 59%
Excretion - Urinary	Males 12% Females 22%	Males 13% Females 24%
Excretion $t_{1/2}$- Urinary	Males (biphasic) 6.2 h, 67.5 h Females (biphasic) 5.6 h, 33 h	Males (biphasic) 7.5 h, 60 h Females (biphasic) 7.6 h, 42 h
Excretion $t_{1/2}$- Faecal	Males (biphasic) 7.7 h, 34 h Females (biphasic) 11.4 h, 30 h	Males (biphasic) 8.6 h, 34.5 h Females (biphasic) 13.6 h, 40 h

Tissue residue concentrations were determined in animals administered endosulfan orally. The highest concentrations were found in the kidneys (1.8 ppm) and liver (0.23 - 0.48 ppm). In the retroperitoneal fat of the females, concentrations of radiolabel ranged between 0.04 and 0.32 ppm (mean 0.16 ± 0.11 ppm), but no residues were found in the fat in males. In all other tissues, tissue concentrations were below 0.1 ppm or undetected. The total tissue residues were 3.74 ± 0.61% of administered dose in males and 4.66 ± 0.94% in females seven days after oral administration.

Based on comparisons between oral and intravenous area under the curve estimations and renal excretion of the radiolabel, the absorption was estimated to be between 60 and 87% in males and 70-92% in females.

Summary

Following administration of ^{14}C endosulfan via oral or intravenous routes to male and female Wistar rats at doses of 2 or 0.5 mg/kg, respectively, excretion was extensive, with greater than 80% (intravenous) or 90% (oral) of the administered dose eliminated in the urine and faeces within the seven days after dosing. Urinary elimination was greater in females than males with both routes of administration, with 11-13% excreted in the urine of males compared with 2-24% of radiolabel excreted in the urine of females. Faecal elimination was 65-82% in males, and 60-72% in females (iv-oral). The highest tissue concentrations were found in the

kidneys (1.8 ppm) and liver (0.23 ppm in males; 0.48 ppm in females), and retroperitoneal fat in females (0.16 ppm). The endosulfan residues were below 0.1 ppm in all other examined tissues. Based on comparison between intravenous and oral AUC data, the absorption of endosulfan was estimated to be 60-70%, and by comparison of elimination of radiolabel the absorption was estimated to be about 90%.

2.2.2 Excretion and Distribution Study

Dorough et al (1978). Fate of endosulfan in rats and toxicological considerations of apolar metabolites. Pesticide Biochemistry and Physiology 8, 241-252, 1978. Hoechst document A14276 (Agrevo 11303).

To obtain quantitative data on the excretion and distribution of α - and β - endosulfan residues in rats, ^{14}C-endosulfan (each isomer separately) was administered as a single oral dose, or as a dietary supplement for 14 days, to albino rats (Laboratory Supply Co, USA; strain not stated). For the single oral dose, female rats (200-250 g) received approximately 2 x 10^6 dpm of endosulfan (2 mg/kg) in corn oil via oral gavage, with urine and faeces collected for 120 h (intervals not stated). In a bile collection study, male rats (400 g) were administered either α - or β -endosulfan at a dose of 1.2 mg/kg, with bile collected via cannula hourly for 48 h.. There is no indication of the number of animals used in the single dose portion of the study, nor for the bile collection study. It is possible that single animals were used for each of these studies.

In the feeding study, an acetone solution of the radiolabeled compounds were added to a ground animal feed and mixed. The homogeneity and stability of the feed was confirmed, then the test feed was added to the diet daily. The test material concentrations were 5 ppm of either α - or β -endosulfan, 25 ppm of α -endosulfan, and 25 ppm of a 7:3 mixture of the α - and β -endosulfan isomers. At 5 ppm, a single animal (for each isomer) was killed after 1, 3, 7, 10, and 14 days of feeding. The remaining animals were returned to a normal diet, and then 2 animals were killed after 1, 3, 7, and 14 days. It is not clear whether this was 2 animals/isomer, or two animals in total. For the diets containing 25 ppm of α -endosulfan and 25 ppm α - and β -endosulfan (7:3), groups of four animals were used, and all rats were killed on day 14, with urine and faeces collected daily. The radiolabel was measured in urine, faeces, bile, and liver and kidney samples.

Cytochrome P-450 and epoxidase enzyme assays were also conducted using livers from female albino rats maintained on a normal diet or a diet containing 50 ppm of α - or β - endosulfan for 28 days. The number of animals used in this portion of the study was not stated.

To test for acute toxicity of endosulfan, female albino mice were administered endosulfan isomers and a number of proposed endosulfan metabolites as a 1:1 mixture of Tween 80 and water, using a range of doses.

Weight gain was also determined in female rats, with weanling rats maintained on a normal diet until their weights reached 100 g, and then groups of rats (6/group) were fed diets containing 5 or 50 ppm of α - or β -endosulfan for 15 days.

The mutagenicity potential of endosulfan and its analogues was tested in a Salmonella typhimurium/reverse mutation assay, at doses of 10, 100, 500, and 1000 µg/plate, using bacterial strains TA98, TA100, TA1535, and TA1978. 2-Acetyaminofluorene was used as the positive control.

Results

After a single dose of radiolabelled α -endosulfan (2 mg/kg), 88% of the radiolabel was excreted within 120 h of administration, with 75% in the faeces and 13% in the urine. The majority of the radiolabel was recovered within 48 h of administration, with 73% (62% faeces, 11% urine) recovered by this time. Biliary excretion following a dose of 1.2 mg/kg/day α - endosulfan in male rats was 47.2%, with an additional 22% excreted in the faeces and 12.5% in the urine. Following administration with 2 mg/kg β -endosulfan, excretion of the radiolabel was almost 87% after 120 h, with 68% in the faeces, and 18.5% in the urine. Elimination was extensive (>70%) within 48 h of administration. Biliary excretion was 28.9% following β -endosulfan administration, with an additional 15% excreted in the faeces and 10% in the urine. These figures suggest that there was little recirculation of radiolabel from bile into the enterohepatic circulation, with biliary radiolabel excreted via the faeces.

Animals administered endosulfan (repeatedly) in the diet displayed a similar pattern of excretion as seen in single dose animals. At 5 ppm α -endosulfan, 56.5% of the administered radiolabel was excreted in the faeces after 14 days, and 7.8% was excreted in the urine in this period. After a further 14 days on a normal diet, excretion had increased to 63% in the faeces and 9.2% in the urine. Almost identical figures were found for β -endosulfan, with faecal and urinary excretion after 14 days (57% and 8%) increasing slightly after the 14-day recovery period (63.5% and 9.3%, respectively). Increasing the dose to 25 ppm made little difference in the excretion patterns, with 56 and 8.7% in the faeces and urine respectively after administration of α -endosulfan, and 54 and 6.8% in the faeces and urine after administration with 7:3 α : β -endosulfan after 14 days. No recovery periods were used at the 25 ppm dose levels.

Analysis of the livers and kidneys of rats 5 days after oral dosing with 2 mg/kg α -endosulfan revealed residues of 0.35 and 1.66 ppm of ^{14}C endosulfan equivalents, respectively. For animals administered 2 mg/kg β -endosulfan, the residues at the same time interval were 0.22 and 1.13 ppm in the liver and kidneys, respectively. For each isomer, the residues detected in the kidneys and liver combined represent approximately 1.5% of the administered dose. After 14 days of administration with endosulfan at 5 ppm in the diet, similar tissue residues were seen with both α - and β - endosulfan. Highest radiolabel residues were in the kidneys, with a maximum of about 3 ppm after 14 days of dietary administration. After dosing ceased, the half life in the kidneys was about 7 days, and kidney residues were about 1 ppm by day 14 following cessation of treatment. Liver residues reached about 1 ppm after 14 days, with a half life of about 3 days following cessation of dosing, with residues falling to 0.1-0.2 ppm by day 14 after cessation of treatment. Residues in the fat were generally below 1 ppm, and no residues were detected in the fat after 3 days following cessation of treatment. Treatment at 25 ppm for 14 days also indicated that residue accumulation was greatest in the kidney (20 ppm) and liver (6 ppm) after 14 days of administration. No recovery period was used in

animals at the 25 ppm dose level. Most of the identifiable compounds in excreta and tissues consisted of polar compounds. A small portion of the radiolabel recovered was identified as apolar metabolites, consisting of the sulfate, diol, α -hydroxy ether, lactone, and ether derivatives of endosulfan.

Administration of 5 or 50 ppm endosulfan in the diet for 28 days did not increase the levels of cytochrome P-450 or epoxidase activity in liver microsomes. The approximate LD50 values for endosulfan and endosulfan metabolites in mice were estimated to be: α -endosulfan 11 mg/kg, β -endosulfan 36 mg/kg, endosulfan sulfate 8 mg/kg, endosulfan α -hydroxy-ether 120 mg/kg, endosulfan lactone 120 mg/kg, endosulfan ether 270 mg/kg, endosulfan diol >2000 mg/kg. Neither endosulfan nor its metabolites showed mutagenicity potential in *Salmonella typhimurium* reverse mutation assays with or without metabolic activation.

Summary

^{14}C-Endosulfan (α - or β - isomers) was rapidly excreted by female rats following single oral administration of 2 mg/kg, or via dietary administration at doses of 5 ppm. After single oral administration, greater than 85% of the administered radiolabel was excreted within 120 h (>70% after 48 h), mainly in the faeces, and to a lesser extent in the urine. After dietary administration for 14 days, followed by a 14 day recovery period, recovery of the radiolabel was > 72% of the administered dose. Biliary excretion of radiolabel in male rats administered 1.2 mg/kg endosulfan as a single dose approached 50% for the α -isomer, and 30% for the β -isomer. There appeared to be little enterohepatic circulation from the bile. Tissue residues were generally greatest in the kidneys and liver, with smaller residues detected in other tissues and fat. After 14 days off the treatment, tissue residues were confined to the kidneys and, to a lesser extent, the liver, with a half life of about 7 days for the kidneys, and 3 days for the liver. Most of the identifiable radiolabelled compounds in the excreta and tissues were very polar, and no bioaccumulation in the fatty tissues was found.

2.2.3 Dermal Absorption Studies

(a) A dermal absorption study in rats with ^{14}C-Endosulfan. Project Number: WIL-39028, Hoechst AG, Craine EM, 1986. GLP, EPA 85-3, Company File No: A35730.

Juvenile (at least 6 weeks old, ca. 260g) male rats (Crl:CD(SD)BR) were treated dermally, 24 animals/dose group, with single doses of ^{14}C labelled endosulfan at 0.10, 0.76 and 10.13 mg/kg. The labelled endosulfan exhibited specific activities of 27.2 and 5.47 μCi/mg for preparations #16014 and #16014 I, and an isomer ratio of 1.87 with radiopurity of 94.6%. Fresh preparations of labelled and unlabelled endosulfan in water and acetone (the acetone was evaporated from the suspension prior to application) were formulated to produce a suspension similar to a field-use 3 EC final spray mix, and applied (0.1 or 0.2 mL) to the shaven and acetone-washed dorsal skin within a rubber ring (diameter = 3.7 cm, area = 10.8 cm^2) cemented to the skin. The animals were maintained in metabolism cages for urine and faeces collection. Four animals from each group was sacrificed by flooding the cages with nitrogen at 0.5, 1, 2, 4, 10 or 24 h after the dose application. Radioactivity was determined in various organs, blood, urine (bladder-urine pooled with the cage urine sample), faeces, the

skin at the application site (dissected away with the rubber ring), the remaining skin, and the whole carcass.

There was no skin irritation at the application site. Recovery of radiolabel was essentially complete. Absorption of the dose into the skin was rapid and substantial at all doses, as skin washings at all sacrifice times removed generally only 20% of the applied dose. Movement through the skin was slow and the 0.10, 0.76 and 10.13 mg/kg groups recorded respectively 73.0%, 73.0% and 88.8% of the absorbed dose still bound to the skin at 24 h. For all three dose groups, excretion was detectable at low levels in urine at 4h, urine>>faeces at 10h and urine<faeces at 24 h. At 10 h each dose group had excreted less than 1% of the applied dose. At 24 h the excretion was ca. 11%, 10%, and 4% of the applied dose for the 0.10, 0.76 and 10.13 mg/kg groups respectively. The amount of label measured in individual tissues increased with the dose and the sacrifice time, and at most sample times was in the order kidney>liver>fat>blood>brain. The percentage absorption of applied dose ranged from 21.5% at a dose of 0.10 mg/kg, to 8.4% at a dose of 10.13 mg/kg.

Distribution of endosulfan equivalents recovered at the 24 h sample time expressed as percentage of the applied dose

Dose mg/kg	Tissues	Excreta	Carcass	Application site	Skin Washing
0.10	2.2	10.8	8.5	57	21
0.76	2.2	9.8	9.5	57.7	21
10.13	1	3.7	3.7	67	25

(b) A dermal absorption study in rats with ^{14}C-Endosulfan with extended test duration. Project Number: WIL-39029, Hoechst AG, Craine EM, 1988. GLP, EPA 85-3, Company File No: A39677.

Young (7-10 weeks old, ca. 240g) female rats (Crl:CD(SD)BR) were treated dermally for 10 h, 16 animals/dose group, with single doses of ^{14}C labelled endosulfan at 0.09, 0.98 and 10.98 mg/kg. The labelled endosulfan exhibited specific activities of 27.2 and 5.47 µCi/mg for preparations #16014 and #16014 I, and an isomer ratio of 1.87 with radiopurity of 94.6%. Fresh preparations of labelled and unlabelled (E4847:27, code 519) endosulfan in water and acetone (the acetone was evaporated from the suspension prior to application) were formulated to produce a suspension similar to a field-use 3 EC final spray mix, and applied (0.1 or 0.115 mL) to the shaven and acetone-washed dorsal skin within a rubber ring (diameter = 3.7 cm, area = 10.8 cm^2) cemented to the skin. The animals were maintained in metabolism cages for urine and faeces collection. After an exposure period of 10 h, the application site was washed by scrubbing with soapy water and gauzes to remove unabsorbed test material. Four animals from each group was sacrificed by flooding the cages with nitrogen at 24, 48, 72, or 168 h after the dose application. Radioactivity was determined in various organs, blood, urine (bladder-urine pooled with the cage urine sample), faeces, the skin at the application site (dissected away with the rubber ring), the remaining skin, and the whole carcass.

There was no skin irritation at the application site, and no signs of systemic toxicity. Recovery of radiolabel ranged from 84-115%. Initial absorption into the skin was dose related but movement through the skin was slow, and by 7 d only 45%, 46% and 20% of the applied dose had fully penetrated the skin for the 0.09, 0.98 and 10.98 mg/kg dose groups respectively. Endosulfan equivalents were present in excreta at 24 h and excretion peaked between 24-48 h, with faeces accounting for about two thirds of the label. The amount of label measured in individual tissues increased with the dose and peaked at the 48 h sacrifice time, and at most sample times was in the order liver>kidney>fat>brain>muscle>blood. Absorption was almost complete by day 7 with little label remaining at the application site. Total residues at 168 h, present mainly in liver and kidney, were 2.5%, 2.3% and 1.3% of the applied dose for the 0.09, 0.98 and 10.98 mg/kg dose groups respectively.

Distribution of endosulfan equivalents recovered at the 24-48-72-168 h sample times expressed as percentage of the applied dose

Dose mg/kg	Tissues & carcass	Excreta	Total penetrated	Application site	Skin Washes at 10 h*	Max. Penetration rate (µg/cm^2/h)
0.09	13-13-7-3	9-23-32-42	22-35-39-45	41-24-7-2	30	0.018
0.98	10-15-7-2	6-21-22-44	16-36-29-46	39-17-12-2	45	0.165
10.98	3-6-4-1	1-6-8-19	4-11-12-20	33-20-13-1	66	0.532

* average of the four sacrifice-time groups

2.3 Goat

2.3.1 Residue study in Lactating Goat

Indraningsih, McSweeney & Ladds (1993). Residues of endosulfan in the tissues of lactating goats. Australian Veterinary Journal, Vol 70, No 2, p 59-62, February, 1993. Hoechst Document A51447 (AgrEvo 11303).

To investigate the accumulation of endosulfan in tissues of lactating goats, endosulfan (source and purity unstated) was administered orally in gelatin capsules to twelve lactating feral goats (25 to 40 kg) for 28 days, at a dose of 1 mg/kg/day. On days 1, 8, 15, and 21 after the last treatment, groups of three animals and their respective kids were killed and necropsied for tissue collection.

Samples of milk and venous blood were taken from each animal before being killed, and the major organs and muscle were removed at necropsy and weighed. Samples of tissues were fixed for microscopic examination, and the remaining tissues were used for residue examination. Clinical biochemical tests were conducted for liver and kidney function and plasma electrolyte profiles were determined.

Results

No clinical signs of toxicity were observed during the study, and the mean feed intake was similar in weeks 1 and 4 of treatment. After administration with endosulfan for four weeks, the greatest tissue concentration of endosulfan residues was observed on day 1 after treatment ceased, with the highest residues detected in the gastrointestinal tract and the liver (0.2 and 0.12 ppm, respectively), and lower residues seen in other tissues. Residues in milk were 0.02 ppm. By day 8 after treatment, endosulfan residues in the kidneys rose from 0.29 ppm to 0.47 ppm, but all other tissue residue levels fell, and residues in the milk became undetectable. In the second week after treatment, the liver and kidney residues were the only ones detectable, and by the third week after treatment, no tissue residues of endosulfan were detected.

Alpha-endosulfan was the major tissue residue in all tissues except for the liver and fat, which contained mainly endosulfan sulfate. The clearance of residues was slowest from the kidneys, but the increase in kidney residues between days 1 and 8 prevented the calculation of a linear disappearance for this organ. The half-lives of endosulfan in the tissues were 3.1 days for liver, 2.6 days for the gastrointestinal tract, 1.1 days for muscle, 1.6 days for brain, and 1.4 days for fat.

Endosulfan residues in organs of goats, 24 h after dosing with technical grade endosulfan at 1 mg/kg/day for 28 days.

Endosulfan residues ppm (std error)	Alpha	Beta	Sulfate
Liver	0.010 (0.007)	0.021 (0.018)	0.097 (0.047)
Kidney	0.220 (0.0001)	0.059 (0.023)	0.012 (0.0004)
Lungs	0.006 (0.002)	<0.001	0.01 (0.0005)
Fat	0.015 (0.014)	0.002 (0.001)	0.040 (0.039)
Muscle	0.033(0.018)	0.009 (0.006)	<0.001
Spleen	0.026 (0.017)	0.010 (0.007)	0.002 (0.002)
Heart	0.005 (0.003)	<0.001	<0.001
G.I. Tract	0.190 (0.017)	<0.001	<0.001

No macroscopic abnormalities were reported at necropsy. Histological examination revealed some hepatic congestion and mid-zonal vacuolation of hepatocytes, but these changes were not consistent, and were considered to be minor in nature by the authors of the report. Severe renal congestion was reported in all animals, and protein was reported to be present in the renal tubules and Bowman's space. No histopathological data were detailed in this study.

Clinical chemistry examination did not reveal any treatment related changes in plasma alanine aminotransferase (ALAT) or aspartate aminotransferase (ASAT) values in the three weeks following treatment. Glutamyl transferase (GGT) and alkaline phosphatase (AP) values were elevated in the two weeks following treatment, but approached normal vales by day 21 after treatment. Other parameters (plasma total bilirubin, total protein, albumin, sodium, potassium, chloride) were considered to be unaffected by treatment with endosulfan.

Summary

Following oral administration of endosulfan to lactating goats at a dose of 1 mg/kg/day for 28 days, the tissue residues were generally low, with the highest tissue concentrations detected on the first day after cessation of treatment being 0.29 ppm in kidney, 0.2 ppm in the gastrointestinal tract, and 0.12 ppm in the liver. Kidney endosulfan residues were increased one week after treatment, reaching 0.49 ppm on day 8 after treatment, but no tissue residues were detected 21 days after treatment ceased. Endosulfan residues did not accumulate in the fat, with tissue concentrations reaching 0.06 ppm on day one after treatment ceased, but no residues were detected in the fat by day 8 after treatment.

2.4 Cow

2.4.1 Repeat Dose Dietary Study

FMC Corporation. (1965) Determination of Thiodan I, and II and sulfate residues in milk and cow tissues. Document No. A14210. Project No. 015. 24 September 1965.

Milk cows were fed a combination of endosulfan isomers (5.0 ppm) and endosulfan sulfate (5.0 ppm) in their diets, daily for 30 days. Two cows were killed at the end of the treatment period. A further 2 animals were maintained on control diet for an additional 30 days prior to being killed. Milk samples were collected at various time intervals throughout the study.

Endosulfan sulfate was the only residue detected in the milk in amounts ranging from 0.01 to 0.16 ppm. The sulfate was also detected in fat (0.89 ppm), liver (0.63 ppm) and kidney (0.07 ppm) tissue samples of cows killed immediately following treatment. Only the fat samples of animals from the withdrawal group contained residues of endosulfan sulfate at low levels (about 0.14 ppm).

2.5 Sheep

2.5.1 Single Oral Dose Study

Gorbach et al (1965) The behaviour of Endosulfan in the metabolism of milk sheep. Hoechst A.G. Document No. 4 030.

A single oral dose of ^{14}C-labelled endosulfan was administered to two lactating East Friesian sheep at a dose level of 0.3 mg/kg. Animals were studied over a 22 day period. Blood radioactivity was determined at 2, 4, 6, 8, 12, 24 and 48 h, then daily up to 22 days following treatment. Radioactivity was determined in milk samples which were taken twice/day for the first 4 days and then daily. Urine and faeces were collected separately and examined at 24 h periods. Tissue radioactivity was determined 40 days after treatment.

The amount of radiolabel in the blood peaked after 24 h with levels equivalent to 0.07 ug endosulfan/mL. After 22 days this amount was reduced by a factor of 10. After 48 h, 0.4% of the administered radiolabel was detected in the milk. The highest concentration measured in milk was equivalent to 0.2 ug/g; this was at 25 h.

Over a 17 day period the total amount of radiolabel eliminated in milk for each sheep was 0.37% and 1.82%, respectively, of the administered dose.

Excretion of radiolabel in the urine peaked at 24 h with 18.5% of the administered dose being eliminated. The concentration steadily fell and on day 22 only 0.01% of the administered radioactivity was still present in the urine. Altogether 41% of the radiolabel administered was eliminated via the urine during the 22 day period. Maximum excretion of radiolabel in the faeces occurred on day 2 with 19% of the administered radioactivity being detected in the faeces. Altogether, 50% of the administered radiolabel was eliminated via the faeces. It was determined that approximately half of this 50% was unmetabolized endosulfan. The organs and tissues of the sheep killed after 40 days revealed concentrations of 0.02-0.03 ug endosulfan/g in fat, kidney and liver. All remaining tissues had considerably lower levels. Total radioactivity found in organs and tissues accounted for less than 1% of the administered label.

2.6 Pig

2.6.1 Dietary Study

Maier-Bode (1966) Investigations on the persistence of the insecticide endosulfan in the vegetable and animal organism. Pharmakologisches Institut der Rheinischen Friedrich Wilhelms - Universitat. Document A4047. July 1966.

Three sows (~ 35 kg in weight) were fed endosulfan (2 ppm) in their diets for 27, 54 or 81 days. For comparative purpose, DDT (7 ppm) was fed to a further 3 sows over the same period. Tissue and organ levels of endosulfan were determined.

Endosulfan was only detected in fatty tissue at levels of 0.07, 0.09 and 0.04 ppm after 27, 54 and 81 days of treatment. In contrast, DDT was found in all tissues and organs examined with a predominance occurring in fatty tissues which had 8.3, 9.1 and 9.7 ppm DDT after 27, 54 and 81 days treatment, respectively. Liver and muscle contained about 15 fold less DDT residues than found in fat. Thus, while endosulfan was found in fatty tissues it does not appear to bioaccumulate as does DDT.

2.7 Primate

2.7.1 Dermal Absorption Study

Lachman G (1987) Dermal absorption of ^{14}C-Endosulfan in Rhesus Monkeys. Hoe 002671-(5a.9a-14-C). Laboratory: Battelle Institut Toxikologie und Pharmakologie Frankfurt. Lab Project ID BieV-V-66.697. Sponsor Hoechst Schering AgrEvo (11482).

Protocol

Two male Rhesus monkeys (supplied by Shamrock Farms, Sheffield) were used. They were housed in metabolism cages for the period of the study under controlled environmental conditions and were fed standard monkey diet, and water was available *ad libitum*. The test compounds used were ^{14}C-Endosulfan (chemical purity 99%, radiochemical purity 98%, relation of isomers α : β = 68:33; Hoe 002671 OI ZE 98 0005; Lot 1797), non-radioactive Endosulfan (Hoe 052618 OI ZB 99 0002; Lot 4157) and 4 reference substances, namely endosufan-lactone, endosulfan-ether, endosulfan-sulfate and endosulfan-diol. All were provided by the sponsor. The study was performed according to GLP, but no indication of the regulations applying was given.

Monkeys were treated with a solution containing 19.025 mg of labelled endosulfan suspended in water immediately prior to application. The suspension was applied to the shaved skin of the neck and shoulders of the animals. The monkeys were then restrained until the application solution had dried. Ten hours after application, the treated skin was washed with a soap solution. The administered dose for each monkey was calculated on the basis of the total radioactivity of the solution, minus radioactivity remaining in the application vial minus radioactivity of the paint brush used to apply the solution.

Blood samples were taken from each monkey at 1, 2, 4, 8, 12, 24, 36, 48, 72 and 96 h after the end of exposure to the test compound. Urine and faeces samples were collected over the 10 h exposure period, and for each of the 24 h periods following the end of administration (up to 96 hours). Tissue distribution was determined 96 h after the end of exposure. The monkeys were euthanised, and the liver, kidneys, brain, fat and treated skin were removed. After weighing, tissues and the residual carcasses were stored until preparation. The skin was prepared immediately. After initial determination of radioactivity, additional tissues were collected for determination, including total muscle below the treated skin, muscle of the hind limb (back - to compare with muscle under treated skin), skin at the inner side of the hind limbs and skin at the back of the hind limbs. The hands of the animals were detached, and the carcasses was separated into smaller portions for determination.

The pattern of metabolites in the urine and faeces were determined, using the 0-24h samples of urine, and the 72-96 h sample of faeces. The samples were chromatographed on silica gel plates, after which the plates were exposed to x-ray films. The autoradiographs were examined qualitatively. For the quantification of metabolites in urine, the urine of monkey 2 was used as this was more concentrated. For metabolites in faeces, equal volumes of samples from both animals were pooled. As the radioactivity of samples was low, they were concentrated prior to chromatography. Three samples of each was used, after being

lyophilised. The first sample was dissolved in methanol and used for chromatography. The second was dissolve in acetate buffer with the addition of glucuronidase and arylsulfatase. The third sample was dissolved in sodium hydroxide and allowed to stand overnight. All samples were then separated by HPLC.

Results

The administered dose was 2.2 mg/kg for monkey 1 and 3.0 mg/kg for monkey 2. The radioactive dose was 11.1 MBq for monkey 1 and 13.6 MBq for monkey 2. Blood and plasma levels of endosulfan increased during the first 24 - 36 hours, after which a steady state level was reached. The ratio of blood levels to plasma levels was approximately 0.65 for the first 8 - 12 h, after which it increased to 0.75 - 0.8.

Total recovery of the radioactive dose applied was 50% . The distribution of this material is indicated below.

Absorption profile following single dermal dose in monkey.

Dose applied	2.6 mg/kg
Total recovered (96 h)	50%
Absorbed	33%
Not absorbed	17%
Bound to skin surface	11%
Total penetrated	22%
Total residue in carcass	10%
Total residue in tissue	1%
Total excreted	11%
Faeces	4%
Urine	4%
Cage wash	3%

Tissue distribution profile for endosulfan in monkey following dermal dose

Tissue	Residue at 96 h post application - ng/g	Residue at 96 h post application - % total recovery
Blood	26	
Brain	8.1	0.01
Liver	478	0.40
Kidneys	83	0.02

Fat	233	
Muscles	159	0.21
Skin	1379	0.04

The main metabolite found in urine was endosulfan-diol, making up 50°/o of the total activity. An unidentified metabolite was also found, contributing to 40% of the activity. This metabolite was also present in faeces

Conclusions

The systemic absorption of endosulfan in the 96 h following dermal administration in monkeys was determined to be 22% of the administered dose, with an additional 11% of the administered dose remaining in the skin. However, only 50% of the administered dose was recovered in this study, and thus the absorption figures calculated in this study may not be an accurate indication of the extent of dermal absorption of endosulfan. A plateau of blood levels was reached at 36 h, and there may not have been significant additional dermal absorption after this time. Levels in the liver, kidneys and fat tissue are highest (0.478, 0.083, and 0.233 ppm, respectively), while there are negligible levels in the brain.

2.8 *In vitro* dermal studies.

Noctor J and John SA (1995) (^{14}C)-Endosulfan: Rate of penetration through human and rat skin determined using an *in vitro* system. Report Number 169/54-1011, Sponsor Number RR06/AZ26. Lab: Hazleton Europe, North Yorkshire England Sponsor: Hoechst, Report Date 10 May l99S. GLP:UK, OECD. (Hoechst Schering Agrevo, 11482)

Protocol

Alpha and beta isomers of radiolabelled endosulfan were obtained from the sponsor. The alpha isomer (batch no 22022II) had a specific activity of 3.051 MBq/mg, a radiochemical purity of 99%; 20.3 mg was received. The beta isomer (batch no 22023II) had a specific activity of 2.935 MBq/mg, a radiochemical purity of 99%; 10.4 mg was received. Additionally 1g each of alpha and beta non-labelled endosulfan was received, with chemical purities of 99.8 and 99.4%, respectively. The radioactive purity and specific radioactivity of the material was determined by the testing laboratory prior to use.

Sprague Dawley Crl:SD(CD)BR female rats (obtained from Charles River (UK) Ltd, Kent) were used, with 28 females of 3 to 5 weeks of age. Rats were housed in groups of up to five in wire floor cages under standard conditions. Food and water were supplied *ad libitum.* Rats were euthanised by asphyxiation and cervical dislocation. An area of the dorso-lumbar skin was clipped without abrading the skin, washed with acetone and excised. The skin was frozen and stored flat until use.

Human skin was obtained from a US supplier (details not supplied), and only skin with intact epidermis, and where the donor had not received medical treatment (if known) were used.

Pieces of skin (human and rat) were partially thawed and cut to a uniform thickness (0.4 mm) using a dermatome. The sample included intact epidermis and a portion of dermis. Skin samples thus prepared were thawed and mounted in a dermal penetration cell. Membrane integrity was checked using tritiated water applied to the epidermal surface of the cell, followed by measuring the radioactivity penetrance. After testing, the epidermal surface of the skin was washed with saline to remove radioactivity, and the sample was maintained in fresh saline. Two human skin samples were not included in the test due to suspected loss of membrane integrity.

Radiolabelled endosulfan was dissolved in a formulation vehicle (emulsifiable concentrate) to produce a concentration of 352 g endosulfan per litre, with a ratio of alpha:beta of 2:1 The formulation was then diluted in PLC grade water to 40, 4.0 and 0.4 mg/mL, providing a nominal application volume of 0.064 mL/preparation, or 0.025 mL/cm^2. Immediately before dose application, the receptor chamber was filled with a known volume of acidified ethanol/water (1:1v/v). The test substance was applied at 1, 0.1 and 0.01 mg/cm^2 to 8 preparations each of human and rat skin. Additionally, 4 preparations from each species were treated at the highest dose and the skin surfaces washed 10 h after application. Samples of the receptor fluid were taken 1, 2, 4, 8, 10, 16, 24, 48 and 72 h after application. At 72 h post application the receptor fluid was removed. The epidermal skin surface was washed, rinsed and dried. The skin section was removed and weighed.

Results

The absorption, penetration and recovery of radiolabelled endosulfan in rat and human are detailed below.

72 hour skin penetration model - rat

Dose (mg/cm^2)	**0.01**	0.1	1.0	1.0 (10 h wash)
% not absorbed	1.7	3.9	23.8	51.1
% present in skin	13.3	14.3	30.7	28.1
% penetrated	95.8	75.9	40.2	9.1
% total recovery	110.8	94.1	94.7	88.3
Penetration rate (ug/cm^2)	0.9	4.4	15.9	15.9

72 hour skin penetration model - human

Dose (mg/cm^2)	**0.01**	0.1	1.0	1.0 (10 h wash)
% not absorbed	25.6	44.3	6.7	58.7
% present in skin	7.4	13.5	49.3	5.1
% penetrated	60.6	29.4	20.0	4.0
% total recovery	93.5	87.2	76.0	67.8
Penetration rate (ug/cm^2)	0.2	0.8	5.2	5.2

Recovery in the human system was lower than in the rat. The penetration of endosulfan in the rat was an average of 4.3 times the penetration of the human skin. The penetration of the compound, as a percentage of that applied, decreased as the concentration of the applied material increased

The metabolites present in the receptor fluid differed for rats and humans. These are detailed in the table below

Endosulfan products present in the Receptor Fluid after 72 h (as % of total radioactivity)

Metabolite	Rat	Human
α-endosulfan	3.1	-
β-endosulfan	81.4	27.3
endosulfan-sulfate	2.9	8.3
endosulfan-diol	8.8	34.0
endosulfan-OH-ether	-	2.7
Unknown	-	17.2

There appears to be an increase in degradation of the compounds following passage through human skin in comparison to rat skin. The components are the products of degradation, either spontaneous, or catalysed by surface bacteria or residual enzyme activity. Given the period of storage in sub-zero temperatures, it seems unlikely that the skin would retain significant metabolic activity. The greater levels of metabolism in human skin may be related to the increased passage time in comparison to the rat.

It can be seen, therefore, that the rate of penetration of endosulfan is lower in human skin in comparison to that seen in the rat. The penetration is dose dependent, and increased in a nonlinear manner with increasing dose. Little degradation of the compound occurred in rat skin, while this was more extensive in human skin.

Summary

The penetration of endosulfan through rat and human skin was studied *in vitro*. The test material consisted of radiolabelled endosulfan formulated as an emulsifiable concentrate (containing 353 g/L endosulfan), which had been diluted to concentrations ranging from 0.4 to 4.0 mg/mL in water. The test material was applied at nominal doses of 0.01, 0.1 and 1 mg/cm^2 to rat and human skin mounted in dermal penetration cells, the rate of penetration was determined. The penetration rate for rats was, on average, 4.3 times that of humans. The percentage of the applied dose varied with concentration with 61% of the lowest dose applied to human skin penetrating (96% in the rat) and 20% of the highest dose penetrating (40% in the rat). When the skin was washed 10 h after application, the amount of endosulfan penetrating decreased to 4% in the human and 9% in the rat. Endosulfan passing through human skin was metabolised or degraded to a greater extent than that passing through rat skin. (Noctor & John, 1995)

2.9 Comparative Pharmacokinetics

Following oral administration of endosulfan, either via single dose or dietary administration, elimination of the parent compound and its metabolites is extensive and relatively rapid in a range of species of experimental animals. In rats and mice, recovery of radiolabelled test material was generally greater than 85% of the administered dose, with a majority of this excretion occurring within a few days of administration. Excretion in rodents was mainly in the faeces, with a smaller amount excreted in the urine. Similarly, elimination of endosulfan was extensive in goats (>90%), with about 50% recovered in the faeces and 40% in the urine. Dermal absorption was reported to be as high as 25% in rats, and about 20% in Rhesus monkeys.

In mice, endosulfan and its sulfate and diol metabolites were the major faecal excretion products, with the diol metabolite excreted in the urine, while in rats, biliary excretion was extensive (up to 50%), and there was little enterohepatic circulation from the bile. There does not appear to be appreciable bioaccumulation of endosulfan residues in body tissues, with only trace amounts of endosulfan residues found in most tissues, including the fat, of most species. In Wistar rats, kidney and liver residues were highest, although the half life for residues in these organs was only 7 days and 3 days, respectively, and kidney residues were also higher than other tissues in goats. In other species, kidney residues were generally low.

3. ACUTE TOXICITY STUDIES

3.1 Lethal Dose Studies

The available lethal dose studies using endosulfan technical and its isomers are summarised below. Most data have been evaluated by the Department previously and are thus only tabulated; data newly submitted (Submission number: 11303) have been tabulated and also evaluated in detail (see below). A comprehensive evaluation of lethal dose studies reported in the open literature (pre 1984) is also listed in the Environmental Health Criteria 40 on endosulfan (EHC 40, World Health Organisation, 1984).

Endosulfan has high acute toxicity in experimental animals, with wide variation in the LD50 of endosulfan depending on the route of administration, species, chemical specification of the test material, dosing vehicle and sex of the animal. Females were generally more sensitive to the acute toxicity effects of endosulfan than males, often by one order of magnitude or more. In many older toxicity studies, the chemical identity of the test material, including impurities, stabilisers and metabolites, was often poorly characterised. The oral LD50 for endosulfan in rats ranged from 9.6 to 160 mg/kg, and in mice from 13.5 to 35 mg/kg. However, the lowest oral LD50 for rats, using technical endosulfan known to conform to current FAO specifications, was 22.7 mg/kg (Diehl and Leist, 1988a). Similarly, the lowest dermal LD50 in rabbits was 106 mg/kg, and in rats was 290 mg/kg, but the lowest dermal LD50 using current FAO specification endosulfan was 500 mg/kg in female rats, and >4000 mg/kg in male rats (Diehl and Leist, 1988b). In a 4-hour, whole body inhalational study the lowest LC50 was 13 mg/m^3 in female rats. The clinical signs of intoxication include piloerection, salivation, hyperactivity, respiratory distress, diarrhea, tremors, hunching and convulsions. The isomers of endosulfan also show acute oral toxicity profiles similar to that of technical endosulfan.

Summary of Lethal Dose Studies for technical endosulfan and its isomers

SPECIES	SEX	ROUTE	VEHICLE	LD50 (mg/kg)	REFERENCE
(a) technical endosulfan					
Rat (SD)	M	po	25% in food	2850	Bracha (1977)
Rat (SD)	F	po	25% in food	45	Bracha (1977)
Rat (CD)	M/F	po	maize oil	43	Lightowler et al (1978)
Rat (albino)	M	po	cottonseed oil	110	Elsea (1957)
Rat	-	po	ethanol	40-50	FAO (1988)
Rat (Haffkine)	M	po		115	Bhide & Naike (1984a)
Rat (Haffkine)	F	po		15	Bhide & Naike (1984a)
Rat (Holtsman)	M	po	corn oil	86.6	Elsea (1958)

Rat (Wistar)	M	po	2% starch	100-160	Diehl & Leist (1988a)
Rat (Wistar)	F	po	2% starch	22.7	Diehl & Leist (1988a)
Rat (SD)	M	po	5% CMC	40	Hazelton (1975a)
Rat (SD)	F	po	5% CMC	9.6	Hazelton (1975a)
Mouse(Balb/c)	-	po	-	15	FAO (1988)
Mouse (Kasauli)	M	po	Tween 80	35	Bhide & Naike (1984b)
Mouse (Kasauli)	F	po	Tween 80	13.5	Bhide & Naike (1984b)
Dog (mongrel)	M/F	po	gelatin capsule	76.7	Nogami (1970)
Rat (NWS)	M	dermal		290	Bhide & Naike (1984c)
Rat (HoeWISKf)	M	dermal		>4000	Diehl & Leist (1988b)
Rat (HoeWISKf)	F	dermal		500	Diehl & Leist (1988b)
Rabbit (NZ)	M	dermal		500-1000	Bracha (1977)
Rabbit (NZ)	F	dermal		1000-2000	Bracha (1977)
Rabbit (NZ)	M	dermal	saline	106	Crown et al (1982)
Rabbit (NZ)	F	dermal	saline	134	Crown et al (1982)
Rabbit (NZ)	M**	dermal	saline	45	Crown et al (1982)
Rabbit (NZ)	F**	dermal	saline	67	Crown et al (1982)
Rat (SD)	M/F	inhal		>21000#	Bracha (1977)
Rabbit (albino)	-	dermal	cottonseed oil	359	Elsea (1957)
Rat (Wistar)	M	inhal	ethanol & PEG	35*	Hollander & Weigand (1983)
Rat (Wistar)	F	inhal	ethanol & PEG	13*	Hollander & Weigand (1983)
Rat	-	ip	ethanol	8	FAO (1988)
(b) endosulfan - alpha isomer					
Rat	-	po	-	76	Goebal et al (1982)
Mouse (albino)	F	po	Tween 80	11	Dorough et al (1978)
(c) endosulfan- beta isomer					
Rat	-	po	-	240	Goebal et al (1982)
Mouse	F	po	Tween 80	36	Dorough et al (1978)

Inhalational LC50 value (mg/m^3) for 4 h (*) and 1 h (#) exposures; dermal exposure for 24 h. PEG = polyethylene glycol. ** indicates dermal studies on abraded skin, all others on intact skin.

3.1.1 Acute Oral Studies: Technical endosulfan

(a) Elsea (1958) Thiodan technical - Acute oral administration - rats. Hazleton Laboratories, Virginia, 28 February, 1958. Hoechst document no. A13686. (AgrEvo, 11303)

In an acute toxicity study in rats, technical endosulfan (lot no. MR 4914, considered to be 100% purity) was administered by oral gavage to groups of five male Holtsman albino rats at doses of 31.6, 46.4, 68.1, 100, 147, and 215 mg/kg body weight. The test material was delivered as a 1% w/v solution or a 10% w/v suspension in corn oil, and food was withheld from the animals for 3-4 h prior to dosing. Animals were observed for gross effects several times on the day of administration, and daily for seven days after administration. Gross autopsies were performed on all animals. No reference was made to the protocol used in this study, or the QA/GLP status of the study.

At the end of the seven day observation period, mortality in the dose groups described above was 0/5, 1/5, 2/5, 2/5, 5/5, and 5/5, respectively. At 215 mg/kg, no animals survived until the 2 h observation period, while at 147 mg/kg, all animals died within 24 h, but 4/5 died in the first hour following administration.

In all groups, signs of intoxication were observed within one hour of administration, including depression, lacrimation, exophthalamia, laboured respiration, ataxia, salivation, tremors, clonic/tonic convulsions, and depressed righting, placement and pain references. Death was preceded by bloody discharge from the eyes, gasping, clonic/tonic convulsions, and coma. Surviving animals at the lower dose levels exhibited essentially normal appearance and behaviour from about 48 h after administration. Gross autopsies on animals that died showed hyperemic or hemorrhagic lungs, irritation of the pylorus and small intestine, and congested adrenals and kidneys.

The acute oral LD50 was 86.6 mg/kg, with confidence intervals from 61.6 to 122 mg/kg.

(b) Diehl & Leist (1988a) HOE 002671 - active ingredient technical (Code: Hoe 002671 0I ZD96 0002). Testing for acute oral toxicity in the male and female Wistar rat. Pharma Research Toxicology and Pathology, Frankfurt am Main, Study no 88.0551, 27 July 1988. Hoechst document no A39680. (AgrEvo, 11303)

Technical endosulfan (96% purity, Hoechst) was suspended in a 2% starch mucilage (potato starch in deionised water) and administered by gavage to groups of male and/or female Wistar rats (strain Hoe: WISKf(SPF71), Hoechst) at doses ranging from 12.5 to 315 mg/kg body weight. At 12.5 and 25 mg/kg, test groups consisted of 5 females only. At 50 mg/kg, the test group consisted of 5 animals/sex, and at 100, 160, 250, and 315 mg/kg, 5 males/group were used. The test material was administered at 10 mL/kg body weight, and animals were observed for clinical signs of intoxication for 14 days postdosing. This study was conducted in compliance with US EPA assessment guidelines 81-1, EPA 540/9-82-025, and OECD guidelines 401, and in compliance with OECD GLP.

A range of clinical signs were observed in either males or females, including: squatting position, high-legged gait, prone or lateral position, reduced spontaneous activity, tonic spasms, saltatory or rolling spasms, tonoclonic spasms, piloerection, mydriasis, clear, bloody and foamy salivation, increased respiratory rate, and irregular breathing. Clinical signs were most marked on the day of treatment, but surviving animals were normal in appearance and behaviour after day 4 post administration.

In females, mortality was 1/5, 2/5, and 5/5 at 12.5, 25, and 50 mg/kg, respectively. In males, mortality was 0/5, 0/5, 5/5, 3/5, and 5/5 at 50, 100, 160, 250, and 315 mg/kg, respectively. The LD50 for females was 22.7 mg/kg, while for males the LD50 was estimated to be in the range 100-160 mg/kg.

(c) Hazleton Laboratories (1975a) Acute oral toxicity study in rats. Endosulfan technical-Final report. 18 December, 1975. Hazleton Laboratories America, project no 915-108. Hoechst document no A33732. (AgrEvo, 11303)

Technical endosulfan (purity and source not stated) was administered via oral gavage to groups of male and female Sprague-Dawley (Charles river, USA) at doses of 15.9, 25.1, 39.8, 63.1, and 100 mg/kg body weight (5 animals/sex/dose), and additionally to female rats at doses of 3.98, 6.31, and 10 mg/kg body weight and to males at 1000 mg/kg body weight (5 animals/dose). The test material was suspended in 0.5% sodium carboxymethyl cellulose, but the total dose volume administered was not stated. The animals were observed for treatment related effects and mortality immediately after dosing, at one and four hours postdosing, and daily thereafter for a total of 14 days. This study was conducted in compliance with the criteria of the Federal Substances Act, 16 CFR, Part 1500.3.

Clinical signs were observed in all treatment groups, ranging from listlessness at the lowest doses, through to tremors, hyperpnoea, prostration, and hunched appearances at the higher doses.

Mortality was observed at doses of 39.8 mg/kg and above in males (ranging from 4/5 at 39.8 mg/kg, to 5/5 at 100 and 1000 mg/kg), and in females at 10 mg/kg (3/5) and above (5/5 in higher dose groups). Generally, mortality occurred within one hour of dosing, and no additional mortality was observed between 24 h and 14 days post dosing. The LD50 in males was 40.38 mg/kg (95% CI 25.94-62.89 mg/kg) and in females was 9.58 mg/kg (95% CI 7.28 - 12.61 mg/kg).

3.1.2 Acute Dermal Toxicity: Technical endosulfan

Diehl & Leist (1988b) Endosulfan - active ingredient technical (Code: Hoe 002671 0I ZD96 0002). Testing for acute dermal toxicity in the male and female Wistar rat. Pharma Research Toxicology and pathology, Germany, study no 88.0552, 22 August 1988. Hoechst report no 88.1331, 1 September 1988, Document A39397 (AgrEvo, 11303).

Technical endosulfan (96% purity, Hoechst) was applied to the skin of male and female Wistar rats (strain Hoe: WISKf(SPF71); Hoechst) aged approximately 8-10 weeks, at doses ranging from 400 to 4000 mg/kg body weight. Groups of five females received doses of 400,

630, or 1000 mg/kg, while groups of five males received 3150 or 4000 mg/kg. The moistened test material was applied once to the shaved intact dorsal skin, and the test site was then covered with an occluded dressing for 24 h, at which time the site was washed with warm water to remove any remaining test material. Animals were observed for clinical signs and mortality for 14 days, during which time the animals were weighed weekly. This study was conducted in compliance with US EPA Guideline Subdivision f, Hazard Evaluation; Humans and domestic animals, Series 81: Acute toxicity and irritation studies, and 81-2, Acute dermal toxicity study, EPA 540/9-82-025. Also with OECD Guidelines for testing of chemicals, 402, updated guideline 24 February, 1987, and OECD Principles for GLP.

Clinical signs of intoxication were observed in males and females in the first three days after administration, and these signs included: squatting position, agitation, increased spontaneous activity, aggression, tonic spasms, saltatory and rolling spasms, piloerection, bloody and foamy salivation, increased respiratory rate and irregular breathing. Dryness and/or redness of the skin was observed in a number of animals, mainly in the high dose males.

No males died at 3150 mg/kg, while 1/5 males died at 4000 mg/kg. In females the mortality was 2/5, 3/5, and 4/5 at 400, 630, and 1000 mg/kg, respectively. No mortality was observed in the first 6 h of administration, with all deaths occurring between days 1 and 4 after administration. The LD50 for males was >4000 mg/kg, and the LD50 for females was 500 mg/kg.

3.1.3 End Use Products

A summary of the acute lethal dose toxicity of a range of end use products is provided in tabular form. Details of these studies are presented below.

EUP	Species	Sex	Route	LD50 mg/kg	Reference
353 g/L EC	Mouse(HoeNMR Kf)	M	po	39	Ebert & Leist (1989a)
353 g/L EC	Mouse(HoeNMR Kf)	F	po	41	Ebert & Leist (1989a)
25 ULV#	Rat (Wistar)	F	po	122	Hollander & Weigand (1975a)
25 ULV#	Rat (Wistar)	F	po	110	Hollander & Weigand (1975d)
25 ULV#	Rat (Wistar)	M	po	251	Hollander &Weigand (1975e)
25 ULV#	Rat (Wistar)	M	po	286	Hollander &Weigand (1975b)
352 g/L EC	Rat (Wistar)	M	po	67	Ebert & Leist (1989b)
352 g/L EC	Rat (Wistar)	F	po	17	Ebert & Leist (1989b)
352 g/L EC	Rabbit (NZW)	M	po	50	Ebert & Leist (1990)

352 g/L EC	Rabbit (NZW)	F	po	34	Ebert & Leist (1990)
352 g/L EC	Rat (Wistar)	M	dermal	412	Ebert & Leist (1989c)
352 g/L EC	Rat (Wistar)	F	dermal	266	Ebert & Leist (1989c)
25 ULV#	Rat (Wistar)	F	dermal	1414	Hollander & Wiegand (1975c)
25 ULV#	Rat (Wistar)	F	inhal	84.4*	Hollander & Wiegand (1976)

* LC50 value (mg/m^3); # Thiodan 25 ULV EUP. An additional acute inhalation study in Wistar rats, guinea pigs , rabbits and cat was provided (Hollander & Weigand, 1975f); the report stated that no animals died following inhalation for 1 h of 0.25% Thiodan 25 ULV. However, The reporting details are insufficent to estimate the actual exposure of animals and hence a LC50 estimate.

3.1.3.1 Acute Oral Studies

(a) Ebert & Leist (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. (AgrEvo, 11303)

The acute oral toxicity of an emulsifiable concentrate solution of endosulfan (containing 33.7 % Hoechst technical endosulfan) was tested in NMRI mice (Strain Hoe:NMRKf(SPF71); Hoechst AG SPF breeding colony) aged approximately 4 weeks at the start of the study. The test material was dissolved in deionized water and administered to fasted animals via gavage at doses of 25, 37.5, and 50 mg/kg body weight. The dose volume was 10 mL/kg body weight, and 5 animals/sex/dose were treated. During a 14-day observation period following treatment, mortality was recorded, and animals were weighed weekly. All animals were dissected and examined macroscopically at the end of the study. This study was conducted in compliance with US EPA Guidelines 81-1: Acute oral toxicity study, EPA 540/9-82-025, revised November 1984; and OECD Guidelines 401 Acute Oral Toxicity, OECD 1981, Updated 24 February, 1987; and Japan MAFF laws and regulations, 1985; and in compliance with OECD GLP Guidelines, 1981.

Clinical signs of intoxication included reduced spontaneous activity, irregular breathing, narrowed palpebral fissures, exophthalmos, straddling of the hind limbs, and tonoclonic spasms. Most signs developed shortly after endosulfan administration, and subsided after one day. Body weights were not affected by treatment, and examination of animals that died during the study revealed discolouration of the liver.

All animals that died during this study died within four hours of treatment. At 25 mg/kg, 1/5 females died, and at 37.5 mg/kg, 2/5 males and 1/5 females died. At 50 mg/kg, 5/5 males and 4/5 females died after treatment. The calculated LD50 doses (and 95% range of confidence)

were 39 (32-47) mg/kg in males and females combined, and 41 (0-infinity) mg/kg in females, while in males the LD50 was 39 mg/kg, with no calculation by probit analysis possible.

(b) 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. (AgrEvo, 11303)

The acute oral toxicity of an emulsifiable concentrate solution of endosulfan (containing 33.7 % Hoechst technical endosulfan) was tested in New Zealand albino rabbits (Hoechst AG conventional breed) aged approximately 3 months at the start of the study. The test material was dissolved in deionized water and administered to fasted animals via gavage at doses of 25, 50, and 80 mg/kg body weight. The dose volume was 5 mL/kg body weight, and 5 animals/sex/dose were treated. During a 15-day observation period following treatment, mortality was recorded, and animals were weighed weekly. All animals were dissected and examined macroscopically at the end of the study. This study was conducted in compliance with OECD GLP Guidelines, 1981.

Clinical signs of intoxication were dose related, and were observed in males and females, and included generalised convulsions, biting convulsions, and salivation. These signs were observed shortly after administration of the test material, and persisted for up to day 6 after treatment. Other signs included increased respiratory rate, hyperactivity, and reddish nasal discharge. In a number of animals, paresis, motor paralysis, and/or dragging of hind limbs were observed, with paresis and/or dragging of hind limb persisting past the end of the observation period in a number of cases. A single male from the 50 mg/kg group was sacrificed on day 10 of the study due to severe intoxication. Reduced body weights were seen in most groups in the first week of the study, and most animals regained their initial body weights by the end of the study.

Mortality was in females at all dose levels, with 2/5, 3/5, and 5/5 deaths at 25, 50, and 80 mg/kg, respectively. Mortality was noted between 1 h and 9 days after treatment. In males, 3/5 animals died at 80 mg/kg. The LD50 (95% confidence range) was 34 (9-58.8) mg/kg in females, 75 (44.1-346) mg/kg in males, and 50 (30.6-90.6) mg/kg in males and females combined.

(c) Hollander& Weigand (1975a) Thiodan 25 ULV Pfl.-Ausl. 1347. Manuf. 1974 Batch No. 4491. Acute oral toxicity to the female SPF-Wistar-rat (Vehicle: Sesame oil). Pharma Research Toxicology and Pathology, Frankfurt am Main, Report no. 549/75, 7 November 1975. Hoechst document no A16751. Translation of document no. A05060 (AgrEvo, 11303)

An end use product containing endosulfan (Thiodan 25 ULV; concentration of active ingredient not stated) was dissolved in sesame oil and administered to fasted female SPF Wistar rats (Hoechst) at doses of 40, 63, 100, 160, 250, and 400 mg/kg body weight, with 10 animals/dose level. The dose volumes were not stated. Animals were observed for 14 days after treatment.

Animals that died after treatment showed disequilibrium and tonoclonic spasms. No animals died at 40 mg/kg, while mortality was 2/10, 4/10, 8/10, 8/10, and 9/10, at 63, 100, 160, 250, and 400 mg/kg, respectively. The acute oral LD50 was calculated to be 122 (93-161) mg/kg.

(d) Hollander & Weigand (1975b) Thiodan 25 ULV Pfl.-Ausl. 1347. Manuf. 1974 Batch No. 4491. Acute oral toxicity to the male SPF-Wistar-rat (Vehicle: Sesame oil). Pharma Research Toxicology and Pathology, Frankfurt am Main, Report no. 548/75, 6 November 1975. Hoechst document no A16750. Translation of document no. A05059 (AgrEvo, 11303)

An end use product containing endosulfan (Thiodan 25 ULV; concentration of active ingredient not stated) was dissolved in sesame oil and administered to fasted male SPF Wistar rats (Hoechst) at doses of 100, 160, 250, 400, and 630 mg/kg body weight, with 10 animals/dose level. The dose volumes were not stated. Animals were observed for 14 days after treatment.

Animals that died after treatment displayed tonoclonic spasms. No animals died at 100 mg/kg, while mortality was 2/10, 3/10, 7/10, and 10/10, at 160, 250, 400, and 630 mg/kg, respectively. The acute oral LD50 was calculated to be 286 (230-356) mg/kg.

(e) Ebert & Leist (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. (AgrEvo, 11303)

The acute oral toxicity of an emulsifiable concentrate solution of endosulfan (containing 33.7 % Hoechst technical endosulfan) was tested in Wistar rats (Strain Hoe:WISKf(SPF71); Hoechst AG SPF breeding colony) aged approximately 7 weeks at the start of the study. The test material was dissolved in deionized water and administered to fasted animals via gavage with a dose volume was 10 mL/kg body weight. Five females/dose were treated at 10, 16, and 25 mg/kg, while 5 males/dose were treated at 40, 63, and 100 mg/kg. During a 14-day observation period following treatment, mortality was recorded, and animals were weighed weekly. All animals were dissected and examined macroscopically at the end of the study. This study was conducted in compliance with US EPA Guidelines 81-1: Acute oral toxicity study, EPA 540/9-82-025, revised November 1984; and OECD Guidelines 401 Acute Oral Toxicity, OECD 1981, Updated 24 February, 1987; and Japan MAFF laws and regulations, 1985; and in compliance with OECD GLP Guidelines, 1981.

Clinical signs of intoxication were similar in males and females, and included reduced spontaneous activity, irregular breathing, narrowed palpebral fissures, straddling of hindlimbs, forward crawling, stilted gait, squatting posture, trembling, twitching, tonic spasms, tonoclonic spasms, blood encrusted eye margins and snout, increased vocalisation, and increased salivation. These signs were observed shortly after administration of the test substance, and generally disappeared before day 2 of the study. Body weights were not affected by treatment. A range of macroscopic effects were observed in animals that died during the study, including discolouration of the kidneys and liver, reddish mucous in the

small and large intestine, and lungs congested with blood. No visible macroscopic changes were observed in animals that survived the treatment.

In females, mortality was 0/5, 2/5, and 5/5 at 10, 16, and 25 mg/kg, respectively. The LD50 in females was 17 mg/kg (nonlinear interpolation between 16 and 25 mg/kg). In males, mortality was 0/5, 2/5, and 5/5 at 40, 63, and 100 mg/kg, respectively. The LD50 in males was 67 mg/kg (nonlinear interpolation between 63 and 100 mg/kg).

(f) Hollander& Weigand (1975d) Thiodan 25 ULV (Hoe 2761 0 I WE026, Op No: 026/74). Acute oral toxicity to the female SPF-Wistar-rat (Vehicle: Sesame oil). Pharma Forschung Toxikologie, Hoechst Aktienesellschaft, Frankfurt am Main, Report no. 546/75, 6 November 1975. Translation of doc No: A05058, Hoechst document no A09979. (AgrEvo, 11218)

An end use product containing endosulfan (Thiodan 25 ULV; concentration of active ingredient not stated) was dissolved in sesame oil and administered by gavage to fasted female SPF Wistar rats (100-152 g bodyweight, Hoechst breeding stock) at doses of 50, 80, 125, 200, and 320 mg/kg, with 10 animals/dose level. The dose volumes were not stated. Animals were observed for 14 days after treatment.

Animals that died after treatment showed tonoclonic convulsions. No animals died at 50 mg/kg, while mortality was 4/10, 5/10, 9/10, and 10/10, at 80, 125, 200, and 320 mg/kg, respectively. Macroscopic pathology of the animals revealed no abnormalities.

The acute oral LD50 was calculated to be 110 (88-136) mg/kg.

(g) Hollander & Weigand (1975e) Thiodan 25 ULV (Hoe 02671 0 I WE026, Op no: 026/74) . Acute oral toxicity to the male SPF-Wistar-rat (Vehicle: Sesame oil). Pharma Forschung Toxikologie, Hoechst Aktienesellschaft, Frankfurt am Main, Report no. 545/75, 6 November 1975. Translation of doc No: A05057, Hoechst document no A09978. (AgrEvo, 11218)

An end use product containing endosulfan (Thiodan 25 ULV; concentration of active ingredient not stated) was dissolved in sesame oil and administered to fasted male SPF Wistar rats (92-155 g bodyweight, Hoechst breeding stock) at doses of 125, 200, 320, 500, and 800 mg/kg body weight, with 10 animals/dose level. The dose volumes were not stated. Animals were observed for 14 days after treatment.

Animals that died after treatment displayed tonoclonic convulsions. Mortality was 1/10, 1/10, 9/10, 9/10, and 10/10, at 125, 200, 320, 500, and 800 mg/kg, respectively. Macroscopic examination of all animal did not reveal any abnormalities.

The acute oral LD50 was calculated to be 251 (205-308) mg/kg.

3.1.3.2 Acute Dermal Studies

(a) Ebert & Leist (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. (AgrEvo, 11303)

The acute dermal toxicity of an emulsifiable concentrate solution of endosulfan (containing 33.7 % Hoechst technical endosulfan) was tested in Wistar rats (Hoechst AG: strain Hoe: WISKf SPF71) aged approximately 9-13 weeks at the start of the study. The test material was administered undiluted at doses of 100, 250, 400, 630, and 1250 mg/kg body weight, with 10 mL/kg body weight applied to the shaved intact dorsal skin of animals, and retained in place under an occlusive dressing for 24 h. After this time, the test site was washed with warm water to remove remnants of the test material. At 100 and 250 mg/kg, 5 females/dose were used; at 630 and 1250 mg/kg, 5 males/dose were used; and at 400 mg/kg, 5 animals/sex were used. During a 14-day observation period following treatment, mortality was recorded, and animals were weighed weekly. All animals were dissected and examined macroscopically at the end of the study. This study was conducted in compliance with US EPA Guidelines 81-2: Acute dermal toxicity study, EPA 540/9-82-025, revised November 1984; and OECD Guidelines 402 Acute dermal toxicity, OECD 1981, Updated 24 February, 1987; and Japan MAFF laws and regulations, 1985; and in compliance with OECD GLP Guidelines, 1981.

The clinical signs of toxicity were seen in males and females, and included straddling of the hind legs, irregular respiration, bizarre movements, stilted gait, ataxic gait, uncoordinated gait, twitching, dilated pupil, decreased spontaneous activity, increased startle reflex, diarrhoea, straub tail, red coloured salivation, increased sound production, clonic spasms, tonic spasms, trembling, motor hyperactivity, increased salivation, and squatting position. The majority of these signs were observed during the first three days after treatment. The test material was irritating to the skin, with dermal findings including erythema, chapping, and scaling. Body weights were not affected by treatment. Macroscopic examination of those animals found dead during the study included discoloured liver and spleen, congestion of lungs, and intestinal tract filled with gas and reddish mucous.

In females, mortality was 0/5, 2/5, and 5/5 at 100, 250, and 400 mg/kg, respectively. In males, mortality was 2/5, 4/5, and 4/5 at 400, 630, and 1250 mg/kg, respectively. Most deaths occurred on days 2-3 of the study. The LD50 in males, calculated by probit analysis, was 412 mg/kg, while in females, the LD50 was approximately 266 mg/kg (between 100 and 400 mg/kg).

(b) Hollander & Weigand (1975c) Thiodan 25 ULV Pfl.-Ausl. 1347. Manuf. 1974 Batch No. 4491. Acute percutaneous toxicity to the female SPF-Wistar-rat. Pharma Research Toxicology and Pathology, Frankfurt am Main, Report no. 550/75, 7 November 1975. Hoechst document no A16752. Translation of document no. A05062 (AgrEvo, 1130)

The acute dermal toxicity of Thiodan 25 ULV, an end use product containing technical endosulfan (concentration not stated) was determined in female SPF Wistar rats (Hoechst). The test material was dissolved in sesame oil and applied once at dose levels of 500, 1000, 2000, and 4000 mg/kg body weight to the shaved intact dorsal skin of the rats, and then

covered for 24 h with an occlusive dressing, after which time the test site was thoroughly washed with tepid water. Animals were kept under constant supervision for the day of treatment, and then observed for a 14 day follow-up period after treatment.

Mortality was 0/6, 2/6, 4/6, and 6/6 at 500, 1000, 2000, and 4000 mg/kg, respectively. Animals that died during the study showed lethargic behaviour and tonoclonic spasms after treatment. The dermal LD50 (95% confidence limits) was determined to be 1414 (994-2012) mg/kg.

3.1.3.3 Acute Inhalational Studies

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 (AgrEvo, 1130)

The acute inhalational toxicity of Thiodan 25 ULV (containing technical endosulfan, concentration unstated) was assessed in a dynamic aerosol exposure apparatus, with female Wistar rats (Hoechst; 10 animals/group) exposed for 4 h (snout only) to doses of 14.23, 84.3, 122, and 443 mg/m^3. Additionally, to simulate field exposure, 10 animals were exposed to a concentration of 4 mg/m^3 for 4 h. The behaviour of animals was closely monitored during exposure, and during a 14 day post exposure monitoring period.

At the low dose of 4 mg/m^3, all rats displayed accelerated respiration after 90 minutes of exposure, but no other treatment related effects were reported. At the higher test doses, signs of intoxication ranged from increased respiration after more than 2 h of exposure at 14.23 mg/m^3, to accelerated respiration (20 minutes exposure), tonoclonic convulsions (50 minutes exposure), and death (98-195 minutes exposure).

Mortality was 0/10, 5/10, 8/10, and 10/10 at 14.23, 84.3, 122, and 443 mg/m^3, respectively. At the simulated field exposure application of 4 mg/m^3, no animals died. The median LC50 established in this study was 84.36 mg/m^3 (95% confidence limits 60-117 mg/m^3).

3.1.4 Oral Lethal studies for Endosulfan Metabolites

A number of studies are available detailing the acute oral toxicity of the metabolites of endosulfan in laboratory and small animals. These data are summarised below.

SPECIES	SEX	ROUTE	VEHICLE	LD50 (mg/kg)	REF
(a)endosulfan lactone					
Mouse (albino)	F	po	Tween 80	120	Dorough et al (1978)

Rat (Wistar)	F	po	starch suspension	290	Hollander &Kramer (1975a)
Rat (Wistar)	M	po	starch suspension	165	Hollander &Kramer (1975b)
Rat	M/F	po	sesame oil	105-115	Goebal et al (1982)
(b) endosulfan sulfate					
Mouse (albino)	F	po	Tween 80	8	Dorough et al (1978)
Rat (Wistar)	F	po	starch suspension	76	Hollander &Kramer (1975c)
Dog (Beagle)	M	po	starch suspension	15	Hollander &Kramer (1975d)
(c) endosulfan hydroxyether					
Mouse (albino)	F	po	Tween 80	120	Dorough et al (1978)
Rat	M/F	po	starch suspension	1750	Goebal et al (1982)
(d) endosulfan ether					
Mouse (albino)	F	po	Tween 80	270	Dorough et al (1978)
Rat	M/F	po	starch suspension	> 1500	Goebal et al (1982)
(**e) endosulfan diol**					
Mouse (albino)	F	po	Tween 80	> 2000	Dorough et al (1978)
Rat	M/F	po	starch suspension	>1500	Goebal et al (1982)

Like endosulfan, the toxicity of the metabolites vary depending upon vehicle and species used. In general the toxicity of the metabolites were similar to the parent compound, except for endosulfan diol which has low acute oral toxicity in the mouse. The clinical signs of intoxication were similar to that of the parent compound and included piloerection, salivation, hyperactivity respiratory distress, diarrhea, tremors, hunching and convulsions.

3.2 Eye Irritation Studies in Rabbits

3.2.1 Technical endosulfan

(a)Hazleton Laboratories. (1975c) Acute eye irritation potential study in rabbits. Endosulfan technical-Final report. 22 December, 1975. Hazleton Laboratories America, project no 915-112. Hoechst document no A33730. (AgrEvo, 11303)

Technical endosulfan (purity and source not stated) was instilled into the conjunctival sac of the left eye of three new Zealand White rabbits (Bunnyville Farms, USA) in aliquots of 0.1 mL (84 mg) to determine the eye irritation potential of the test material. The treated eyes were washed with tap water after 20 seconds of treatment. The right eye of each rabbit was not treated and served as a control. The treated eyes of each rabbit were examined for gross signs of irritation at 24, 48 and 72 h post installation, and evidence of corneal damage was assessed following staining at 24 and 72 h. Irritation was graded and scored according to the Draize system. No indication was given as to the GLP status of the study, or the test guidelines used.

No evidence of eye irritation or corneal damage was reported in this study. Under the conditions of this study, technical endosulfan was not considered to be an eye irritant in rabbits.

(b) Elsea (1957) Acute oral administration in rats, acute dermal application to rabbits, and acute eye irritancy in rabbits. Hazleton Laboratories. Document No. A13683. 11 January 1957.

Endosulfan technical (3.0 mg) was instilled into the conjunctival sac of the left eye of 3 albino rabbits. The untreated right eye served as a control. Animals were observed for signs of irritancy immediately following application and at 1, 4 and 24 h and then daily for a further 6 days.

Immediately following application slight erythema and vascularization of the sclera and nictitating membrane and lachrymation were seen. These effects were transient and all eyes appeared normal by 24 h. Systemic toxicity from the mucous membrane absorption was not observed and is unlikely given that endosulfan technical is virtually insoluble in aqueous medium.

Under the conditions of this study, endosulfan technical showed none to only very slightly irritancy.

(c) Bracha (1977) Thionex Tech: Acute oral toxicity study in rats, skin irritation study in rabbits, acute dermal toxicity study in rabbits, eye irritation study in rabbits and acute inhalation study in rats. Report No. 6111820. Warf Institute Inc. Madison, USA, 2 February 1977.

Endosulfan (100 mg) was instilled into one eye of each of 6 New Zealand White rabbits; the untreated eye served as a control. Reaction to test material was noted on instillation and at 24, 48 and 72 h.

No irritation of the cornea or iris were seen. There was mild chemosis and redness of the conjunctiva in 4 animals within 24 h; this had subsided at 48-72 h. Under the conditions of this study, endosulfan showed no to only slight ocular irritancy potential.

3.2.2 End Use Products

Ebert & Leist (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. (AgrEvo, 11303)

The acute eye irritation of the undiluted emulsifiable concentrate solution of endosulfan (containing 33.7 % Hoechst technical endosulfan) was tested in New Zealand albino rabbits (Hoechst AG conventional breed) aged approximately 3-5 months at the start of the study. The test material (0.1 mL) was applied once to the conjunctival sac of the left eye of 6 rabbits, with the untreated eyes serving as controls. The treated eyes of the animals were left unwashed for 24 h. If there were signs of irritation at 72 h, the test substance was applied to the conjunctival sac of the left eye of 3 other animals, and left unwashed for 2 minutes, at which time the treated eyes were thoroughly washed with physiological saline solution. The eyes were examined at 1, 24, 48, and 72 h after test substance administration, and further examinations were performed at 7, 14, and 21 days. Lesions in cornea, iris, and conjunctivae were scored numerically. This study was conducted in compliance with US EPA Guidelines 81-4: Primary eye irritation, EPA 540/9-82-025, revised November 1984; and OECD Guidelines 405 Acute eye irritation/corrosion, OECD 1981, Updated 24 February, 1987; and Japan MAFF laws and regulations, 1985; and in compliance with OECD GLP Guidelines, 1981.

In eyes washed after 24 h, irritation was observed from the 1 h examination to the end of the observation period. At 1 h, moderate clear colourless discharge, slight to moderate chemosis, and slight to diffuse crimson conjunctival redness were observed in all animals. In three animals, iridial hyperaemia was also observed. The mean irritancy score at 1-2 h was 13.5. At 24 h, chemosis was generally moderate, but all animals showed moderate to severe, white-yellowish and viscous discharge and diffuse crimson to diffuse beefy red colouring of the treated eye. Iridial inflammation, and corneal opacity with visible iris were observed in most animals, and generally involved more than three quarters of the eye. The eyes were irrigated after 24 h. The mean irritancy score at 24 h was 38.3. At 48-72 h after treatment, the irritation was similar to that seen at 24 h, except that the corneal opacity was denser, with easily discernable translucent areas, and details of iris slightly obscured, and the area affected was reduced to one quarter to one half of the surface in some animals, but remained at greater than three quarters in other animals. The mean irritancy scores at 48 and 72 h were 36.7 and 34.2, respectively. At 7 days, the majority of animals still had irritation similar to that seen at 72 h, while the irritation was greatly reduced in one animal, and had disappeared in another. The mean irritancy score at 7 days was 25.2. At 14 days, three animals still had signs of irritation, one with areas of corneal opacity, and at 21 days, several animals still displayed signs of irritation similar to those seen at 14 days.

In eyes washed out after 2 minutes, similar progression and severity of irritation was observed to that seen in the eyes washed after 24 h, with a maximum irritancy score of 38 after 7 days. Corneal opacity was observed from day 1 until day 21, and at 72 h, diffuse beefy redness was still observed.

Under the conditions of this study, the test material was considered to be a severe eye irritant to rabbits, as the corneal opacity was not reversible after 7 days. This effect was seen in treated eyes washed either 2 min or 24 h after treatment.

3.3 Dermal Irritation Studies in Rabbit

3.3.1 Technical endosulfan

(a)Hazleton Laboratories. (1975b) Primary skin irritation study in rabbits. Endosulfan technical-Final report. 12 November, 1975. Hazleton Laboratories America, project no 915-112. Hoechst document no A33731. (AgrEvo, 11303)

Technical endosulfan (0.5 g; purity and source unstated) was applied to the premoistened intact or abraded shaved skin on the flanks of six new Zealand White rabbits, then covered with gauze and occlusive dressings for 24 h post application, at which time the test site was rinsed with water to remove the test material. Observations for skin reactions were made 24 and 72 h post application, and graded and scored according to the system of Draize. The study was conducted in accordance with criteria of the federal Hazardous Substances Act, 16 CFR, Part 1500.41.

At 24 h, very slight to severe erythema at the abraded sites, and very slight to slight erythema at the intact sites, were observed. Necrosis along an abrasion was also noted in one animal at 24 h. At 72 h, very slight erythema was noted at the abraded sites in two animals, and at the intact site of one animal. The primary irritation score of was 0.9, calculated from erythema and oedema scores.

Under the conditions of this study, technical endosulfan was not considered to be a skin irritant to rabbits.

(b) Elsea (1957) Acute oral administration in rats, acute dermal application to rabbits, and acute eye irritancy in rabbits. Hazleton Laboratories. Document No. A13683. 11 January 1957.

During acute dermal toxicity testing, endosulfan, applied to the shaved abdomen of albino rabbits for a 24 h period under an occlusive bandage, induced slight to mild erythema which subsided within 1 to 4 days. Endosulfan was applied as 10 or 20% solutions in cottonseed oil at doses of 40, 100, 215, 404 or 1000 mg/kg. The dose also produced slight atonia and/or slight desquamation 3 to 4 days after exposure.

Under the conditions of thsi study, endosulfan technical was a slight skin irritant.

(c) Bracha (1977) Thionex Tech: Acute oral toxicity study in rats, skin irritation study in rabbits, acute dermal toxicity study in rabbits, eye irritation study in rabbits and acute inhalation study in rats. Report No. 6111820. Warf Institute Inc. Madison, USA, 2 February 1977.

Endosulfan technical (500 mg) was applied to the clipped intact and abraded, back and flanks of New Zealand White rabbits. The treated areas were covered with a gauze patch and a semi-occlusive bandage for 24 h. Erythema and oedema were scored after the 24 h contact period and at 72 h.

No primary skin irritation was seen in any of the animals tested at any of the observation times. Endosulfan appears to lack potential to produce skin irritancy in rabbit under the conditions of this test.

(d) Dikshith (1984) Skin irritation studies of endosulfan technical in female rabbits. Industrial Toxicology Research Centre, India. submitted to Department. October 1984.

Endosulfan (50 mg/kg) was applied as a single application to the clipped intact skin of adult (1.2-1.5 kg) albino female rabbits under semi-occlusive bandage for 24 h. Control animals were treated with the acetone/ethanol solvent Animals were observed for up to 7 days post application for erythema and oedema.

No signs of erythema or oedema were seen in any of the test animals. Endosulfan lacked primary skin irritation potential under the conditions of this test.

3.3.2 End Use Products

Ebert & Leist (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. (AgrEvo, 11303)

The acute dermal irritation of the undiluted emulsifiable concentrate solution of endosulfan (containing 33.7 % Hoechst technical endosulfan) was tested in New Zealand albino rabbits (Hoechst AG conventional breed) aged approximately 3-5 months at the start of the study. The test material (0.5 mL) was applied to the shaved intact skin on the dorsal region of six animals, and then covered with surgical plaster under a semi occlusive bandage for 4 hours. After the exposure period, all remnants of the test material were removed from the skin with warm tap water, and the skin was examined for irritation after 30-60 minutes, and 24, 48, and 72 h after removal of the patches. If abnormalities were present after 72 h, further examinations were conducted at 7, 14, and 21 days. Erythema, eschar formation and oedema were evaluated numerically according to the methos of Draize. This study was conducted in compliance with US EPA Guidelines 81-5: Primary dermal irritation, EPA 540/9-82-025, revised November 1984; and OECD Guidelines 405 Acute dermal irritation/corrosion, OECD 1981, Updated 24 February, 1984; and Japan MAFF laws and regulations, 1985; and in compliance with OECD GLP Guidelines, 1981.

After 30-60 minutes from removal of the plaster, very slight but well defined erythema was observed in all animals, and very slight but well defined oedema was observed in 5/6 animals. After 24-48 h, all animals displayed well defined erythema, while very slight oedema persisted in several animals. After 72 h, erythema had progressed to moderate to severe in 3/6 animals, while oedema had disappeared in most animals. After 7 days erythema was still moderate-severe in one animal, and moderate in another, and all signs of dermal irritation did not disappear until 28 days after treatment, with well defined erythema still seen in several animals after 21 days.

Under the conditions of this study, the test material was considered to be a moderate skin irritant to rabbits.

3.4 Dermal Sensitisation Studies in Guinea Pigs

3.4.1 Technical Endosulfan

Jun & Weigand (1983) Dermal sensitization in guinea-pig. Test for sensitizing properties in female Pirbright-White Guinea pigs according to the method of Buehler. Hoechst AG. Project No. Tx117/06.04-20, Report No. 83.0339. 15 July 1983.

Endosulfan technical was administered, epicutaneously, to the shaved flank of 20 female Pirbright-White guinea-pigs at a dose of 0.5 mL of 40% endosulfan in polyethylene glycol 400, 3 times/week for 3 weeks. A vehicle control group of 10 females received 0.5 mL of polyethylene glycol 400 over the same treatment regime. For each treatment, endosulfan was held under a semiocclusive patch for 6 hours when excess compound was washed from the flank. Following the last application, animals remained untreated for 16 days when they were challenged with two treatments of endosulfan at a 48 h interval; both treated and control animals received 0.5 mL of the 40% endosulfan and skin reactions evaluated at 24 and 48 h.

No signs of toxicity or dermal irritation were seen in any of the animals tested. Endosulfan did not induce skin sensitization in the guinea-pig under the conditions of this assay.

3.4.2 End Use Products

Ullmann (1986) Delayed contact hypersensitivity to Endosulfan-emulsifiable concentrate 352 (g/L) (Code: Hoe 002671 0I EC33 B310) in Albino Gunea Pigs. Buehler test. Research and Consulting Company Switzerland, Project 074935, Report dated 5 November 1986. Hoechst document A34194. (AgrEvo 11303)

The dermal sensitisation potential of a 33.5% emulsifiable concentrate of endosulfan (Hoechst) was studied in Himalayan white spotted guinea pigs (outbred) aged 8-9 weeks at start of treatment. Ten animals/sex/group were used in vehicle control (physiological saline) and test article groups. One flank of each of ten test and control animals was shaved and an adhesive patch containing 0.5 mL of the selected test article was applied to the skin for six

hours, three times/week, for three consecutive weeks (nine applications in total/animal). No test material was applied for a further two weeks, after which time the untreated flanks of each animal were depilated and then challenge doses of test and control materials were made. The challenge doses were applied for six hours, and the treated skin sites were examined for erythema at approximately 24 and 48 h after the challenge dose. A second challenge was applied two weeks after the first using a similar procedure to that described above.

No positive reactions were observed in control or treated animals at either of the challenge periods. Under the conditions of this study, the test material was not sensitising to the skin in guinea pigs.

3.5 Antidote Studies

Ebert, Weigand (1984). Testing of the therapeutic effect of diazapam and phenobarbital in the event of acute poisoning with endosulfan-active ingredient technical (Code: Hoe 002671 OI ZD97 0003) in Wistar rats. Pharma Forshung Toxikologie, report no 84.0062, 2 May 1984. Hoechst document A29211 (AgrEvo 11303).

To test the therapeutic effect of diazepam and phenobarbital after acute endosulfan poisoning, 75 female Wistar rats (Hoe: WISKf SPF71, Hoechst) were administered technical endosulfan (97.2% purity, Hoechst) via oral at a dose of 80 mg/kg body weight. The test material was suspended in a 2% starch solution and administered to the animals as a 1% concentration. Twenty animals received the test material alone, 5 animals /group received subsequent intraperitoneal injections of diazepam (5% preparation), either as a 2 mg/kg injection 10-20 minutes after the endosulfan, 2 mg/kg after 10-20 minutes and 60-75 minutes, or 20 mg/kg injections after 10-20 minutes and 105-165 minutes. In addition, a group of 10 animals received a single diazepam injection of 60 mg/kg after 10-20 minutes. Groups of 10 animals received injections of phenobarbital (2% solution in physiological saline) at 50 mg/kg (10 to 20 minutes and 10 mg/kg (105-165 minutes; or 70 mg/kg (10-20 minutes) and 20 mg/kg (105-165 minutes); or 70 mg/kg (10-20 minutes) and 10 mg/kg (60-75 minutes and 105-165 minutes, and 210-315 minutes, and daily from days 1-6).

After treatment, signs of intoxication were recorded, and animals were weighed weekly during the 14 day observation period.

All animals that did not receive antidote treatments died within 1 day of treatment (19/20 within 6 h of endosulfan administration). Clinical signs of intoxication included sedation, hyperactivity, trembling and tremors with tonoclonic convulsions, and jumping and rolling spasms. Salivation was frequently observed, and most animals died between 1 and 4 h after endosulfan administration.

All animals that received endosulfan followed by diazepam died within 1 day of treatment, with the exception of a single animal given 20 mg/kg diazepam plus 2 mg/kg diazepam, which survived the 14 day observation period. The clinical signs observed above were also seen in animals administered endosulfan and diazepam, with increased sedation seen depending upon the dose of diazepam.

For animals that were administered endosulfan followed by phenobarbital, the death rates were 7/10, 6/10, and 5/10, with survival increasing with the dose of phenobarbital. There was a marked reduction in the clinical signs of intoxication compared with those animals that did not receive antidote treatment, and reduced tonoclonic convulsions and spasms were seen in this group, particularly in those animals that survived the observation period.

Phenobarbital administration proved to be an effective therapeutic measure against an absolute lethal dose of endosulfan in rats, with reductions in the clinical signs of intoxication and in the mortality rate. Diazepam did not have a therapeutic effect against endosulfan intoxication in rats.

4. SHORT TERM REPEAT DOSE STUDIES

4.1 Rat

4.1.1 21-Day Dermal Study

Ebert, Weigand & Kramer (1985). Endosulfan - active ingredient technical (Code: Hoe 002671 0I ZD97 0003). Testing for subchronic dermal toxicity (21 applications over 30 days) in SPF Wistar rats. Pharma Forshung Toxicologie study number 83.0118 completed 5 October 1983. Hoechst report number 84.0321, 11 March 1985, Hoechst document number A30754, translation of document A30751. (SB:KI, A3162/8; AgrEvo 11303)

Technical endosulfan (Hoechst, 97.2% purity) was applied to the shaved nape skin of Wistar rats [strain Hoe: WISKf (Spf71); Hoechst] aged 8-10 weeks at the initiation of the study. At the beginning of the pretreatment period, 110 rats were assigned to treatment groups, with 5 animals/group. The test material was applied to the shaved skin 21 times over a 30 day period (5 days/week) in the form of 0.15, 0.3, 0.6, 2.4, 4.8, or 9.6% solutions (w/v) in sesame oil, with 2 mL/kg as the dose volume. Control animals were treated with the vehicle alone in the same proportion as for the highest dose test group. At each treatment, the test material was applied for 6 h under an occlusive bandage, after which time the test site was washed with a 20% aqueous solution of polyethylene glycol 400. For males, the dose levels were 0, 12, 48, 96, and 192 mg/kg/day, and for females the doses were 0, 3, 6, 12, and 48 mg/kg/day, with 6 animals/main group (terminated one day after the final dermal treatment), and 5 animals/recovery group (observed for 14 days after treatment).

Behaviour and general health were observed twice daily, and the animals were examined weekly for neurological disturbances, and other conditions. The test sites were examined for changes before each application, and irritation was scored according to the method of Draize. Bodyweights, and food consumption were measured twice weekly, and a haematology examination was conducted at the end of the study. The following haematology parameters were determined: haemoglobin, erythrocytes, leucocytes, haematocrit, reticulocytes, Heinz bodies, differential blood count, thrombocytes, coagulation time, methaemoglobin. Clinical

chemistry examinations were conducted at the end of the study, with the following parameters determined: sodium, potassium, inorganic phosphorous, uric acid, total bilirubin, direct bilirubin, creatinine, serum glucose, urea nitrogen, calcium, chloride, SGOT, SGPT, alkaline phosphatase, LDH, erythrocyte, serum and brain cholinesterase, cholesterol, total lipids, total protein. These parameters were measured in all main group animals, while in recovery groups, examination was confined to parameters which were affected by treatment in the main groups (ie cholinesterase). Urinalysis examination comprised the following parameters: appearance, colour, protein, glucose, haemoglobin, bilirubin, pH, sediment.

Absolute and relative organ weights of the following were determined: heart, lungs, liver, kidneys, spleen, brain, testes/ovaries, adrenals, pituitary, thyroid, seminal vesicles. A range of organs and tissues were preserved, with kidneys and liver subsequently examined histopathologically.

This study was conducted in accordance with US EPA Pesticide Assessment Guidelines, Subdivision F, Series 82: Subchronic testing and 82-2, Repeated Dose Dermal Toxicity: 21 day study, EPA 540/9-82-024, November 1982. Also OECD Guidelines for Testing of Chemicals, Section 4: Health effects, No 410, Repeated Dose Dermal Toxicity: 21/28 Day Study, 12 May 1981, and OECD Principles of GLP.

Results

No clinical signs of intoxication or mortality was reported in males at any dose level up to 96 mg/kg/day. At 192 mg/kg/day, 2/6 animals in the main group had tremors and/or hypersalivation, and one of these animals died on day 6 of the study. In the recovery group, 1/4 animals had hypersalivation and died on day 9 of the study. In females, no clinical signs of intoxication were observed at 3 or 6 mg/kg/day. At 12 mg/kg/day, one animal displayed piloerection and slight lacrimation on days 16-18. While these effects are slight, they are considered to be treatment related. At 48 mg/kg/day, a number of animals displayed signs of intoxication, including hypersalivation, crusted eyes, dacryohemorrhea, tonic/clonic convulsions, marked salivation, bloody exudate, bloody encrusted nose. Most of these signs were confined to a single animal that died on day 4 of the study. In females, mortality was mainly confined to the 48 mg/kg/day group, with 4/11 animals dying between days 2 and 22 of the study, while at 3, 6, and 12 mg/kg/day, single animals died on day 18 of the study, though none of these animals had previously displayed any signs of endosulfan intoxication.

On occasion, some slight changes in the skin were reported, including slight erythema, and dryness, but the irritation was confined to a small number of animals, and generally occurred early in the study, after which time the effects were reversed. No consistent dose related effects were observed on bodyweights and food consumption.

Haematology and urinalysis examinations did not reveal any changes in parameters which were considered to be treatment related. In the clinical chemistry examinations conducted on day 1 after the last treatment, statistically significant changes in a number of parameters were observed in males at all test doses. At 192 mg/kg, an isolated decrease in serum cholinesterase activity of about 33% in males was considered to be unrelated to treatment with endosulfan. The changes to other parameters (eg increased sodium and potassium at 96 mg/kg, decreased SGPT at 12 mg/kg, decreased erythrocyte cholinesterase activity (28%) at 48 mg/kg, and decreased brain cholinesterase activity (17%) at 96 mg/kg) were not

considered to be treatment related, as the effects were confined to a single dose and there was no dose relationship, and/or the changes were slight, and within normal biological variability. Similarly in females, a single statistically significant change in protein at 12 mg/kg was not considered to be treatment related. After a 14-day recovery period following treatment, clinical chemistry parameters were similar in control and treated groups, with the exception of erythrocyte cholinesterase activity in females, where reduced enzyme activity was observed at 3, 6, and 12 mg/kg. These reductions in activity, however, ranged from 11-13% compared with controls, and are not considered to be biologically significant.

No statistically significant changes in absolute organ weights resulted from treatment with endosulfan. Some slight changes in relative organ weights were observed, including slight increases in relative kidney weights at 12, 96, and 192 mg/kg in males. In the absence of other effects on the organs at gross and/or histopathological examination, these slight changes in relative organ weight are not considered to be treatment related.

Gross and histopathological (liver and kidneys) examinations were conducted on male and female animals from control groups, males at 12 and 192 mg/kg/day, and females at 48 mg/kg/day. No effects were observed that were attributed to treatment with endosulfan.

In males, the no observed effect level was 96 mg/kg/day, based on mortality, and clinical signs of intoxication (tremors and/or hypersalivation) at 192 mg/kg/day. In females, clinical signs of intoxication were observed at doses of 12 mg/kg/day and above, while mortality was mainly confined to the group receiving 48 mg/kg/day. However, at 3, 6, and 12 mg/kg/day, single animals also died, possibly due to poor application technique. As such, the sponsors followed this study immediately with another study using a modified method of test material application, to determine whether the mortality seen at the lower doses in females was related to treatment, and the report of that study is reported below.

4.1.2 21-Day Dermal Study

Ebert, Leist, Kramer, (1985). Endosulfan - active ingredient technical (Code: Hoe 002671 0I ZD97 0003). Testing for subchronic dermal toxicity (21 applications over 30 days) in Wistar rats. Pharma Forshung Toxicologie study number 83.0508 completed 11 November 1983. Hoechst report number 84.0223, 22 February 1985, document number A30753, translation of document A30750. (AgrEvo 11303)

Technical endosulfan (Hoechst, 97.2% purity) was applied to the shaved nape skin of Wistar rats [strain Hoe: WISKf (Spf71); Hoechst] aged 8-10 weeks at the initiation of the study. The test material was applied to the shaved skin 21 times over a 30 day period (5 days/week) in the form of 0.05, 0.15, 0.45, 1.35, or 4.05% solutions (w/v) in sesame oil, with 2 mL/kg as the dose volume. Control animals were treated with the vehicle alone in the same proportion as for the highest dose test group. At each treatment, the test material was applied for 6 h under an occlusive bandage, after which time the test site was washed with a 20% aqueous solution of polyethylene glycol 400, and with warm water. The dose levels were 0, 1, 3, 9, 27, and 81 mg/kg/day, with 6 animals/sex/group (males only at 81 mg/kg/day).

Behaviour and general health were observed twice daily, and the animals were examined weekly for neurological disturbances, and other conditions. The test sites were examined for

changes before each application, and irritation was scored according to the method of Draize. Bodyweights, and food consumption were measured twice weekly, and a haematology examination was conducted at the end of the study. The following haematology parameters were determined: haemoglobin, erythrocytes, leucocytes, haematocrit, reticulocytes, Heinz bodies, differential blood count, thrombocytes, coagulation time, methaemoglobin. Clinical chemistry examinations were conducted at the end of the study, with the following parameters determined: sodium, potassium, inorganic phosphorous, uric acid, total bilirubin, direct bilirubin, creatinine, serum glucose, urea nitrogen, calcium, chloride, SGOT, SGPT, alkaline phosphatase, LDH, erythrocyte, serum and brain cholinesterase, cholesterol, total lipids, total protein. Urinalysis examination comprised the following parameters: appearance, colour, protein, glucose, haemoglobin, bilirubin, pH, sediment.

Absolute and relative organ weights of the following were determined: heart, lungs, liver, kidneys, spleen, brain, testes/ovaries, adrenals, pituitary, thyroid, seminal vesicles. A range of organs and tissues were preserved, and examined histopathologically, namely: heart, lungs, liver, kidneys, spleen, stomach, jejunum, colon, urinary bladder, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, thyroid, pancreas, adrenals, thymus, pituitary, brain, eye with optic nerve, bone marrow, treated skin areas, untreated skin areas.

This study was conducted in accordance with US EPA Pesticide Assessment Guidelines, Subdivision F, Series 82: Subchronic Testing and 82-2, Repeated Dose Dermal Toxicity: 21 day study, EPA 540/9-82-025, November 1982. Also OECD Guidelines for Testing of Chemicals, Section 4: Health effects, No 410, Repeated Dose Dermal Toxicity: 21/28 Day Study, 12 May 1981, and OECD Principles of GLP.

Results

No clinical signs of toxicity or mortalities were observed at 1 or 3 mg/kg/day. At 9 mg/kg/day, one male had piloerection, another male had hypersalivation, blood encrusted nose, stagger, passivity and dyspnoea and died on day 8 of treatment, while a third male died on day 5 of treatment, in the absence of any clinical signs of intoxication. The males that died at 9 mg/kg/day had reduced or immature testes and/or sex organs (including undescended testes), and the livers of these animals has accentuated lobular markings. The study investigators reasoned that these effects resulted from a non-substance-related developmental disturbance already present prior to treatment. No mechanism has been proposed as to the cause of such an effect, but impaired development of the males in this group, including underdeveloped livers, may have made these animals more susceptible to the toxic effects of endosulfan, due to impaired metabolism.

No treatment related mortality or clinical signs were reported in females at 9 mg/kg/day. At 27 mg/kg/day, no mortality or clinical signs were reported in males, while in females, 5/6 animals died between days 2 and 6, with no clinical signs observed. At 81 mg/kg/day, 2/6 males died between days 2 and 3 with no clinical signs, while a third male displaying tonic-clonic convulsions, hypersalivation, accelerated breathing and nervous disposition died on day 3 of treatment.

On occasion, a number of animals were seen with very slight erythema at the test sites, often accompanied by dry and/or chapped skin. These effects were transient, usually seen in the

first few days of the study, and were reported in control and treated animals. As such, these effects are not considered to be treatment related.

In males, a substantial decrease in body weights was observed at 9 mg/kg/day (about 15% reduction), and to a lesser extent at 81 mg/kg/day (about 5% reduction), but in the absence of a dose relationship at 27 mg/kg/day, these reductions were not considered to be related to treatment. In females, bodyweights were unaffected by treatment. In males, food consumption in all groups was similar at Day 30 of the study, but food consumption was reduced at 9 and 81 mg/kg/day until about Day 10 of the study. There was not dose relationship, and food consumption was not affected at 27 mg/kg. No consistent, dose related effect on food consumption was observed in females.

Statistically significant changes in haematology parameters were observed only rarely, with an increase in reticulocytes in males at 3 mg/kg/day, and a decrease in thrombocytes at 27 mg/kg/day in females. In the absence of any consistent relationship with dose, these effects are not considered to be treatment related. No treatment related effects on urinalysis parameters were noted during this study.

Clinical chemistry examination revealed statistically significant decreases in serum cholinesterase activity in males, with reductions of 70-80% at doses of 9-81 mg/kg/day. These changes were not consistently dose related, and statistically significant changes in serum cholinesterase were not seen in females in this study, nor in males or females in the previous study, with the exception of males at 192 mg/kg/day. In females, serum cholinesterase activity was reduced by about 40% at 9 mg/kg/day, but this decrease was not statistically significant. In males, brain cholinesterase activity was statistically significantly reduced at 9 (21%), 27 (28%), and 81 (24%) mg/kg/day, but these changes were not dose-related, and the biological significance of these changes is questionable. In females, brain cholinesterase was statistically significantly reduced at all treatment doses, but as the reduction was in the range 13-18%, this effect is not considered to be biologically significant. No biologically significant reduction in cholinesterase activity was seen in males or females at 3 mg/kg/day. The decreases seen in cholinesterase activity in this study are not normally associated with endosulfan administration, and long-term administration of endosulfan in experimental animals has not been shown to result in cholinesterase activity. The large decreases in serum cholinesterase inhibition seen in males in this study were not observed in females, which are generally more sensitive to the toxic effects of endosulfan than males. In the previous 30-day dermal study in rats, conducted by the same testing facility (Ebert et al., 1985), no serum cholinesterase activity inhibition was observed at doses up to 96 mg/kg/day in males. On the weight of toxicological evidence, it seems unlikely that endosulfan administration results in toxicologically signifiant cholinesterase inhibition, and the changes seen in this study are considered to be incidental to treatment. On occasion, statistically significant changes in a few other clinical chemistry parameters were observed, but these effects were not considered to be treatment related, due to the isolated nature of these effects, and the lack of a dose response relationship.

On occasion, statistically significant changes in absolute and/or relative organ weights were observed, but these effects were not considered to be treatment related due to the isolated nature of these effects, and the small magnitude of the changes.

Macroscopic examination of the animals that died during the study revealed that the principal factor leading to death was a treatment related failure of circulation and acute heart failure, accompanied by oedema or acute congestion of blood in the lungs and an agonal release of lipids in the adrenal cortex. Macroscopic examination of the animals that survived the treatment period did not reveal any effects related to endosulfan administration. Microscopic examination revealed some slight cellular changes in the liver, with enlargement of parenchymal cells in the periphery, and loss of cytoplasmic basoplilia, noted at doses of 9 mg/kg/day and above. However, these effects were isolated in nature, a dose dependency was not demonstrated, and these findings are not considered to be treatment-related.

The no-observed-effect-level (NOEL) for this study is 9 mg/kg/day based on mortality in females at 27 mg/kg/day.

4.1.3 30-Day Gavage Study

Nath et al. (1978) 30 Day oral administration in rats. Interaction of Endosulfan and Metepa in Rats. Industrial Toxicology Research Centre. Document No. A17906.

Endosulfan was administered to male albino rats (10/group), by gavage, at a dose level of 11 mg/kg/day for 30 days. A vehicle control group received peanut oil over the same treatment period. In addition, the possible interaction between endosulfan and the chemosterilant, metepa, was investigated with further groups of animals receiving either metepa alone (30 mg/kg/day for 30 days) or in combination with endosulfan at the above mentioned dose levels. Animals were observed for signs of toxicity and morbidity. Upon termination of treatment clinical chemistry parameters and residue levels were determined and histopathological examinations were carried out.

There were 3 deaths in the endosulfan-treated group, one of which showed signs of endosulfan induced toxicity. Endosulfan administration produced no significant changes in organ weights or body weight, did not alter clinicochemical parameters and was without histopathological effects.

Metepa, alone, produced severe testicular changes including necrosis of the tubule and deformed spermatids. In addition, there was a slight increase in DNAase activity in the testis and significant elevations in the levels of succinic dehydrogenase in liver, kidney and testis and marked increases in testicle ATPase levels and alkaline phosphatase levels. When administered in combination, no potentiation of toxicity was seen. No NOEL was established.

4.1.4 29-Day Inhalation Study

***Hollander Dr, Wigand Dr, and Kramer Professor* (*1984)Endosulfan TGAC. Study on subacute inhalational toxicity in SPF Wistar rats*: 21 exposures in 29 days. Collaborative work between Makhteshim Chemical Works and Hoechst AG, Frankfurt. Study No. 83.0103, Report No. 84.0539, Documentation No. 726, Hoechst document No A29823 Report date:15 August 1984.**

Groups of Wistar rats (Hoe:WISKf SPF71) 15/sex/group were exposed 6 h/day, 5 days/week for 3 weeks in head/nose-only exposure chambers to aerosols of technical endosufan (Batch Number Hoe 002671 01 ZD97 0003; Purity 97.2%). Endosulfan was diluted with ethanol-polyethylene glycol 400 (1:1) :ethanol-polyethylene vehicle and concentration tested were 0 (air control), 0 (vehicle control), 0.5, 1, and 2.0 mg/m^3. Mean exposure concentrations achieved were 0, 0, 0.53, 0.88 and 2.21 mg/m3. The study methodology was in accordance with the OECD Test Guideline 412.

Animals were observed daily for general health and behavioural changes, body weights and food consumption twice weekly, water consumption weekly, and haematological and blood chemistry parameters at the end of the exposure period.

The clinical parameters determined on 10 animals/sex/group consisted of haematology (haematocrit, haemoglobin, red blood cell (RBC) and white blood cell (WBC) counts, differential blood count, coagulation tim , Heinz bodies, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and thrombocyte count)**;** and blood glucose, urea, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase, sodium, potassium, bilirubin, creatinine, calcium, cholride, methaemoglobin, lactate dehydrogenase (LDH), total protein and cholesterol.

One day after the last exposure 10/sex/group were killed and necropsied, the remaining animals were sacrificed at the end of the 29 day observation period. After sacrifice animals were examined macroscopically, and any abnormal findings recorded.

Absolute and relative organ weights (heart, testicles/ovaries, liver, lung, spleen, adrenals, kidneys, brain, pituitary, thyroid and seminal vessicles) were determined. Histopathology examination was carried out on 37 different regions of the animals.

Animals exhibited no clinical signs during the treatment or observation period, except for one male rat in the high dose group (2.0 mg/m^3) which showed clinical signs of emaciation, pale skin, squatting position and high-legged position. No deaths occurred during the treatment period and no neurological disturbances, opacity of the refractive media, impairment of the dental growth or changes of the oral mucosa were observed during the treatment period.

Body weight gain was depressed (-10%) in the high dose males from day 20 of exposure until the end of the recovery period, however, this was not statistically significant. Female rats in one group (vehicle control) showed a statistically significant decrease in body weight (-10%) on day 36 (recovery period). There was a marked decrease in food consumption (-63%) in high dose males on day 20, returning to control values at the end of the study period. No other changes in food consumption were observed in male or females during the treatment period.

Haematological parameters recorded one day after the end of the exposure period showed some transient increases in RBCs, haemoglobin and haematocrit, however, there was no dose relationship. These returned to normal values day 29 post treatment and were considered to be within the normal historical range for this strain of rat.

Clinical chemistry parameters recorded one day after the end of the exposure period showed a transient non-dose related increase in creatinine (+17%) and a decrease in SGOT (-23%) levels in the high dose female rat group. No other treatment related changes were noted in blood chemistry when measured at this time, and in addition when recorded at day 29 post exposure. No pathomorphological changes were seen in any of the animals. Whilst creatinine can be a marker of kidney dysfunction, the transient nature of the effect, together with the lack of morphological change in the kidney, is such that this finding was not considered to be toxicologically significant.

The NOEL for the study was 2.0 mg/m^3, as no significant effects were seen at any of the doses tested.

4.1.5 30-Day Feeding Study

Leist KH and Mayer D (1987) Endosulfan - active ingredient technical (Code: Hoe 002671 0I ZD97 0003). 30-Day feeding study in adult male Wistar rats. Pharma Research Toxicology and Pathology, Project ID study no. 84.0585. Study completed 15 November 1984. Hoechst report 87.0129 dated 27 March 1987. Hoechst document no. A37112. [AgrEvo; Submission]

This study was reportedly conducted in compliance with OECD GLP Guidelines. In a number of earlier reports, findings of yellow discolouration of the proximal tubules of the kidneys and/or granular pigmentation within the tubular cells were reported in rats exposed to endosulfan in the diet for up to 13 weeks (Edwards et al., 1984; Offer 1985), or in F0 animals in a reproduction study (Barnard et al., 1985). These kidney effects were largely reversible following the withdrawal of endosulfan administration; similar findings were not seen in other species, or in rats exposed to endosulfan for longer periods of time; and these effects were not associated with any toxicologically significant effects in the kidneys. It was considered that these findings were consistent with adaptive changes associated with the storage and lysosomal metabolism of the test material prior to excretion.

This study was conducted in male rats at high doses of endosulfan, with particular attention given to the nature of the discolouration and pigment deposits, and the mechanism of pigment storage.

The test material (endosulfan; stated purity 97.9%; Hoechst) was received as a 60-fold concentrated dietary premix, and 1 kg lots of this premix were blended for 30 minutes with 59 kg of a commercial animal feed (Altromin 1321) as necessary to prepare the final test diet. Dietary mixes were prepared at 2-week intervals, based on previous stability testing information. Homogeneity and test material concentration were tested and found to be acceptable. The test material was administered to male Wistar rats (100/dose; Hoe: WISFf SPF71; Hoechst) for 30 days at dietary concentrations of 360 and 720 ppm. These dietary levels were calculated to be equivalent to 34 and 67.8 mg/kg/d, respectively. After administration of the test material, 50 animals/dose were allowed to recover for 30 days, and received a normal diet during this recovery period. Control animals (20 animals) received a diet without endosulfan for 30 days, with 10 of these animals maintained on a control diet during the 30 day recovery period, similar to the test group animals.

The behaviour and general health condition of all animals were observed twice daily (once on weekends and holidays), and the animals were examined weekly for neurological disturbances and ocular opacities. Body weight and food and water consumption were determined weekly. After treatment (and recovery where appropriate) the animals were killed, and all external and internal tissues were examined macroscopically. Livers, kidneys and brains were removed for weighing and portions of these selected organs were used for histopathological examination (6 animals/group) and semi-thin section and electron microscopy examination (2 animals/group).

Results

During the treatment period, mortality was confined to one animal in each of the 360 and 720 ppm groups. Neither of these animals displayed clinical signs of intoxication, nor were any clinical signs reported in any other animals in the study. Statistically significant ($p>0.05$) decreases in group mean body weights were seen at all sample intervals during treatment in treated animals at 360 and 720 ppm, but no group mean body weights were statistically significantly reduced during the recovery period. The rate of growth (body weight gain) was similar in animals of all groups during the study, with the exception of delayed growth in the first week of treatment. At 360 ppm, the group mean body weights were about 7-10% lower than controls from days 8-15 of the study, and at 720 ppm the group mean body weights were about 10-12% lower than controls from days 8-22 of the study. These changes were considered to be biologically significant and related to treatment. Body weight gains at 360 and 720 ppm were 15 and 35% lower than controls, respectively, at day 8, but similar to controls at other sample intervals. Food consumption was also slightly decreased compared with controls in treated groups at days 15-30, but food consumption was similar in all groups during the recovery period of the study.

Statistically significant ($p>0.05$) increases in group mean absolute and relative organ weights were observed at 360 and 720 ppm at the completion of treatment. Absolute liver weights were increased by 10-17% (360-720 ppm), and kidney weights by 14% (720 ppm) compared with controls. Relative to body weights, liver and kidney weights increased by about 25% at 720 ppm, and liver weights increased by about 13% at 360 ppm. After the 30-day recovery period, no statistically significant differences were seen between group mean organ weights in control and treated animals.

The results of pathological examinations were only reported qualitatively, and the pathological findings of individual animals were generally not reported in detail. Macroscopic examination of tissues reportedly revealed brown discolouration of the kidneys in all treated animals at the end of the 30-day administration period, but these signs had disappeared by the end of the recovery period. Microscopic examination (6 animals/treatment regime) of animals treated at 360 ppm for 30 days revealed finely distributed yellowish pigment in isolated cells in the proximal convoluted tubules of the kidneys of all animals. At 720 ppm, all sections showed yellowish pigment deposits in cells of the proximal nephron, more so in the pars convoluta than the pars recta. These deposits were larger and more strongly marked than the deposits seen at 360 ppm, but not all proximal tubules showed pigment to the same degree, and pigment was reportedly absent from most of the proximal tubules. At 720 ppm, a single animal displayed signs of cholangitis and cholangiolitis in the liver, but other animals displayed only sporadic Kupffer cell nodulation, similar to that seen in control animals.

In animals allowed to recover after treatment, all animals from the 360 and 720 ppm groups showed isolated brownish pigment deposits in the proximal tubule region. These deposits were reportedly slight, and occurred to a lesser extent than that seen in animals after 30 days of treatment at 360 ppm. No other treatment-related histopathological findings were reported after recovery.

Semi-thin section examination of tissues from 2 animals from each group revealed an increase in the size of lysosomes in the cells of the proximal convoluted tubules compared to controls, and there was also an apparent increase in the lysosome number in isolated segments. These findings were similar at 360 and 720 ppm following 30 days of treatment. After the recovery period, these animals still displayed an increase in the number and size of lysosomes in isolated segments of proximal nephrons.

Electron microscopy examination revealed an increase in the number of lysosomes at 360 ppm, and the shape of lysosomes at this dose level was generally similar to that of controls. The exception was a small number of bizarrely shaped lysosomal structures containing fine granular material. At 720 ppm, the number of lysosomes was more numerous and larger than at 360 ppm, with lysosomal content again being electron-dense, and containing fine granular material. The size of these lysosomes, though increased compared with controls, did not exceed the normal 0.4-3µm range. After the recovery period, lysosomes from treated animals were generally similar to controls, with occasional findings of small aggregates of finely granular material. No evidence of adverse cellular effects or tissue damage were seen in any samples, either following treatment or recovery.

Summary

The administration of endosulfan to male Wistar rats at dietary levels of 360 and 720 ppm for 30 days was carried out to determine the nature and toxicological significance of pigment deposition in the kidneys of rats observed in previous experiments. In this study, slight but statistically significant, dose-related reductions in group mean body weights were observed during the treatment period at both of the test doses. No clinical signs associated with endosulfan administration were reported, and the incidence of mortality was low. Statistically significant, dose-related increases in absolute and relative organ weights were observed in the kidney and livers of treated animals. Body weights and organ weights returned to control levels during the 30-day recovery period. Pathological examination revealed kidney effects in treated animals, including dark discolouration, granular pigmentation and increases in size and number of lysosomes in cells of the proximal convoluted tubules, with the extent of pigment deposition related to dose. These effects were largely reversible, with very few effects still observed after the recovery period. This study supports the suggestion from two other studies that the kidney pigment deposition observed following endosulfan administration in rats is a reversible adaptive change related to lysosome induction, and the pigmentation is unlikely to be associated with any short- or long-term toxicological effects.

4.1.6 30-Day Dermal Study

Dikshith SS, Raizada AB, Kumar SN, Srivastava MK, Kaushal RA, Singh RP, & Gupta KP (1988) Effect of repeated dermal application of endosulfan to rats. Veterinary and Human Toxicology, Vol 30, No. 3, June 1988, pp 219-224.

The dermal toxicity potential of endosulfan (source and purity not stated) was assessed in Wistar rats (Industrial Toxicology Research Centre, India), by applying the test material to the clipped lateral abdominal skin of each animal once daily for 30 days, at doses of 9.83, 19.7, and 32 mg/kg/d (females; 6/group) or 18.75, 37.5, and 62.5 mg/kg/d (males; 6/group). Neither the concentration and stability of the test material nor details of the vehicle were stated. Control groups (6/sex) received applications of acetone only. No details were provided on the length of exposure, whether the test sites were washed between daily applications, whether or not animals were restrained during the study, or if the test sites were occluded. The liver, kidney, adrenal, brain, spleen, testes, epididymis, uterus, ovary, cervix and vagina were removed from animals at the end of the dosing period and weighed. Histopathological examinations were conducted on sections of skin, liver, kidney, spleen, brain, testes, epididymis, adrenal, ovary and cervix. Freshly removed livers and blood serum were used for estimation of enzyme activity (GOT, GPT, AP, LDH) and protein determinations. Red and white blood cells were counted, haemoglobin was measured, and differential leukocyte counts determined. Levels of endosulfan in liver, kidney, testes, brain, fatty tissue and blood of all treated animals were analysed by gas-liquid chromatography.

Results

No treatment-related deaths were observed during the study, and clinical signs associated with treatment (hyperexcitation, tremor, dyspnoea, and salivation) were confined to the first week of treatment (no details were provided on the incidence, severity or group distribution of these findings). Body weights were reportedly unchanged as a result of treatment, but no data were reported to support this finding. On occasion, statistically significant changes in organ weight/body weight rations were reported, but these effects were generally slight and not dose-dependent. The data were suggestive of decreases in testes/body weights in a dose-related manner, but the relevance of this finding was unclear, due to some data-reporting anomalies in the paper. It was reported that histopathological examination did not reveal any adverse findings associated with treatment.

Liver biochemical determinations revealed a number of changes in treated groups that were statistically significantly different to controls. Decreases in GOT and GPT activity were seen in most treated groups, and this finding was not considered to be toxicologically significant. An increase in alkaline phosphatase activity was seen in low- and high-dose females, but in the absence of a strong dose-relationship and with no similar findings in males, this effect was not considered to be related to treatment. LDH activity was also increased in all treated female groups, but there was no dose-relationship, and this finding was attributed to a particularly low LDH activity reported in the female control group. Statistically significant increases in protein were reported in mid- and high-dose males, but the toxicological significance of this finding is unclear.

In the serum biochemical determinations, there were also a range of parameters that were statistically significantly different from control values. These findings were not considered to

be treatment-related, as there was a general absence of a dose-dependence, and the variation in the magnitude of some of the measurements was suggestive of difficulty in the determination of these parameters. Haematology determinations did not reveal any consistent, treatment-related findings.

Under the conditions of this study, dermal application of endosulfan resulted in some clinical signs of toxicity in the absence of mortality. Biochemical determinations revealed some changes in enzyme activity that may have been associated with treatment, reportedly in the absence of pathological findings. The lack of detail in this report makes it unsuitable for regulatory purposes.

5. SUBCHRONIC TOXICITY

5.1 Mouse

5.1.1 13-Week Dietary Study

***Barnard A. W. et al (1984) Endosulfan TGAC (Code: Hoe 002671 OI ZD97 0003). 13 Week dietary study in mice.* Hoechst AG, Frankfurt. Huntingdon Research Centre. Report No. HST 229/831052, Report date:25 September 1985.**

Male and female CD-1 mice (20/sex/group) were administered endosulfan technical (Code. No. Hoe 002671 0I ZD97 0003; 97.2% purity) via the diet at 0, 2, 6, 18 or 54 ppm for 3 months. These concentrations are equivalent to doses of 0, 0.24/0.27, 0.74/0.80, 2.13/2.39, or 7.3/7.5 mg/kg/day for males/females, respectively. Control animals were administered normal diet mixed with acetone and maize oil. Mice were supplied by Charles River Breeding Laboratories and were 45 days old at initiation of treatment. This study was conducted in accordance with US EPA/FIFRA draft guidelines, November 1982, the principles of Good Laboratory Practice(GLP) as set out in the OECD guidelines for testing of chemicals and the GLP guidelines of the USFDA.

Animals were observed twice daily for clinical signs and morbidity and body weights and food consumption measured weekly. During weeks 6 and 13 of treatment, haematological and blood chemistry parameters were measured. Haematological parameters measured included packed cell volume, haemoglobin (Hb), red cell count (RBC), reticulocyte count, mean corpuscular haemoglobin concentration (MCHC), mean cell volume, white cell count, differential white cell count and platelet count. Blood chemistry recorded consisted of analysis of glucose, total proteins, urea nitrogen, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic oxaloacetic transaminase, gamma-glutamyl-tranpeptidase, total lipids and total bilirubin.

After sacrifice animals were examined macroscopically, and any abnormal findings recorded. Histopathological examination was conducted on all surviving mice. Organs weighed at terminal sacrifice were adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes and uterus.

Histopathology examination was carried out on 26 different anatomical regions of the animals.
Clinical signs attributable to treatment consisting of convulsions and salivation were seen in one male and one female from the high dose group. Other incidental clinical findings observed in control animals and at doses of 2 ppm upwards were testes withdrawn, hairloss, damaged tail, swollen penis, ulceration of selected regions, scabs, and swelling in the urogenital area; however, these are not considered to be treatment related. In addition, ulceration of the left ear pinna and cervical region in one 6 ppm male rat (possibly due to cannibalism), lethargy in one male rat at 6ppm, and piloerection at 18 ppm in one male rat was seen. These effects are considered to be spontaneous in nature and unrelted to treatment. There was a marked (50%) treatment-related decrease in the survival rate of male and female animals from the high dose group. Incidental deaths occurred in one male rat at 2 ppm, and in two male rats at 6ppm, however, no deaths occurred in females at the lower doses.

The mean food intake of males and females receiving the highest dose was significantly reduced ($p<0.05$) for the first 2 weeks of the study (16% reduction and 11% reduction, respectively, compared with controls), whereas, over the remaining 11 weeks of treatment, food consumption returned to values similar to control animals. Mean bodyweight gain was reduced in high dose males compared to controls (85% reduction), during the first week of treatment, however, from week 2 and over the remaining 11 weeks weight gain was similar to control animals. Females at all doses were unaffected by treatment. Due to the transient nature of the changes in body weight gain, this effect is not considered to be toxicologically significant.

Male rats receiving the high dose showed statistically significant ($p<0.01$) neutrophil reductions (72% reduction) compared to controls at week 6, while at week 13, a non-statistically significant of 62% was seen. Slight non-dose related reductions ($p<0.05$) in MCHC was observed in females receiving doses of 6 (4% reduction), 18 (4%) and 54 ppm (1%). Although these were statistically significant, these changes are not considered to be biologically significant.

Significant reductions ($p<0.01$) in blood glucose were observed at 6, 18 and 54 ppm (reduced by 11% at all doses) in females at 6 weeks. Significantly increased serum lipids in females were seen at week 13 (15 % increase) at the high dose level.

Macroscopic examination and organ weight analysis revealed reductions ($p<0.05$) in spleen weights of males at 13 weeks (24 % reduction compared to controls) at the high dose, and slight congestion of the lungs in high dose male and female rats. Histopathological examinations did not reveal any treatment-related effects at any of the doses tested.

The NOEL was 18 ppm (2.13 mg/kg/day for males and 2.39 mg/kg/day for females), based on clinical signs (convulsions, salivation), decreased survival, and increased serum lipids seen at 54 ppm (7.3 to 7.5 mg/kg/day).

5.1.2 12-Month Dietary Study

Arai et al (1981) Life span chronic toxicity study of endosulfan in mice- 12 month interim report. Sumitomo Chemical Co Ltd, Japan, Ref No: 0285, Document No. AT-10, 28 April 1981.

Endosulfan technical (91.4% pure) was administered daily to 4 week old ddY mice (10/sex/group) at dietary levels of 0, 10, 30, 100 and 300 ppm for 12 months. The endosulfan was dissolved in corn oil and formulated into the diet; the dietary concentration of corn oil was 2%. Actual achieved doses were 0, 1.17, 4.08, 15.2 and 41.7 mg/kg/day in males and 0, 1.41, 4.74, 13.5 and 42 mg/kg/day in females. Animals were observed twice daily for clinical signs and morbidity and were palpated for masses monthly. Body weights and food consumption was determined weekly. Ophthalmological examinations were carried out on all test mice at 12 months and haematological and clinical chemistry parameters were determined at 0 and 12 months. Gross and histopathological examinations were carried out upon termination of the study.

There were no apparent treatment related clinical signs or deaths. Some transient increases in body weight gain were seen in males at 10 and 300 ppm but overall there were no adverse effects on body weight gain associated with treatment. Food consumption and water intake levels were similar in treated and control animals. Ophthalmological examination revealed a few incidences of granulation on the corneal surface, white spots on the lens and opacity, however these were not dose related in their incidence or severity and do not appear to be related to treatment with endosulfan.

In the high dose males, a small but significant decrease in mean corpuscular volume was noted (1% reduction compared to controls). In addition, some transient non dose related increases in haemoglobin (11% increase compared to controls), haematocrit (4% increase) and eosinophils (33% increase) were seen in males at 30 ppm. Due to the transient nature, and/or small magnitude of this effects, they are not considered to be treatment related.

Clinical chemistry changes consisted of a significant decrease in serum glutamic oxaloacetic transferase (SGOT) in males at 100 (40% reduction) and 300 (40% reduction) ppm and a decrease in bilirubin (26% reduction) in high dose males. Some small non dose related changes seen were an increase in blood urea nitrogen (BUN) in females and an increase in bilirubin at 10 ppm. A non significant increase in SGOT was seen in high dose females. These findings were not associated with any adverse pathological changes.

Organ weight changes were confined to a dose related increase in the relative adrenal weights in females; this was significant at 300 ppm, with an increase of about 30% compared to controls. There were no treatment related effects on gross pathology. Histopathological effects consisted of dose related granulomatous changes in the liver and lymph nodes; these findings are summarised below.

Incidence of histopathological findings in the liver and lymph nodes

FINDING	SEX	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	
lymphocytic infiltration	M	3/10	6/10	2/10	2/10	2/10	
	F	4/10	5/10	8/10	2/10	4/10	

giant cell infiltration	M	0/10	0/10	0/9	1/10	6/10
	F	1/10	1/10	1/10	0/10	8/10
nodular hyperplasia	M	0/10	0/10	0/9	0/10	1/10
pigmented histocytic	M	0/10	1/10	0/9	1/10	5/10
cells	F	1/10	2/10	0/10	2/10	8/10
granuloma	M	1/10	2/10	1/9	5/10	8/10
	F	3/10	3/10	3/10	4/10	10/10

In liver, granuloma, giant cell infiltration and/or large histocytic cells filled with brown pigment were found in treated mice; these effects were significant in the high dose groups (100 and 300 ppm). In lymph nodes, giant cell infiltration and/or reticuloendothelial cell proliferation were found in the 100 and 300 ppm groups but not at lower dose levels.

The histological findings in the kidney are summarised below.

Incidence of histopathological findings in the kidney

FINDING	SEX	0ppm	10ppm	30ppm	100ppm	300ppm
interstitial lymphocytic						
infiltration	M	9/10	6/10	6/10	3/10	5/10
	F	6/10	8/10	7/10	7/10	9/10
cystic dilatation of						
cortical tubules	M	0/10	0/10	0/9	1/10	0/10
	F	2/10	2/10	1/10	1/10	0/10
vacuolation tubular						
epithelial cells	M	4/10	2/10	2/9	3/10	1/10
	F	0/10	1/10	1/10	1/10	0/10
glomerulonephritis	M	0/10	0/10	1/9	0/10	0/10
	F	1/10	0/10	0/10	0/10	0/10
chronic nephrosis	M	0/10	0/10	0/9	0/10	0/10
	F	0/10	1/10	0/10	1/10	0/10

In the kidney, interstitial lymphocyte infiltration was found at a high incidence in mice of both sexes, including controls. In addition, a very low incidences of cystic dilatation of cortical tubules, vacuolation in tubular epithelial cells, glomerulonephritis and/or chronic nephrosis were seen. Due to the isolated nature of these findings, and lack of dose-response relationship, these effects are not considered to be related to treatment.

Testicular atrophy occurred in 3/10 (30%) control, 7/10 (70%) 10 ppm, 3/9 (33%) 30 ppm, 5/10 (50%) 100 ppm, and 8/10 (80%) 300 ppm male rats. Spermatic retention occurred in 1/10 (10%) control, 2/10 (20%) 10 ppm, 0/10 30 ppm, 3/10 (30%) 100 ppm, and 1/10 ppm male rats. Due to the lack of a consistent dose response relationship in these testicular

findings, they are not considered to be treatment related. No treatment related effects were noted on the reproductive organs in female rats.

The NOEL is 30 ppm (4.1mg/kg/day in males; 4.7 mg/kg/day), based on histological findings in the liver and lymphatic system at 100 ppm (13.5 mg/kg/day in females; 15.2 mg/kg/day in males) and 300 ppm (42 mg/kg/day, males and females).

5.2 Rat

5.2.1 13-Week Dietary Study

***Barnard A. W. et al (1985) Endosulfan TGAC (Code: Hoe 002671 OI ZD97 0003). 13 Week dietary study in rats.* Hoechst AG, Frankfurt. Huntingdon Research Centre. Report No. HST 230/84176, Report date:25 September 1985.**

Male and female CD rats (25/sex/group) were administered endosulfan technical (Code. No. Hoe 002671 0I ZD97 0003; 97.2% purity) via the diet at 0, 10, 30, 60 or 360 ppm for 3 months. Dietary dosages were equivalent to 0, 0.64, 1.92, 3.85, and 23.41 mg/kg/day in males, ans 0, 0.75, 2.26, 4.59, and 27.17 mg/kg/day in females, respectively. Five animals of each sex from each group were maintained on a control diet for a further 4 weeks; the remaining animals were killed upon termination of treatment. Control animals were administered normal diet mixed with acetone and corn oil. Rats were supplied by Charles River Breeding Laboratories and were 34 days old at initiation of treatment. This study was conducted in accordance with US EPA/FIFRA draft guidelines, November 1982, the principles of Good Laboratory Practice(GLP) as set out in the OECD guidelines for testing of chemicals and the GLP guidelines of the USFDA.

Animals were observed twice daily for clinical signs and morbidity and body weights and food consumption were measured weekly. Opthalmoscopic examinations were conducted prior to commencement of dosing and before terminal sacrifice. A neurological examination was carried out on 10 rats/sex/group at 0 , 2, 6, and 13 weeks of treatment.

During weeks 6 and 13 of treatment, haematological and blood chemistry parameters were measured. Haematological parameters measured included packed cell volume, haemoglobin (Hb), red cell count (RBC), reticulocyte count, mean corpuscular haemoglobin concentration , mean cell volume (MCV), white cell count, differential white cell count and platelet count. Blood chemistry recorded consisted of analysis of glucose, albumin, globulin, total proteins, urea nitrogen, creatine, alkaline phosphatase, glutamic-pyruvic transaminase (GPT), Glutamic oxaloacetic transaminase, Gamma-glutamyl-tranpeptidase, total lactate dehydrogenase, total lipids, total bilirubin, sodium, calcium, inorganic phosphorous, chloride and cholesterol. Plasma and erythrocyte cholinesterase levels were measured at 5 and 12 weeks and after the withdrawal period. Urinalysis was performed at week 4 and 13 and pH, Specific Gravity (SG), protein, glucose, ketones, bile pigments, urobilinogen and haemoglobin were determined.

On completion of treatment 20 animals/sex were sacrificed and examined macroscopically for abnormal findings, including all the animals from the withdrawal group. Organs weighed at

terminal sacrifice were adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, epididimides, thyroids, and uterus. Brain cholinesterase was measured from 10/sex/group at the end of treatment, and 5 females of each group sacrificed at the end of the withdrawal period. Histopathology examination was carried out on 25 different anatomical regions of the animals.

Clinical signs attributable to treatment consisted of hair loss in high dose females**,** however this regressed during the withdrawal period. Other incidental clinical findings observed in control animals and at doses of 10 ppm upwards were red staining around eyes and a watery discharge, and hair loss from the side of the face.

Three deaths occurred in female rats during the treatment period**;** one control animal and one administered endosulfan at 60 ppm, the other animal at 360 ppm. The actual cause of death was unknown.

A transient, but statistically significant ($p<0.001$) reduction in food consumption compared to controls was seen at 360 ppm, with a 12% reduction in females, and a 3% reduction in males, for the first 2 weeks of the study only. Similarly, mean bodyweight gain was significantly ($p<0.001$) reduced at 360 ppm in females (-32%), during the first 2 weeks of treatment only. Food consulption and body weight gains were similar to controls for the remaining 11 weeks of the study. No treatment-related ophthalmoscopic abnormalities were noted at term, nor any treatment related findings observed from the neurological assessment.

No haematological changes occurred in the 10 ppm treated animals that were considered to be treatment-related, with only a slight, transient, non dose related decrease in Hb count seen at this dose. In the 30 ppm group and above there was a slight, but statistically significant ($p<0.01$) dose related reduction in RBC and Hb count after 6 weeks of treatment in both sexes, and an increase in MCV in both sexes at 30 ppm and above. These haematology changes persisted in females at week 13, with the most significant being a continued decrease in RBC count at 360 ppm. The reduction in RBC and Hb were slight, and the haematological changes were within normal biological variation, and within the historical historical control range for animals from this testing facility.

Statistical evaluation of the chemistry parameters measured after 6 weeks of treatment revealed a significant ($p<0.05$) increase in globulin levels in 30 ppm males only, a decrease in albumin levels at and above 60 ppm, and a decrease in GPT at 360 ppm in male rats. The increase in globulin levels was still evident after 12 weeks treatment although there was no dose relationship. Statisitically significant ($p<0.05$) decreases in Na and K occurred at doses of 60 ppm and above in males when measured at week 12, however, there was no dose relationship. In females statistically ($p<0.01$) significant increases in phosphorous, cholesterol and lipids were found at a dose of 360 ppm at 6 and 12 weeks, and decreases in creatine in week 12 at 60 ppm. All parameters were within normal range following the withdrawal of treatment.

Measurements of plasma and erythrocyte cholinesterase (ChE) levels showed that males were unaffected by treatment. However, in females at 5 and 12 weeks a statistically significant decrease ($p<0.01$) in plasma ChE levels occurred at a dose of 360 ppm, and a decrease ($p<0.05$) in erythrocyte ChE levels was seen at 12 weeks. All levels were within control values 4 weeks after withdrawal of treatment. At necropsy, brain ChE levels were slightly

elevated (by 16%) at 60 and 360 ppm in females, whilst, males and females sampled from the withdrawal period had no changes in brain ChE levels.

Urinalysis parameters were generally unaffected by treatment except in males at 360 ppm where urine volume was increased (35% greater than controls), and specific gravity decreased, at week 4; and, urinary protein levels were significantly ($p<0.05$) elevated (47% above controls), after 13 weeks treatment. All parameters were within normal range following the withdrawal of treatment.

Histopathologic examination revealed, in males, enlargement of the liver at 360 ppm (15 % of animals) and of kidneys at 60 ppm (10% animals) and 360 ppm (55 % animals). Similar findings were not observed in females. At high doses, statistically significant ($p<0.01$) increases were observed in absolute liver, kidney and epididymides weights of males (18%, 20% and 8% increase, respectively, compared to controls); and, similarly in females (liver 21% increase, kidneys 10% increase). The kidney weights remained significantly ($p<0.01$) elevated in the male rats (15% increase) at the end of the withdrawal period in rats treated at 360 ppm, however, this was not observed in females.

Histopathological examination revealed traces of brown pigment in scattered hepatocytes in 25% of male rats, and minimal centrilobular enlargement of hepatocytes in 25% of females at 360 ppm. These changes were not observed in rats at the end of the withdrawal period. Yellowish discolouration of the kidney proximal tubule cells was seen in males at 10 ppm to 360 ppm; the degree of pigmentation increased in a dose-related manner. No cell death was associated with this finding. A similar finding was observed in females at 30 to 360 ppm. In addition, granular pigmentation, which possibly indicates lysosomal storage of the compound and/or its metabolite/s, was seen in proximal tubular cells in males at 60 and 360 ppm. The discolouration of the kidney tubules in male rats decreased at the end of the withdrawal period, however, the pigmentation persisted at doses at and above 30 ppm. At 30 ppm, the pigmentation was not seen during the treatment period, and in the recovery period was only seen in trace amounts in a small number of animals, and was not considered to be toxicologically significant at this dose level. In females pigmentation persisted at and above 60 ppm. In addition, males at 360 ppm also had yellow coloured protein aggregation in the proximal convoluted tubules and intracytoplasmic eosinophilic droplets in the tubules.

The increase in incidence and severity of the yellowish discolouration of proximal tubular cells appears to be treatment related, with no control animals displaying these effects. However, this effect alone does not appear to be toxicologically significant, with no adverse effects associated with these findings alone. At 30 ppm, all animals displayed either trace or minimal discolouration. At doses of 60 and/or 360 ppm, when the pigmentation was present, other treatment related effects were also seen, including enlarged kidneys and centrilobular hepatocyte enlargement. No treatment related increase in the incidence of other kidney related effects were reported at 30 ppm or below.

While the increase in incidence of the cellular discolouration is related to the administration of endosulfan, these findings were not considered to be toxicologically significant, as they were not associated with any adverse effects on the cells, and the yellow pigment was considered likely to be endosulfan and metabolites being stored and metabolised in lysosomes prior to excretion (JMPR, 1989). The presence of the discolouration is an indication of endosulfan exposure, rather than an index of toxicity, and in longer term studies in rats

(Ruckman, 1989), where the induction of lysosome enzymes is complete, the lysosomal degradation of endosulfan becomes effective and the test substance is fully eliminated (Canada PMRA, 1993), and thus this discolouration is not observed in the kidneys.

The NOEL was 30 ppm (1.92 mg/kg/day) based on increases in kidney weight and granular pigment formation in kidney proximal tubule cells at 60 ppm (3.85 mg/kg/day).

6. CHRONIC TOXICITY/CARCINOGENICITY STUDIES

6.1 Mouse

6.1.1 78-Week Dietary Study

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.

Male and female B6C3FI mice were administered endosulfan technical (Thiodan R, Purity 98.8%) via the diet, with the low and high time-weighted average concentrations being 3.5 and 6.9 ppm for the males, and 2 and 3.9 ppm for the females.

The B6C3FI mice were from the Charles River Breeding Laboratories, and were 6- 7 weeks old at initiation of treatment. No mention was made of specific adherence to any international toxicological gudielines.

Animals were observed daily for clinical signs and mortality and body weights and food consumption were measured weekly for the first 10 weeks, and at monthly thereafter. A histopathological examination consisting of a gross and microscopic examination (of major organs, and tissues) was carried out on every animal. Thirty one different anatomical regions were selected.

There were no definite compound-related effects on appearance or behaviour in any of the treated groups, and the subsequent clinical signs that were observed also occurred in control animals. These included alopecia, hunched appearance, penile, anal or vulvar irritation, rough fur, bloated appearance. Body weights in both males and females were unaffected by treatment**.**

There was an increase in the mortality rate with high dose males early in treatment and survival at termination of the bioassay was 15% (3/20) in control males, 38% (19/50) in low dose males and 10% (5/50) in high dose males. These early deaths were not tumour-related. In contrast, the survival rate of females was unaffected by treatment.

Hepatocellular carcinomas were found in 1/20 (5%) control males, 6/49 (12%) low dose males, 2/50 (4%) high dose males, and 1/50 (2%) high dose females. None of the control female rats demonstrated hepatocellular carcinomas. It can be concluded that there were no treatment related neoplastic lesions seen in the females and it may be concluded that endosulfan lacks oncogenic potential in female $B_6C_3F_1$ mice.

The NOEL for female mice was 3.9 ppm (0.58 mg/kg/day). Due to the high early mortality, no conclusion as to the oncogenic potential of endosulfan in males could be drawn. No non-neoplastic changes in the kidneys or sex organs of male and female mice could be atrributed to treatment with endosulfan.

6.1.2 24-Month Dietary Study

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 (AgrEvo 11303). and

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 (AgrEvo 11303).

In a combined chronic toxicity/carcinogenicity study in NMRI mice (Hoe:NMRKf SPF71; Hoechst), technical endosulfan (97.9% purity, Hoechst) was incorporated in the diet at concentrations of 0, 2, 6, and 18 ppm for up to 24 months, with 60 animals/sex/dose. The intake of endosulfan for males was calculated to be 0.28, 0.84, and 2.51 mg/kg/day, at dietary concentrations of 2, 6, and 18 ppm, respectively. The corresponding doses in females were 0.32, 0.97, and 2.86 mg/kg/day, respectively. In addition, satellite groups of animals (10/sex/dose) were killed after 12 and 18 months. For the 12-month treatment period, the intake of endosulfan was calculated to be: 0.28, 0.87, and 2.55 mg/kg/day for males, and 0.32, 1.03, and 2.75 mg/kg/day for females. For the 18-month treatment period, the intake of endosulfan was calculated to be 0.28, 0.82, and 2.39 mg/kg/day for males, and 0.32, 0.94, and 2.79 mg/kg/day for females. The stability, concentration and homogeneity of endosulfan in the diet was tested weekly and guaranteed. Behaviour and general health conditions were observed twice daily, and the animals were tested for neurological disturbances, opacity of the refracting media of the eyes, and dental growth and changes in oral mucosa once a week. Body weights and food consumption were determined weekly and survival checks were conducted twice daily.

Haematological examinations were performed on 10 animals/sex/group for the following parameters: erythrocyte count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, platelet count, total leukocyte count, and differential leukocyte count, with a first intermediate value at 6 months (in animals of the 12 month study, final value at 12 months (interim

sacrifice), final value at 18 months (interim sacrifice), and final value at 24 months (terminal sacrifice). Clinical chemistry examinations were conducted at 12, 18, and 24 months on 10 animals/sex/group for the following parameters: ALAT, ASAT, alkaline phosphatase.

At the scheduled sacrifice intervals, autopsies were conducted, and included a macroscopic examination of integumenta, orifices, eyes, and internal organs, and the following organs were removed and weighed: heart, lungs, kidneys, spleen, brain, adrenals, testes/ovaries. Other organs removed and preserved for histopathological examination were: eyes with optic nerves, nasal septum, pituitary, thyroid, salivary glands (parotid and mandibular), trachea, aorta, oesophagus, stomach (fundus and pyloric region), intestine (duodenum, jejunum, ileum, caecum, colon, rectum), pancreas, thymus, lymph nodes (mesenteric, iliac, and cervical), gall bladder, urinary bladder, epididymides, seminal vesicles, prostate, uterus, skeletal muscle, skin with mammary gland, bone marrow (femoral), sciatic nerve, spinal marrow, tumours (detected macroscopically), and other organs with macroscopic findings.

Measurement of tissue endosulfan concentrations were made using liver and kidney samples of 10 animals/sex/group at terminal sacrifice.

This study was conducted in accordance with : OECD Guidelines for testing of chemicals no 451 “Carcinogenicity Studies”, May 12, 1981; EPA Pesticide Assessment Guidelines subdivision F Hazard Evaluation-Human and domestic animals, series 83: chronic and long term studies, 83-5 Combined chronic toxicity/oncogenicity studies, November 1984; and OECD Principles of GLP 1981.

Results

The behaviour and general health conditions of animals in this study were not affected by treatment with endosulfan. In male mice, reductions in body weight were seen throughout the study at the high dose level compared with control animals, and while these changes were generally slight, they were frequently statistically significant, and are considered to be treatment related. The body weights of animals in other groups were not adversely affected by administration with endosulfan. Food consumption was similar in control and treated groups during this study. A statistically significant increase in mortality was observed in females at 18 ppm, but the intercurrent mortality in other treatment groups was similar to that seen in control animals.

No statistically significant changes were observed in haematology or clinical chemistry parameters between control and treated animals during the study. Macroscopic examination did not reveal any findings that were related to treatment with endosulfan. At the terminal sacrifice, no statistically significant changes in organ weights were seen in treated animals. On occasion, slight but statistically significant changes in organ weights were observed at the 12 or 18 month interim sacrifices (decreased lung and ovary weights in females at 12 months; decreased liver weights in males and ovary weights in females at 18 months) at the high dose level, and while the magnitude of the effects was not great, they are considered to be related to the administration of endosulfan.

Histopathological examination did not reveal any effects that were related to the administration of endosulfan in this study, with a range of neoplastic and non-neoplastic lesions seen in control and treated mice, consistent with the spontaneous tumours seen in

aging laboratory rodents. There was no treatment related increase in the incidence of any tumour type or in the total number of animals with benign or malignant tumours in any group. A slight increase in the incidence of minimal focal epithelial thickening and minimal epithelial thickening was observed in the urinary bladders of treated females. However, as there was no progression to any further proliferation (with no evidence of epithelial hyperplasia seen in treated animals), and no clear dose dependence for this effect, it is not considered to be toxicologically significant.

Summary

The no-observed-effect-level (NOEL) for this study is 6 ppm endosulfan (approximately 0.84 mg/kg/day in males and 0.97 mg/kg/day in females), based on decreased body weights in males (24-month sacrifice) and decrease organ weights (liver, ovaries, lung) in males and females at the 12 and /or 18 month interim sacrifices at 18 ppm (approximately 2.51 mg/kg/day in males and 2.86 mg/kg/day in females). No increase in the incidence of neoplastic or non-neoplastic lesions was observed in mice administered endosulfan at dietary levels of up to 18 ppm for 24 months.

6.2 Rat

6.2.1 2-Year Dietary Study

Ruckman et al (1989). Endosulfan, active ingrediant 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 (AgrEvo 11303).

Technical endosulfan (Hoechst batch 381 A-D, 97.1% purity) was administered to rats (Charles River Crl:CD (SD) BR, 5-6 weeks of age at initiation of treatment) at dietary concentrations of 0, 3, 7.5, 15, and 75 ppm for 104 weeks, with 50 animals/sex/group. These dietary concentrations were calculated to be equivalent to achieved intakes of endosulfan of 0, 0.1, 0.3, 0.6, and 2.9 mg/kg body weight/day for males, and 0, 0.1, 0.4, 0.7, and 3.8 mg/kg body weight/day for females, respectively. In addition to the main groups of animals (50/sex) which were intended primarily for tumourigenic evaluation, there were satellite groups each consisting of 20 animals/sex/dose which were intended for blood sampling at intervals and sacrifice after 104 weeks.

A test material premix was prepared weekly by dissolving endosulfan in acetone and then mixing in corn oil. This mixture was ground directly into Labsure Animal Diet No 2 and mixed, then the required dietary concentrations were prepared using further quantities of untreated diet in a double cone blender. Control animals received similar quantities of acetone and corn oil to treated animals. The stability, homogeneity and accuracy of mixing were tested prior to treatment, and diets were analysed in week 1, and months 3, 6, 9, 12, 15, 18, 21, and 24 to confirm the accuracy of preparation.

Observations for clinical signs of ill health and behavioural changes were made daily for the first four weeks of the study and weekly thereafter. During these examinations, all rats were

palpated for appearance, location, and dimensions of any masses. Observations for mortality were conducted twice daily while body weights and food consumption were recorded weekly. Ophthalmoscopic examinations were preformed before treatment commenced, during week 50 and at termination in all animals from control and 75 ppm groups.

Prior to termination, samples of blood, urine and faeces were collected from 5 animals/sex/dose (satellite animals where possible) for terminal laboratory examinations. Blood samples were obtained during weeks 13, 26, 52, 78, and 103 from 10 animals/sex/dose from satellite groups. At week 103, animals were obtained from the main groups also, if necessary to maintain the sample size of 10 animals/sex/dose.

The following haematological parameters were determined: packed cell volume (PCV), red cell count (RBC), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), total white cell count (WBC), platelet count, thrombotest, differential white cell count (neutrophils, lymphocytes, eosinophils, basophils, monocytes), cell morphology.

The following biochemical parameters were determined: total protein, albumin, globulin, urea nitrogen, creatinine, sodium, potassium, calcium, inorganic phosphorous, chloride, glucose, alkaline phosphatase (AP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), cholesterol, total bilirubin.

Urinalysis examinations were performed at the same intervals as the other laboratory investigations, with the following parameters determined: total reducing substances, glucose, ketones, bile pigments, urobilinogens, haem pigments, epithelial cells, polymorphonuclear leucocytes, erythrocytes, organisms, renal tubule casts, sperm.

At 104 weeks, all animals were killed and examined. All superficial tissues, including the urogenital orifices and tail, pinna, eye, and external auditory meatus, were examined visually and bu palpation, and similar examinations were made to the mammary tracts and the subcutaneous structures. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, testes, thyroid.

Histopathological examinations were performed on samples from the following tissues: adrenals, alimentary tract (oesophagus, stomach, jejunum, duodenum, ilium, caecum, colon, rectum), aorta, brain (medullary, cerebellar, and cortical regions), eyes, femur, heart, kidneys, liver, lungs, lymph nodes, mammary gland, ovaries, pancreas, prostate, salivary gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal column, spleen, sternum, testes, thymus, thyroid (with parathyroid), trachea, urinary bladder, uterus, ureter. Microscopic examinations were made on all the listed tissues from: all control and 75 ppm animals from main and satellite groups; animals from other main or satellite treatment groups dying or killed in a moribund state during the study; macroscopically abnormal tissues from animals from other main or satellite treatment groups. Lung, liver, and kidney samples were examined microscopically from all main or satellite group animals from 3, 7.5, or 15 ppm groups.

Kidney, liver and fat samples were used for analysis of alpha and beta-endosulfan residues. This study was designed in accordance with the OECD 'Short term and long term toxicology group guidelines', 14 August 1981, and EPA FIFRA 'Pesticide assessment guidelines', subdivision F, November 1982. This study was conducted in compliance with GLP standards

of: US EPA, 1983; OECD, 1982; Japanese MAFF, 1984; and UK Department of Health and Social Security, 1986.

Results

No clinical signs attributed to treatment were observed in animals during the study, and mortality was similar in control and treated groups during the study. The major factors contributing to death included mammary tumours in females, and pituitary tumours in males and females, both of which normally occur at relatively high frequency in aging laboratory rats. The incidence of these as causes of mortality were similar in control and treated animals, and were not considered to be related to the administration of endosulfan. Another major contributory factor for mortality in main and satellite group males was renal lesions. While renal lesions appeared to contribute more to the mortality of males administered endosulfan at doses of 7.5 ppm and above than for control animals, there was no dose dependence for this factor, and overall mortality was not increased in treatment groups.

Factors contributing to death (no of animals with factor/no of animals dead)

	Males					**Females**				
Dose (ppm)	0	3	7.5	15	75	0	3	7.5	15	75
Pituitary Tumour-										
Main*	11/32	10/31	5/31	8/30	11/32	16/37	16/31	13/32	19/29	17/33
Satellite**	1/9	5/12	2/15	5/16	2/14	8/16	8/12	6/10	9/14	5/11
Total#	12/41	15/43	7/46	13/46	13/46	24/53	24/43	19/42	28/43	22/44
Mammary Tumours-										
Main*						14/37	14/31	14/32	8/29	6/33
Satellite**						2/16	4/12	4/10	3/14	2/11
Total#						16/53	18/43	18/42	11/43	8/44
Renal Lesions-										
Main*	9/32	7/31	15/31	12/30	14/32	1/37	2/31	1/32	3/29	3/33
Satellite**	1/9	5/12	5/15	3/16	9/14	0/16	3/12	1/10	2/14	2/11
Total#	10/41	12/43	20/46	15/46	23/46	1/53	5/43	2/42	5/43	5/44

* Main groups consisted of 50 animals/sex/dose
**Satellite groups consisted of 20 animals/sex/dose
#Combined groups of 70 animals/sex/dose

Statistically significant ($p<0.01$) decreases in group mean body weight gains were seen in high dose males from weeks 6-18 (10% below controls), weeks 18-64 (18%), weeks 0-64 (9%), and over the entire study period (weeks 0-104, 17%), and in males at 15 ppm for weeks 6-18 (9% reduction). In females, statistically significant reductions in group mean body weight gains were also seen at a number of intervals at 75 ppm, namely 0-6 weeks (8% reduction), weeks 18-64 (18%), weeks 0-64 (13%), and in the study overall (weeks 0-104, 18% reduction, $p<0.05$). Over the period of the study, group mean bodyweights were reduced by 13-14% in males and females at 75 ppm compared with controls, but at other dose levels, group mean body weights in treated animals were >90% of control values. The effects on body weight and body weight gain at 75 ppm are considered to be treatment related. However, the reduction in group mean body weight gain in males at 15 ppm from weeks 6-18 was slight (9%), though statistically significant, and as the group mean body weights for males at this dose level over this interval was similar to controls, this transitory slight change in body weight gains in males at 15 ppm was not considered to be toxicologically significant.

Food consumption was generally similar in control and treated animals. Statistically significant ($p<0.05$) decreases in group mean consumption were seen in males at 15 and 75 ppm, between weeks 65-104 only. These changes were approximately 5-6% lower than controls, and were not considered to be toxicologically significant.

Ophthalmoscopic examinations did not reveal any effects related to the administration of endosulfan. Haematology examination revealed a number of parameters that were, on occasion, statistically significantly different in treated animals compared with controls. At 13 weeks, reductions in PCV and eosinophil count (75 ppm only), and RBC and MCV (all doses), were seen in females, while reduced thrombotest results were seen in males at all doses. In males at 75 ppm, reduced total WBC (26 weeks) and reduced lymphocyte counts (26 and 52 weeks) were noted. At the terminal examination, reduced MCV and increased platelet count were seen in males at 75 ppm, while in females, increased MCHC and reduced thrombotime were seen at 7.5, 15, and 75 ppm. These effects were not considered to be related to administration with endosulfan, as the magnitude of these changes was small compared with control values, there was no relationship with increasing dose, and the effects were not dependent upon the length of administration of the test material.

Clinical chemistry examination revealed a number of parameters in treated animals that were statistically significantly different to controls. These consisted mainly of protein/globulin measurements and electrolyte values. However, these changes were not considered to be related to endosulfan administration as they were not dependent upon dose, and the magnitude of these effects was small, and not considered to be biologically significant.

Urinalysis examination revealed statistically significant differences between control and treated groups for a number of parameters (including specific gravity and pH) at several sample intervals. These effects were slight, and not dependent upon dose, and are not considered to be treatment related. At the terminal examination, males had statistically significant increases in urinary protein at 15 and 75 ppm. The protein concentration was 375, 400, 533, 600* and 620** mg/dL, at 0, 3, 7.5, 15, and 75 mg/kg/day, respectively (*$p<0.05$;**$p<0.01$). This effect was not associated with any histopathological findings, there was no marked increase in proteinuria between the 15 and 75 ppm levels, considerable intergroup variation (neither dose related nor statistically significant) in this parameter was seen at other intervals, and the protein concentration was within the testing facility's historical

control range for this parameter. As such, this effect is not considered to be related to treatment with endosulfan.

Macroscopic pathology examination revealed: an increase in the incidence of enlarged kidneys in females at 75 ppm; an increase in the incidence of blood vessel aneurysms in (mainly satellite) males at 75 ppm; and an increased incidence of enlarged lumbar lymph nodes in (satellite) males at 75 ppm. These effects are considered to be related to the administration of endosulfan.

Blood vessels:	In the main study, the incidence of aneurysms in blood vessels in male rats was 9/50, 3/50, 10/50, 5/50, 12/50, at 0, 3, 7.5, 15, and 75 ppm, respectively. In the satellite males, the incidence was 1/20, 2/20, 2/20, 3/20, and 6/20, respectively. The combined main/satellite group incidence was: 10/70, 5/70, 12/70, 8/70, and 18/70, respectively.
Kidneys:	In females in the main study, the incidence of bilaterally enlarged kidneys was 8/50, 12/50, 14/50, 13/50, and 18/50, at 0, 3, 7.5, 15, and 75 ppm, respectively. In the satellite females, the incidence was 2/20, 6/20, 5/20, 4/20, and 8/20, respectively. The combined main/satellite group incidence was: 10/70, 18/70, 19/70, 17/70, and 26/70, respectively.
Lumbar Lymph Nodes:	In the satellite males, the incidence was 2/20, 2/20, 3/20, 2/20, and 5/20, at 0, 3, 7.5, 15, and 75 ppm, respectively. The combined main/satellite group incidence for this finding was: 14/70, 10/70, 8/70, 7/70, and 19/70, at the dose levels outlined above.

For all of these findings, there is considerable intergroup variation in incidence, and, in the case of the lymph nodes, the macroscopic findings were not accompanied by any increased incidence of adverse findings at histopathological examination. However, no historical control data are provided for these effects, and in the absence of any evidence to the contrary, these effects are considered to treatment related at the high dose level. A range of other macroscopic lesions were seen in all groups, including controls, and as the incidence of these findings was similar in control and treated groups, these effects were considered to be unrelated to the administration of endosulfan.

Statistically significant ($p<0.01$) decreases in group mean absolute testes weight were seen in main group males at 15 and 75 ppm (3.94 and 4.04 g, respectively, compared with 4.78 g in controls). As these testes weights were within normal historical control ranges (1.34-6.11 g), and the decreases were not dose-related in degree, they are not considered to be toxicologically significant. Treatment with endosulfan did not have any effect on the group mean weights of other organs in this study.

Histopathological examination did not reveal any treatment related increase in incidence of any particular tumour types, nor were there differences in the incidence of animals bearing tumours between control and treated groups. A high incidence of pituitary and mammary tumours was seen in both control and treated groups, typical of tumour types commonly seen in aging laboratory rats. Progressive glomerulonephrosis (PGN) was a common finding in control and treated animals at histopathological examination.

PGN is a common, age-related renal lesion in rats, and occurs in nearly all 2 year old male laboratory rats (Haschek and Rousseaux, 1991), with evidence that the development of PGN is influenced by diet, with standard laboratory diets containing excess protein for maintenance needs after maturation has been reached. The disease has been reported in most rat strains, with considerable difference reported among strains, and within strains from different testing laboratories (Benirschke et al, 1978). Microscopically, there are tubular cysts filled with proteinaceous material, degeneration of tubular cells may vary from vacuolation changes to hyalin-droplet formation, markedly thickened tubular basement thickening, and glomerular changes (ranging from marked cystic dilation of Bowman's space with atrophic glomerular tufts to occasional glomeruli with proliferation of epithelial cells). The pathogenesis of PGN in rats is uncertain, but may be related to basement membrane dysfunction, and a higher incidence of PGN is generally seen in males than females, though some studies have shown a high incidence of this effect in females also (Benirschke et al, 1978).

In this study, the lesion has been recorded into three grades of severity, namely minimal, moderate and marked. Minimal is used when the lesions characteristic of PGN affected up to 15% of the nephrons with large areas of unaffected renal tissue. Moderate is similarly when 15-50% of the nephrons were affected. Marked was when the majority of the nephrons were involved with characteristic changes associated with PGN, leaving progressively lesser amounts of normal tissue.

Historical control data from six studies indicate an incidence of marked progressive glomerulonephrosis in male Sprague-Dawley rats ranging from 10-38% (mean 23%). In this study, the incidence of all grades of PGN were similar in control and treated animals, but in the satellite males there appeared to be an increase in the severity of the lesion at 75 ppm. When the main and satellite groups were combined, the incidence of marked PGN in males ranged from 26-34% at doses of 3-15 ppm, and the incidence of marked PGN at these doses was similar to control males (28% incidence). However, at 75 ppm, the incidence of marked PGN was 43% (30/70 animals), and while this incidence is only slightly higher than the historical control range, it is an increase of 50% over the concurrent control incidence for this study, and is considered to be related to the administration of endosulfan.

Non-Neoplastic Findings-Kidney

	Males					**Females**				
Dose (ppm)	0	3	7.5	15	75	0	3	7.5	15	75
Progressive glomerulonephrosis: Main Group (50/sex/dose)										
Minimal	15	11	14	9	10	10	12	8	18	9

Moderate	10	11	12	18	13	13	8	14	8	10
Marked	16	13	18	18	19	1	3	4	3	6
Progressive glomerulonephrosis : **Satellite Group** (20/sex/dose)										
Minimal	5	6	6	9	3	3	6	7	7	5
Moderate	5	6	10	2	3	2	5	7	1	5
Marked	4	5	4	6	11	0	3	2	2	2
Total (70 animals/sex/dose)										
Minimal	20	17	20	18	13	13	18	15	25	14
Moderate	15	17	22	20	16	15	13	21	9	15
Marked	20	18	22	24	30	1	6	6	5	8

Histopathological examination also revealed an increase in the incidence of blood vessel aneurysms in male rats at 75 ppm, with an incidence of 27% in the combined main and satellite groups (19/70 animals), compared with an historical control incidence range of 4-18% (mean 10%), and a concurrent control incidence of 14%. This effect in high dose males was considered to be related to treatment with endosulfan. Macroscopic examination revealed an increase in enlarged lumbar lymph nodes in satellite group males at 75 ppm, but histopathological examination did not reveal any microscopic changes associated with this finding.

Non-Neoplastic Findings: Other Tissues

	Males					**Females**				
Dose (ppm)	0	3	7.5	15	75	0	3	7.5	15	75
Blood vessel: Main Group (50/sex/dose)										
aneurysms:	9	4	12	7	13	1	1	4	4	3
Blood vessel : **Satellite Group** (20/sex/dose)										
aneurysms	1	2	2	3	6	0	1	1	0	0
Total aneurysms (70 animals/sex/dose)	10	6	14	10	19	1	2	5	4	3
Lumbar lymph nodes: Cystic node(s)										

Main group	23	17	20	13	23		2	1	3	2	4
Satellite group	4	5	3	5	8		0	1	0	0	0
Total	27	22	23	18	31		2	2	3	2	4

Summary

In Sprague-Dawley rats administered endosulfan in the diet at up to 75 ppm (2.9-3.8 mg/kg/day) for two years, there was no evidence of increased carcinogenicity findings at any dose tested. Reductions in body weights and body weight gains were observed in males and females at 75 ppm, but no clinical signs of intoxication were observed at any treatment dose. No increase in mortality was observed in treated groups. 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. The increase severity of PGN suggests that the kidney is the target organ for endosulfan toxicity, although this is complicated by the fact that PGN is a common lesion in aging laboratory rats and occurs at a high incidence in control animals. The no-observed-effect-level (NOEL) for this study was 15 ppm (approximately 0.6 mg/kg/day), based upon the reduced body weights and pathological findings at 75 ppm (2.9 mg/kg/day).

6.2.2 78-Week Dietary Study

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.

Male and female Osborne-Mendel rats were administered endosulfan technical (Thiodan R, Purity 98.8%) via the diet, with time-weighted average doses of 0 ppm (20/sex), 223 ppm (50 females), 408 ppm (50 males), 445 ppm (50 females) and 952 ppm (50 males) for 78 weeks with a return to control diets for a further 4 weeks. Subsequently, the time-weighted average low and high dietary concentrations of endosulfan were, respectively, 408 and 952 ppm for male rats, and 223 and 445 ppm for female rats. Control animals were administered normal diet mixed with corn oil. The Osborne-Mendel rats were obtained from Battelle Memorial Institute, and were 6- 7 weeks old at initiation of treatment. No mention was made of specific adherence to any international toxicological guidelines.

Animals were observed daily for clinical signs and mortality and body weights and food consumption were measured weekly for the first 10 weeks, and at monthly thereafter. A histopathological examination consisting of a gross and microscopic examination (of major organs, and tissues) was carried out on every animal. Thirty one different anatomical regions were selected.

Clinical signs observed were hunched appearance, reddened or squinted eyes, rough fur, alopecia, sores on the body and/or extremities, eye or nasal discharge, and swollen areas.

None of these were specifically treatment related as they were observed in control animals and would reflect normal ageing of rats.

Statistical analysis on survival rates in male rats showed a highly significant ($p<0.001$) morbidity rate in male rats at all doses, and by week 54, 52% of the high dose rats had died. Due to the high mortality rates no conclusion could be drawn on analysis of tumour rates in male rats. Survival rates in female rats were unaffected, except in the low dose group where (7/50) 14% of rats died in week 21, of which, six were found to have cerebral haemorrhage.

These cerebral lesions were not found in any other groups and were not considered related to treatment.

Body weights of female rats were not apprecibly reduced compared to controls, other than a marginal decrease in the high dose group from weeks 35-100; however, these returned to control values at the end of the study. A dose related reduction in body weights was found at all treatment concentrations, specifically, in male rats from weeks 22-78; (-28% cf controls) at week 60 in the high dose group. As no specific statistical analysis was performed, it could not be determined whether this was significant.

Histopathological examination revealed toxic nephropathy in 47/50 (94%) low dose and 43/47 (91%) high dose males; 27/50 (54%) low dose and 29/50 (58%) high dose females; however, none of the control animals exhibited nephropathy. Chronic renal inflammation was observed in 8/20 (40%)male controls, 42/50 (84%) low dose males and 34/47 (72%) high dose males. Renal calcium deposits were observed in 1/20 male controls (5%), 29/50 (58%) low dose males, and 22/47 (47%) high dose males. The female rats exhibited some chronic inflammation and calcium deposits but this did not vary from control incidences.

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. Some tubules had hyalin casts, and infrequent enlarged dark-staining regeneartive tubular epithelial cells were observed (at this stage the kidney often had infiltration of inflammatory cells, fibrosis, and focal mineralisation. Parathyroid hyperplasia possibly associated with renal lesions occurred in 0/20 controls, 21/48 (44%) low dose males, 18/47 (38%) high dose males, and 1/49 (2%) low dose females.

Male rats showed medial calcification of the aorta; 29/50 (58%) in the low dose group, 22/49 (45%) in high dose; and medial calcification of the mesenteric artery, 28/50 (56%) in the low dose and 23/49 (47%) in the high dose group. Calcium deposits were noted in the stomach of 31/50 (62%) of low dose, and 21/47 (45%) high dose males. Female rats showed low incidences of arterial calification and stomach calcium deposits which did not vary from control incidences.

Testicular atrophy occurred in 3/19 (16%) control, 18/47 (38%) low dose, and 24/47 (51%) high dose male rats. This was 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.

The NOEL for male or female rats could not be established due to the renal effects observed at low dose in both sexes. Due to the high early mortality, no conclusion as to the oncogenic potential of endosulfan in male rats could be drawn. There were no treatment related neoplastic lesions seen in female rats and it may be concluded that endosulfan lacks oncogenic potential in this sex. Treatment related non-neoplastic changes in the kidneys of male and female rats and the testes of males were observed at the low doses of 408 ppm for male rats (approximately 20 mg/kg/day), and 223 ppm for female rats (approximately 10 mg/kg/day).

6.2.3 2-Year Dietary Study.

Hazelton Laboratories. (1959a) 2 Year dietary study in rats. Hazleton Laboratories, Ref: A-199-119, 22 May 1959.

Endosulfan technical was fed to Wistar rats (25/sex/group) in their diets at dose levels of 0, 10, 30 or 100 ppm for 2 years. Clinical signs, body weights and food consumption were determined weekly. Haematological examinations were carried out at 0, 26, 52 and 78 weeks on 3/sex/group and on remaining animals at 104 weeks of treatment. An interim kill (5/sex/group) was carried out at 52 weeks and gross and histopathological examinations performed for all groups except the high dose group, where a high incidence of intercurrent deaths significantly reduced the group size. All surviving animals were autopsied at 104 weeks.

There were no treatment related clinical signs, and body weights were unaffected except for a non significant decrease in body weights and food consumption in the high dose males. Survival of female rats treated with 10 and 30 ppm was reduced during the second year of treatment. Females from the high dose group had a reduced survival rate after 26 weeks (93% in controls, 74% in high dose) and 104 weeks (88% in controls, 46% in high dose). The deaths were predominantly associated with respiratory infections. Survival rates in males were unaffected by treatment.

Haematological parameters were within normal limits with the exception that the percentage of segmented neutrophils were elevated in both control and all treated groups of both sexes. There were no consistent gross pathological changes associated with treatment.

Upon necropsy, the testes weights of males from the 10 ppm group only were reduced by 7% with respect to controls at 104 weeks ($p<0.05$) and kidney weights were significantly ($p<0.001$) elevated by 16% in the high dose males at 104 weeks.

Histopathologic changes observed in kidney of the high dose males at 104 weeks consisted of enlarged kidneys, mild to severe renal tubule dilatation (12/12 animals), mild to moderate formation of irregular albuminous casts (10/12 animals), pronounced focal nephritis (7/12 animals) and mild to severe degeneration of the renal tubule epithelium (11/12 animals). At 104 weeks, female rats at the high dose showed some minimal degeneration of renal tubules (2/3 animals) and some focal nephritis (1/3 animals), but no extensive pathological renal tubule changes as observed in the male rats; however, the low survivial rate due to the respiratory infections procluded any definite conclusions in female rats. At 10 and 30 ppm, a number of animals displayed kidney effects also, but these findings were not considered to be

related to treatment as the incidence of these findings was similar to controls, and there was no consistent dose response relationship at the lower doses.

At histopathological examination at 104 weeks, findings in the livers of 50% of high dose males consisted of microscopic cellular alteration, namely focal areas of hydropic cells. These hydropic cells were pale and swollen with the nuclei surrounded by a clear zone, and a few cells appeared to have eosinophilic cytoplasmic inclusions. In comparison high dose female rats did not show any liver cell changes. At 52 weeks and 104 weeks liver sections of male and female rats at doses of 10 and 30 ppm were comparable to controls.

The tumour incidence is summarised in below.

Summary of tumour incidence in rats after 2 year treatment with endosulfan

Dose (ppm)	0 ppm		10 ppm		30 ppm		100 ppm	
	M	F	M	F	M	F	M	F
Thyroid								
adenoma	0	0	1	0	0	0	0	0
fibroadenocarcinoma	0	0	0	0	0	2	0	0
Thymus								
adenofibroma	0	0	0	0	0	1	0	0
fibroadenosarcoma	0	0	0	0	0	1	0	0
lymphosarcoma	0	0	0	0	1	0	1	0
Liver								
malignant lymphoma	0	0	0	1	0	0	0	0
granuloma	0	0	0	0	0	0	0	1
Kidney								
carcinoma (metastasis)	0	0	0	0	1	0	0	0

None of the tumours noted occurred consistently or in a dose related manner and are not considered to be treatment related. Thus, under the conditions of this assay, endosulfan lacked carcinogenic potential at doses up to and including 100 ppm. The NOEL was 30 ppm (1.5mg/kg/day), based on kidney effects at 100 ppm (5mg/kg/day).

6.3 Dog

6.3.1 1-Year Oral Study

Brunk (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 (AgrEvo 11303).

Brunk (1990). Addendum to report 89.0188. Hoechst report 89.0188. Hoechst document no A44605 (AgrEvo 11303).

Technical endosulfan (96.5% purity, Hoechst) was administered in the diet to groups of Beagle dogs (Hoechst breed, Hoe:BEAK strain; 6/sex/dose; aged 6 months at the beginning of the study) at dietary concentrations of 0, 3, 10, 30 ppm for one year. These dietary concentrations approximate 0, 0.23, 0.77, and 2.3 mg/kg/day. For the 10 ppm group only, the testing facility has calculated the test substance intake to approximate 0.65 mg/kg/day in males and 0.57 mg/kg/day in females. In addition, a group of animals (6/sex) was administered diets containing 30-60 ppm, increasing in stages from 30 ppm (54 days) to 45 ppm (52 days) and 60 ppm (19-40 days) as the study progressed. This group shall be designated as 30/45/60 ppm.
The test material was received by the testing facility in the form of cornmeal premixes, then stirred into the mixed feed diet (Vipromix) daily immediately before feeding, with control animals receiving cornmeal premix base in the same proportion as the highest treatment group. The final dietary concentrations were achieved by adding 0.8-1.0 g of the cornmeal premixes to the daily feed rations of 1000 g (males) and 800 g (females) of wet feed. The stability, content and homogeneity of the test substance in cornmeal was tested and found suitable for the purposes of this study.

Checks were conducted for death and behaviour at least twice daily, for general health and food consumption daily, and for body weights weekly. Tests for neurological status (flexor, patellar, anal, cutaneous, corneal, pupillary and blink reflexes; visual and tactile placing and righting reactions) were conducted before the initial treatment and before study termination, and/or after 6 weeks or 3 months. Ophthalmological examinations, hearing tests and dental inspections were conducted at the same intervals as the tests for neurological status.

Haematological examinations were conducted before the start of the study, after approximately 6 weeks and every 3 months, and at the termination of the study, with the following parameters being tested: erythrocytes, haemoglobin, haematocrit, leucocytes, thrombocytes, differential blood count, reticulocytes, Heinz bodies, thromboplastin time, MCV, MCH, and MCHC. Methaemoglobin was measured at the terminal examination only.

Clinical chemistry examinations were conducted at the same intervals as for haematology, with the following parameters determined: sodium, potassium, inorganic phosphorous, uric acid, total bilirubin, direct bilirubin, creatinine, glucose, urea nitrogen, calcium, chloride, iron, magnesium (terminal value only), ASAT, ALAT, alkaline phosphatase, LDH, gamma-

GT (terminal value only), cholinesterase, cholesterol, triglycerides, total lipids (terminal value only), total protein, electrophoresis, creatinine phosphokinase.

Urinalysis examinations were conducted at similar intervals to haematology and clinical chemistry, with the following parameters determined: appearance, colour, pH, glucose, haemoglobin, bilirubin, ketone bodies, urobilinogen, specific gravity, sediment, volume.

Hepatic function testing and renal function testing were performed prior to the start of the study, and at the end of dosing, and/or at 6 weeks and 3 months. At pathological examination, the weights of the following organs were determined: heart, lungs, liver, kidneys, spleen, brain (without medulla), pituitary, pancreas, ovaries/testes, uterus/epididymides, prostate, thyroid with parathyroid, thymus and adrenals.

The following organs or parts of organs were removed for microscopic examination, and histopathological examinations were performed on all of the specified tissues and all gross lesions: heart, lungs, liver, kidneys, spleen, adrenals, thyroid with parathyroid, pancreas, thymus, pituitary, cerebral cortex, brain stem, cerebellum (cortex and medulla), medulla, cervical, thoracic and lumbar regions of the spinal cord, bone marrow (middle sternal segment), caput femoris, eyes (each with optic nerve), urinary bladder, testes/epididymides, ovaries/uterus, prostate, stomach (fundus and prepyloric region), intestine (duodenum, jejunum, ilium, caecum, colon and rectum), skeletal muscle, diaphragmatic muscle, gallbladder, tonsils, trachea, oesophagus, aorta (thoracic region), lymph nodes (cervical and iliac), sciatic nerve, skin with mammary gland, and salivary glands (parotid and mandibular).

This study was conducted in accordance with the following guidelines: EPA Pesticide Assessment Guidelines Subdivision F, Hazard Evaluation: Human and Domestic Animals. Series 83: Chronic and long-term studies, 83-1 “Chronic Toxicity Studies”, November 1984; OECD Guidelines for testing of Chemicals no 452, “Chronic Toxicity Studies” Adopted: 12 may 1981; and OECD principles of Good laboratory Practice Annex 2 of “OECD Guidelines for testing of Chemicals”, OECD 1981.

Results

No spontaneous deaths occurred during the study. At 30/45/60 ppm, one male was killed in extremis after 125 doses of endosulfan, and the remaining animals in this group were killed on days 146 or 147 due to marked nervous conditions. One male in the 30 ppm group was killed on day 276 to prevent suffering as the animal was in very poor condition with extensive preputial oedema and oedematous swellings in the knee joints.

With the exception of the animals that were killed during the course of the study, no other animals displayed impairment of physical condition during the study. Body weights and body weight gains did not appear to be adversely affected by administration with endosulfan in most groups, with the group mean body weights in treated groups closely following the course of body weights seen in control animals at doses up to 30 ppm. At 30/45/60 ppm, the body weights again were similar to controls, but the animals at this dose level were removed from the study at an early stage; group mean body weight gains were also similar in control and treated animals. In males at 30 ppm, the body weight gain decreased after week 43, resulting in a group mean body weight approximately 8% lower than controls (15.6 kg versus 14.4 kg). Body weight gain was also reduced in males at 30 ppm (by 28%) with a group

mean figure of 3.3 kg versus 4.7 kg in controls. At 30 ppm, 1/6 males only gained 1.0 kg over the course of the study, but even if this animal is removed from the calculation, the group mean body weight gain was 16% lower at this dose than in controls (3.9 kg versus 4.7 kg). Marginal reductions in group mean food consumption were observed at 30 ppm in the early phase of the study, and also in the first two weeks of administration at 30/45/60 ppm.

At 30 ppm, observations were made in 3 males and 2 females (2.5-6 h after dosing) of sudden and violent contractions of the abdominal muscles with contraction of the upper abdomen, and also convulsive movement of the chaps, though not followed by vomiting. All animals at 30/45/60 ppm had pronounced clinical signs after dosing at 60 ppm endosulfan, including increased sensitivity to noise, frightened reaction to optical stimuli, and tonic contractions of the muscles in the extremities and face. No signs of intoxication were observed in animals at 3 or 10 ppm. In 1 male and 4 females at 30/45/60 ppm, signs of impairment of the central nervous system (impaired righting reflexes) were seen at the terminal examination, but no such signs were seen in animals at other dose levels during the study. No adverse effects associated with treatment were observed in the ophthalmoscopic, hearing or dental inspections.

Haematological examination did not reveal any effects that were considered to be treatment related. On occasion, statistically significant changes in a number of parameters were measured (including reticulocytes, erythrocytes, haemoglobin, and haematocrit). These effects were sometimes seen at the low dose only, or at the preliminary examination before administration with endosulfan, and were not dependent upon dose or length of time administered. As such, these effects were not considered to be related to treatment.

Clinical chemistry examination revealed a number of statistically significant changes in parameters compared with control values, including differences between control and treated groups at preliminary examination, i.e. before endosulfan administration. For most parameters, changes in clinical chemistry parameters were not consistently related to dose or length of administration, and were not considered to be treatment related. A statistically significant increase in alkaline phosphatase and LDH activity was observed at the 30 ppm dose level at the final examination, and on a number of intermediate examinations during the study; these effects may be related to the administration of endosulfan. No gross or histopathological findings associated with these elevations in enzyme levels were observed after the terminal examination. It should be noted that the reporting and statistical analysis for clinical chemistry parameters in this study is variable, with a number of parameters (i.e. alkaline phosphatase) having separate statistical analyses for male and female groups, while other parameters (i.e. LDH) statistically analyses in a combined male/female group. This makes comparison between group mean and/or individual animal data with the statistical analysis difficult.

Serum and erythrocyte cholinesterase activity appeared to be similar in control and treated animals, although the reporting of statistical analysis for these data is also questionable. For brain cholinesterase activity, large variations in activity were measured between groups, with males at 30 ppm having >50% activity compared with controls. However, there were very large intra-group variations in the measurement of this parameter, and none of the differences were reported to be statistically significantly different to controls. Given the reporting of the statistical analysis, and the large variations between animals in this study, it is not possible to

determine whether treatment with endosulfan significantly affected cholinesterase activity in dogs.

Urinalysis examination did not reveal any effects that were considered to be treatment related. At preliminary (before endosulfan administration) and intermediate examinations during the study, a number of statistically significant differences between control and treatment group urinalysis parameters were reported. In the absence of any consistent dose or time dependence, these effects are not considered to be treatment related. At the final examination, no statistically significant changes were seen in the urinalysis parameters of treated animals.

No treatment related changes in absolute or relative (to body weight) organ weights were seen during this study. A single statistically significant increase in absolute liver weights was reported for males and females combined at 10 ppm, but in the absence of any effects at the high dose, this effect was not considered to be treatment related. Hepatic and renal function tests did not reveal any impairment related to the administration of endosulfan.

Pathological examination did not reveal any neoplastic or non-neoplastic lesions that were attributed to the administration of endosulfan. At the 30/45/60 ppm dose level, while the animals were killed in extremis due to neurological disturbances, no pathological abnormalities were observed, including in the brain and nerves. All findings in animals treated at doses of 3, 10, and 30 ppm were spontaneous changes unrelated to the administration of the test material.

Summary

Dogs that were administered 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. Some animals that were administered endosulfan at 30 ppm (estimated to be 2.3 mg/kg/day) throughout the 12 month study were observed with violent muscular contractions of the abdominal muscles, and males at this dose level had reduced body weight gains throughout the study, and slightly reduced body weights in the latter stages of the study, compared with control animals. No other effects related to treatment were observed, and no increase in incidence of neoplastic or non-neoplastic lesions were observed in treated animals. Based on these clinical signs and reductions in body weights, the NOEL for this study is 10 ppm (calculated to be equivalent to 0.65 mg/kg/day for males, and 0.57 mg/kg/day for females).

6.3.2 1-Year Oral Study

Hazelton Laboratories. (1959b) One-year oral study in dog. Hazleton Laboratories, 12 May 1959.

Endosulfan technical was administered orally, via gelatin capsules, to adult mongrel dogs (2/sex/group) at dose levels of 0, 3, 10 and 30 ppm (0, 0.075, 0.25 and 0.75 mg/kg/day) on 6 days/week for one year. The group receiving 3ppm originally was treated at 100 ppm for the first 3 days of treatment however clinical signs of vomiting, tremors, convulsions, rapid

respiration and mydriasis, salivation, tonic-clonic convulsions and rapid respiration in one male and both female dogs led to the dose being reduced to 3ppm for the rest of the study. Endosulfan was administered as a weight/weight mixture with lactose. Control dogs received lactose only. Dogs were weighed and observed for clinical signs at weekly intervals. Haematological and clinical chemistry parameters were determined at 0, 3, 6, 9 and 12 months, kidney function was assessed at 6 and 12 months and BUN was determined at 6, 9 and 12 months. All dogs were necropsied at the end of the study period.

No clinical signs or treatment related effects on body weight gains were seen, other than one female dog at 10 ppm exhibiting an eczema-like irritation on the nose (which responded to treatment with medication), another female showing a 12% reduction in body weight with respect to its initial weight at 52 weeks, and one male dog at 30 ppm also showing a 12% reduction in body weight.

Clinical chemistry, haematology were within normal limits and kidney function was unaffected by treatment.

No gross or histopathologic changes associated with treatment were noted. Incidental findings of mild congestion and atelectasis of the lungs, mild cytoplasmic disturbances of the liver (chiefly an increase in granularity), free and unphagocytosed pigment in the spleen, mild congestion and focal interstitial nephritis of the kidneys were found in control and treated animals of both sexes. These findings were not considered related to tratment.

The NOEL for this study was 30 ppm (0.75 mg/kg/day).

7. REPRODUCTIVE TOXICITY

7.1 Rat

7.1.1 2-Generation Dietary Reproduction Study.

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.

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 (AgrEvo 11303).

To identify the reproductive toxicity potential of endosulfan, the test material (Hoechst, 97% purity) was administered in the diet to rats (CrL: COBS CD (SD) BR; Charles River UK) at concentrations of 0, 3, 15, and 75 ppm for two mating generations, with two mating phases in each. In the premating period for the F0 generation, these dietary concentrations were calculated to be equivalent to 0.2, 1.0, and 4.99 mg/kg/day for males, and 0.24, 1.23, and 6.18 mg/kg/day for females, at 3, 15, and 75 ppm, respectively. In the premating period for the F1B generation, these dietary concentrations were calculated to be equivalent to intakes of 0.23, 1.18, and 5.72 mg/kg/day for males, and 0.26, 1.32, and 6.92 mg/kg/day for females, at 3, 15, and 75 ppm, respectively. Group sizes were 32/sex/group for F0, and 28/sex/dose for F1B. Diets were prepared freshly every two weeks, with the test material dissolved in a small amount of acetone, mixed with corn oil, and dispersed through the diet after the acetone was evaporated from the dispersant. Control animals received the same powdered diet, without the endosulfan. The homogeity and stability of endosulfan in the diet was established and was acceptable for the purposes of this study.

Diets containing endosulfan were administered to animals throughout the study. In the F0 generation, (animals nominally 6 weeks of age at initiation of treatment) were fed throughout a 12 week premating period, a 20-day mating period, and through gestation and lactation until day 2 postpartum, when F1A young were killed and the organs of one male and one female per group preserved for possible histopathological examination. After a rest period (approximately 10 days), males and females were mated with alternative mates for 20 days, followed by gestation and lactation, with females allowed to rear their young to day 21 post partum. At this time, 28 male and 28 female pups from each group were selected to form the basis of the F1B generation. F0 adults were killed and used for gross and histopathological examination.

In the F1B generation, a similar dietary regimen was used as for the F0 generation, with animals maintained for a premating period until they were approximately 18 weeks of age, then through mating, gestation, and lactation, for two generations, with all F1B adults and F2B pups sacrificed at or soon after day 21 post partum. Tissues of all adults and the selected pups from the control group and the 75 ppm group were examined histopathologically, and testes and the accessory sex organs of all males failing to produce pregnancy at the second mating, and ovaries of females without young at the second mating, were also examined histopathologically.

All animals were regularly examined for signs associated with treatment, and determinations of body weights, food consumtption, and water consumption were made at least weekly. Offspring were examined for external abnormalities, and were sexed, weighed and counted. The following tissues were used for histopathological examination: adrenals, bone marrow, brain, epididymides, eye, heart, ilium, kidneys, liver, lungs, lymph nodes, mammary glands, seminal vesicles, skin, testes, thymus, thyroids, urinary bladder, uterus, vagina, mid colon, ovaries, pancreas, pituitary, prostate.

This study was conducted in compliance with GLP regulations (Title 21 of the US Code of Federal Regulations, Part 58), and a Quality Assurance Statement was issued for this study.

Results

No clinical signs or mortality related to endosulfan administration were observed during the study. Single mortalities occurred in the F0 females at 0, 3, and 15 ppm, and in F1B control females.

In the F0 adults, body weights and body weight gains in control and treated animals were generally similar during the study. Statistically significant decreases in body weight gain were seen in females only, at 75 ppm from weeks 0-4 of endosulfan administration. The magnitude of this effect was small, and transient, and was not considered to be related to treatment. In the F1B animals, statistically significant differences in body weight gains between control and treated groups were observed only at 3 ppm. However, as a decrease in body weight gain was seen only up to week 8 of treatment, and as these changes were slight, this effect is not considered to be related to endosulfan administration. In F1B males, no statistically significant decreases in body weight gains were observed during the study. The group mean body weight of males at 75 ppm was consistently lower than controls during the study, but as the magnitude of this effect was slight (generally 5% or lower), it was not considered to be toxicologically significant.

Mating performance and pregnancy rates were not affected by treatment during the study. The incidence of total litter loss was low in both generations, and was not related to the dose of endosulfan, and pup mortality and litter size were similar in control and treated groups. In the F0 generation, statistically significant decreases in litter weight were seen on days 12 and 21 post partum at 75 ppm, while in the second mating in this generation, similar decreases in litter weight were seen on days 4 (15 ppm), 8, 12 and 21 (15 and 75 ppm). No statistically significant decreases in litter weight were seen in the F2 offspring. The decreases in litter weight at 15 ppm were not considered to be toxicologically significant, as they were infrequent, and generally the weight decreases at this dose were less than 8% compared with controls. At 75 ppm, the decreases in litter weight were generally 10-12% compared with controls, but there was no effect on the mean pup weights during the study, nor on the litter sizes. No treatment related effect on sex ratios was seen at any dose tested.

Statistically significant ($p<0.01$) increases in kidney weights (relative to body weights) were seen at 75 ppm in F0 and F1B males, with relative kidney weights increasing about 8-9% compared with controls. Relative kidney weights in males at 3 and 15 ppm, and in females at all doses, were not increased compared with controls. Statistically significant increases in relative liver weights were observed in F0 males ($p<0.05$) and females ($p<0.01$) at 75 ppm, and in F1B females at 15 ($p<0.01$) and 75 ppm ($p<0.001$). The effect at 15 ppm in F1B was not seen at this dose level in any other matings during the study, and is considered incidental to treatment. On occasion, statistically significant increases in relative organ weights were seen during the study (F0 male hearts, F0 female brains, F0 first mating female offspring pituitaries, F1B first mating females uteri), but in the absence of any consistant relationship with dose, these effects are considered to be incidental to treatment with endosulfan.

The original histopathological examination did not reveal any difference in incidence of microscopic effects related to treatment between control and high dose groups. However, in a 13-week dietary study in rats (Barnard, 1984), yellow discolouration of some proximal cells were seen in males at all doses at histopathological examination (10-360 ppm), and in females at doses of 30-360 ppm, with the degree of pigmentation increasing with dose.

While no adverse toxicological findings were associated with this discolouration, in the light of the findings in the 13-week study, renal histopathological examinations were conducted on adult rats from the 3 ppm and 15 ppm groups of the reproduction study. The histopathological findings for the kidneys of F2B weanling rats from control and high dose groups were also reviewed.

Yellowish discolouration of cells in the proximal convoluted tubules were observed in male F1B rats at 3, 5, and 75 ppm, and in female F1B rats at 75 ppm. The incidence and extent of this effect was dose related, with the incidence of traces of discolouration being 0/28, 11/28, 12/28, and 18/28 for males at 0, 3, 15, and 75 ppm, respectively, while 9/28 females had traces of discolouration at 75 ppm only. Minimal discolouration was seen in male rats at 15 ppm (1/28) and 75 ppm (10/28) only. Granular/clumped pigment were seen in proximal convoluted tubular cells in high dose males only. These findings were not associated with any histopathological evidence of renal damage at any dose level tested. No evidence of discolouration of the proximal tubular cells was observed in F2B weanlings at any dose level.

The increase in incidence and severity of the yellowish discolouration of proximal tubular cells in F1B adult rats appears to be treatment related, with no control animals displaying these effects. However, this effect does not appear to be toxicologically significant, with no adverse effects associated with these findings at any dose level. At 75 ppm, all animals displayed either trace or minimal discolouration, and at this dose level, signs of granular/clumped pigment were also detected. No treatment related increase in the incidence of progressive chronic glomerulonephrosis or other kidney related effects were reported.

Dose ppm Sex	0 M	0 F	3 M	3 F	15 M	15 F	75 M	75 F
Yellowish discoloured cells in proximal convoluted tubules								
- Minimal	-	-	-	-	1	-	10	-
- Traces	-	-	11	-	12	-	18	9
Granular/clumped pigment in proximal convoluted tubular cells								
- Minimal	-	-	-	-	-	-	3	-
- Traces	-	-	-	-	-	-	11	1
Early progressive glomerulonephrosis	5	-	6	1	3	-	6	2

While the increase in incidence of the cellular discolouration is related to the administration of endosulfan, these findings were not considered to be toxicologically significant, as they were not associated with any adverse effects on the cells, and the yellow pigment was considered likely to be endosulfan and metabolites being stored and metabolised in lysosomes prior to excretion (JMPR, 1989). The presence of the pigment is an indication of endosulfan exposure, rather than an index of toxicity, and in longer term studies in rats (Ruckman, 1989), where the induction of lysosome enzymes is complete, the lysosomal degradation of endosulfan becomes effective and the test substance is fully eliminated (Canada PMRA, 1993), and thus this discolouration is not observed in the kidneys.

The no-observed-effect-level (NOEL) for this study was 15 ppm (approximately 1.0 mg/kg/day), based on the increase in liver and kidney weights at 75 ppm (approximately 6 mg/kg/day). 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.

7.1.2 2-Generation Dietary Study- Addendum to Study 7.1.1 above

Edwards JA (1993). Endosulfan-technical (Code: Hoe 02671 OI AT209). Effect on reproductive function of multiple generations in the rat. Huntingdon Research centre report 204/931464, 25 November 1993; Supplement to HRC report HST 204/83768. Hoechst document A51721 (AgrEvo 11303).

This report consists of individual pup weight data, which was not originally included with the submission of the original report of a 2-generation reproduction study in rats, HRC report HST 204/83768, 1984.

8. DEVELOPMENTAL TOXICITY

8.1 Rat

8.1.1 PO Teratology Study

Gupta PK, Chandra SV, Saxena DK (1978). Teratogenic and embryotoxic effects of endosulfan in rats. *Acta pharmacol. et toxicol*., 1978, 42, 150-152. (AgrEvo 11303).

In this study, female albino rats (Strain and age unstated; Industrial Toxicology Research Centre, India) were mated with males, then were orally administered endosulfan (technical; source not stated; suspended in corn oil) from days 6-14 of gestation at doses of 0, 5, and 10 mg/kg body weight/day. At the beginning of the study, group sizes varied, with 20, 26, and 32 females/group at 0, 5, and 10 mg/kg/day, respectively. The number of pregnancies at these dose levels was 18, 20, and 21, respectively. Animals were killed on day 21 of gestation for examination of the dams and fetuses. Half the number of live fetuses from each litter were fixed and stained for examination of skeletal malformations, while the remaining fetuses were fixed and used for inspection of gross visceral and internal malformations.

Results

No marked changes in behaviour and appearance were reported in females administered endosulfan, and body weights in treated animals were similar to those seen in controls. 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/day, and 22.8% at

10 mg/kg/day), and an increase in fetal mortality, though this effect was slight and not dose related (two fetal deaths at 5 mg/kg/day, one at 10 mg/kg/day, compared with no fetal deaths in controls). Slight increases in the incidence of cerebral hypoplasia and enlargement of the renal pelvis were observed during visceral examination, but these effects were not considered to be related to treatment, as the magnitude of these increases was small, the effects were also seen in control animals, and the effects were not dose dependent. No other increases in visceral abnormalities were reported.

Skeletal examination revealed a statistically significant increase in incidence of absent 5 th sternebrae (incidence of 3, 5, and 6, at 0, 5, and 10 mg/kg/day, respectively), and in fetuses with incomplete ossification (incidence of 6, 9, and 0, at 0, 5, and 10 mg/kg/day, respectively). A slight increase in the incidence of absent 5th metacarpus, though not statistically significant, was also noted in treated animals compared with controls. 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.

Summary

Under the conditions of this study, the administration of endosulfan to female rats at doses up to 10 mg/kg/day during organogenesis did not result in an increase in developmental effects in offspring. No maternotoxicity was evident at any dose level. The level of reporting in this published paper is not adequate for the purposes of defining an NOEL for developmental toxicity.

8.1.2 Oral Teratology Study

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. (AgrEvo 11303)

Technical endosulfan (Hoechst; 97.3% purity) was dissolved in sesame oil and administered daily via oral gavage (dose volume 5 mL/kg body weight) to groups of 20 to 24 mated female Wistar rats (Hoe: WISKf SPF 71; aged approximately 65-70 days) on days 7-16 of gestation, at doses of 0 (vehicle control), 0.66, 2, and 6 mg/kg body weight/day. The homogeneity and stability of the solutions were analysed and were considered suitable for the purposes of this study. Behaviour, food consumption and general health condition of the animals were observed daily, while body weights were recorded weekly. The surviving dams were killed on day 21, and offspring delivered by caesarean section. The live and dead foetuses, the conceptuses undergoing resorption, the placentae, and the corpora lutea on the ovaries were counted and examined macroscopically. The diameter of conceptuses undergoing resorption and placental weights were also determined. The fetuses were examined for outward appearance, external anomalies, and the fetal weights measures. About half of the fetuses were used for skeletal examination, and the remaining fetuses used for visceral examination. The study was conducted in compliance with: OECD Guidelines for testing of Chemicals, section 4, Health effects, 414, Teratogenicity, adopted 12 May 1981; EPA Pesticide

Assessment Guidelines, Subdivision F, Hazard evaluation series 83, November 1984; and in compliance with current principles of GLP.

Results

No clinical signs of toxicity were reported in females at 0.66 or 2 mg/kg/day. At 6 mg/kg/day, four dams died, after 6-10 doses of endosulfan, and 3/4 of these animals displayed tonoclonic convulsions for several days prior to death. In the surviving animals, 13 had tonoclonic convulsions for a number of days, generally around day 10 of gestation. A number of these animals also displayed hypersalivation on a number of days during treatment. Food consumption was not affected by treatment with endosulfan at 0.66 and 2 mg/kg/day, but there was a marked decrease in food consumption in animals at 6 mg/kg during days 7-14 of gestation. Statistically significant ($p<0.05$) decreases in body weight (days 14-17 of gestation) and bodyweight gain (days 7-14 of gestation) were observed at 6 mg/kg/day. No statistically significant changes in reproductive or pup parameters were observed at any dose level in this study. The fetal sex ratio was relatively balanced, with a slightly increased percentage of males at 0.66 and 2 mg/kg (55% and 50% males, respectively), and slightly more females than males in controls and 6 mg/kg groups (52% males in both groups). No statistically significant increase in the incidence of abnormalities was observed in fetuses during examination. A single oedematous, retarded fetus in the 6 mg/kg group presented with brachygnathia superior with a relatively small alveolar cavity in the upper jaw combined with cleft palate, bending of both hind feet in the tarsal joint, wavy clavicles, and bent and shortened scapula. These findings were considered to be spontaneous in nature, given that no other limb or head defects were observed in any pup in any of the litters at this dose level.

Skeletal examination revealed a statistically significant ($p < 0.05$, Fisher's Exact Test) increase in fragmented thoracic vertebral centra at 6 mg/kg, with seven fetuses from three litters seen with this effect (6.3% incidence versus previous control values 3.9% maximum incidence). This effect was considered to be treatment related, and reflects the frank maternotoxicity of endosulfan seen at the high dose level, as no other significant skeletal abnormalities were seen at 6 mg/kg in this study.

Summary

The NOEL for maternotoxicity in this study was 2 mg/kg/day, based on clinical signs (tonoclonic convulsions and hypersalivation) and decreased bodyweights seen at 6 mg/kg/day. The NOEL for embryo/fetotoxicity was 2 mg/kg/day based on increased incidence of fragmented thoracic vertebral centra seen at 6 mg/kg/day. No treatment related major malformations were observed in this study.

8.1.3 Oral Teratology Study

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

Mated CD Sprague Dawley rats (25/group) were administered endosulfan technical (in corn oil), by gavage, on gestation days 6-19 at dose levels of 0, 0.66, 2 and 6 mg/kg/day. Animals

were necropsied on gestation day 20 and all fetuses examined for external, skeletal and soft tissue anomalies and developmental variations.

In the treatment of the high dose group, poor gavage techniques led to the death of 30% of the dams. As a consequence an additional 10 mated rats were retested at 6 mg/kg/day; an additional five, untreated, rats were used as controls for this group.

Maternotoxicity was evident in dams treated with 6 mg/kg/day with clinical signs including placidity, rough coat, alopecia and hyperactivity being observed. A dose-related decrease in maternal body weight gain was seen at 2 and 6 mg/kg/day.

The number of implantations and litter size were unaffected by endosulfan treatment. There was a slight reduction in fetal weight and length in the high dose group. A non dose related reduction in the percent of live fetuses and an increase in the number of resorbed fetuses were seen at 2 mg/kg/day. No statistically significant treatment related effect on sex ratios was observed, with the percentage of males ranging from 50.5% in controls to 45.8% at 6 mg/kg/day. No external variations or malformation were seen at 0.66 or 2 mg/kg/day however, at the high dose, 5 (of 405) fetuses exhibited lordosis and 6 fetuses had oedema. All five of the fetuses with lordosis (anteroposterior curvature of the spine), and 5/6 of the fetuses with oedema were from a single litter from the one dam (animal 109). One fetus (also from the same litter) had the skin of the upper forelimb webbed to the chest.

No significant treatment-related effects were seen on soft tissue development. Common minor skeletal variations were present in all groups. The incidence of poorly ossified sternebrae (6th) in the high dose group was significantly greater than for the control group. Two fetuses had clubbed left hind limbs in the high dose group. The five fetuses from dam no 109 which had oedema and lordosis also had wide and thickened vertebral arches, ribs, and clavicles, and the clavicles were also shortened, curved, and twisted. Four of these fetuses had shortened pubes and two had an unossified hyoid bone.

No indication is given of the historical control incidence of these adverse findings. However, the incidence of these effects was generally under 1%, the effects were largely related to delayed development, and mainly confined to a single litter from a single dam. The dam in question displayed numerous signs of intoxication related to endosulfan administration, including face rubbing, alopecia, flaccidity and hyperactivity, and the developmental effects are probably related to the maternotoxicity of endosulfan at the high dose level.

The NOEL for maternotoxicity was 0.66 mg/kg/day based on decreases in body weight gain at 2 mg/kg/day and decreased body weight gain and clinical signs at 6 mg/kg/day. Evidence of delayed development and isolated low incidence of skeletal variations were seen at the maternotoxic dose of 6.0 mg/kg/day. Based on these effects, the NOEL for developmental toxicity was 2 mg/kg/day.

8.2 Rabbit

8.2.1 Oral Teratology Study

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

Mated New Zealand White rabbits (20-26/group) were administered endosulfan technical, by gavage, on gestation days 6 to 28 at dose levels of 0, 0.3, 0.7 or 1.8 mg/kg/day. The vehicle control received corn oil. All animals were observed daily for signs of toxicity. On gestation day 29, does were killed and all fetuses examined for external, skeletal and soft tissue anomalies and developmental variations.

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. In addition, 4/20 animals in the high dose group died during treatment. While the cause of death was not established one animal had vacuolisation of hepatocytes indicative of systemic disturbance. This was attributed to bleeding into the bowel. As a consequence of these deaths a further 6 animals were treated with 1.8 mg/kg/day endosulfan.

The number of implantations, litter size, sex ratio, mean fetal weight and length and the number of live and resorbed fetuses were unaffected by endosulfan treatment. There were no dead fetuses in any of the treated or control groups. No gross external observations were reported. The only soft tissue anomaly occurred in 6/167 high dose fetuses and consisted of the left carotid arising from the innominate; 1/141 control fetuses also showed this abnormality. Common skeletal variations and minor anomalies occurred with a similar incidence in control and treated fetuses.

Endosulfan did not produce any teratogenic or developmental effects even at the maternotoxic dose of 1.8 mg/kg/day. The NOEL of maternotoxicity was 0.7 mg/kg/day based on clinical signs seen at 1.8 mg/kg/day.

9. GENOTOXICITY STUDIES

9.1 Gene Mutation Assays

9.1.1 Microsomal Reverse Mutation (Ames) Test.

Shirasu et al. (1978) Microbial mutagenicity testing on endosulfan. Institute of Environmental Toxicology, Japan, Report No A21215, 1978.

The mutagenic potential of endosulfan (0, 5, 10, 50, 100, 500, 1000 and 5000 ug/plate) was tested in the Ames Test using six bacterial strains (*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100 and E.coli WP2 hcr) in the presence and absence of metabolic activation (S9). Positive control substances were 2-amino-anthracene (10 µg/plate, with and without S9; all strains), AF-2 (-S9, 0.25 µg/plate, TA100), 2-nitrofluorene (-S9, 50 µg/plate,

TA1538), -propiolactone (-S9, 50 µg/plate, TA1535), 9-aminoacridine (-S9, 200 µg/plate, TA1537), AF-2 (-S9, 0.1 µg/plate, TA98). The vehicle control was DMSO.

In all cases uniformly negative results were obtained for endosulfan. Positive controls produced expected results. Under the conditions of this study, endosulfan was not mutagenic in bacteria at doses up to 5000 µg/plate, with or without metabolic activation.

9.1.2 In vitro Forward Mutation Assay in *Schizosaccharomyces pombe*

Milone & Hirsch (1984a) Mutagenic activity in vitro of the compound Endosulfan - technical with Schizosaccharomyces pombe. Instituto Di Recherche Biomediche. Experiment No. M 708. 18 June 1984.

Endosulfan was tested in the forward mutation assay using *S.pombe* and was used at dose levels of 0, 62.5, 125, 250 and 500 ug/mL. A vehicle control (DMSO, 27.5 mg/mL) was also included. Assays were carried out both in the absence and presence of metabolic activation. The positive controls used were methylmethane sulfonate (MMS) (84.5 ug/mL) in the absence of metabolic activation and dimethylnitrosamine (DMNA), 375 ug/mL) in the presence of metabolic activation.

The maximum concentration (500 ug/mL) of endosulfan assayed produced 46% and 75% survival rate in *S. pombe* in the absence and presence of metabolic activation, respectively. Endosulfan did not induce any significant increases in gene mutation of *S. pombe* under the conditions of this assay. Positive controls produced expected results.

9.1.3 Mouse Lymphoma Forward Mutation Assay.

Cifone (1984a) Mutagenicity evaluation of HOE 002671 - substance technical in the mouse Lymphoma Forward Mutation Assay. Litton Bionetics Inc. Genetics Assay No. E9294. LBI Project No. 20980. November 1984.

The ability of endosulfan technical to induce forward mutation at the TK locus in L5178 TK +/-mouse lymphoma cell line was examined. Endosulfan was tested, in duplicate, at doses of 0, 6.25, 12.5, 18.8, 25.0, 37.5, 50.0 and 75.0 µg/mL in the absence of metabolic activation and at 0, 6.25, 12.5, 50.0, 75.0 and 100 µg/mL with metabolic activation. Appropriate solvent (DMSO) and positive controls were included in the assay.

Endosulfan treatment did not increase the mutant frequency above that of the solvent control, both with and without metabolic activation. In the presence of metabolic activation, the minimum criterion for mutagenesis in this assay was a mutant frequency exceeding 70×10^{-6}. One of the 100 µg/mL treatments induced a mutant frequency that exceeded this criterion (95.6×10^{-6}). However, this increase in mutant frequency was caused by a decrease in cloning frequency (8.6% relative growth) due to toxicity of the testmaterial at this dose level. The duplicatetreatment at 100 µg/mL had a mutant frequency of 55.4×10^{-6}, and a relative growth of 18.8%. There was no increase in the number of mutant colonies at 100 µg/mL compared with solvent controls. As such, the single increase in mutant frequencies at 100

µg/mL was considered to be erroneous. Positive controls (ethylmethane sulfonate 0.25 to 0.5 µg/mL, -S9; 3-methylcholanthrene 1 to 4 µg/mL, +S9) produced expected results. Under the conditions of this assay, endosulfan lacks mutagenic potential in the mouse forward mutation assay.

9.2 Chromosomal Effects Assays

9.2.1 Mouse Micronucleus Test

Müller (1988). Endosulfan-substance technical (Code: Hoe 002671 0I ZD95 0005). Micronucleus test in male and female NMRI mice after oral administration. Pharma Research Toxicology and Pathology, Laboratory Project no 87.0041, 7 January 1988. Hoechst report no 88.0012, 2 February, 1988. Hoechst document A38059. (AgrEvo 11303).

In an *in vivo* micronucleus test in NMRI mice (Hoe: NMRKf SPF71, Hoechst; 5 animals/sex/dose/sample interval), the clastogenic potential of endosulfan technical (Hoechst, 95.5% purity) was investigated, with the test material administered via oral gavage at doses of 0 (solvent control; sesame oil), 2.5, 5, and 10 mg/kg body weight. Animals received a single dose of test material, with animals killed after 24, 48, or 72 h. The positive control, Endoxan (cyclophosphamide), was administered to 5 animals/sex, at a single dose level of 50 mg/kg body weight, with animals killed after 24 h. The volume of administration was 10 mL/kg body weight for all doses. Bone marrow smears were examined for the occurrence of micronuclei in red blood cells. This study was conducted in compliance with GLP regulations (no further details provided), and a QA statement was issued for the study.

All animals survived after administration of endosulfan. A range of clinical signs of intoxication were reported at 10 mg/kg, including increased spontaneous activity, stilted gait, clonic convulsions, trembling, back arched position, and forward crawling.

No statistically significant increases in the incidence of micronucleated polychromatic micronuclei were observed in animals treated with endosulfan at any dose level, while the positive control compound induced a marked and statistically significant increase in the number of polychromatic erythrocytes with micronuclei in males and females.

Under the conditions of this study, endosulfan technical was negative for genotoxicity in an *in vivo* micronucleus assay at doses up to 10 mg/kg in mice.

9.2.2 Mouse Micronucleus Test

Jung et al. (1983) Mouse micronucleus test following oral administration. Hoechst AG, Report No. 83.0458, Project No. Tx 117/06.04-20, 3 October 1983.

Endosulfan technical was tested for its ability to induce micronuclei in mouse bone marrow cell *in vivo.* NMRI mice (5/sex/group) were administered endosulfan, by gavage, at single doses of 0, 0.2, 1.0, and 5.0 mg/kg. The doses were repeated after an interval of 24 h. The

animals were killed 6 h after the second dose and bone marrow smears prepared from each animal. The number of cells with micronuclei was recorded out of 2,000 polychromatic erythrocytes counted for each animal.

While the positive control, cyclophosphamide (100 mg/kg) induced a marked and significant increase in the number of cells with micronuclei in both sexes, endosulfan was without cytogenic activity at all doses tested.

9.2.3 In vivo Cytogenetic Assay in Rats

Dikshith & Datta (1978) Endosulfan: Lack of Cytogenetic Effects in Male Rats. Industrial Toxicology Research Centre. Doc No. A17140. 1978.

Male albino rats (8/group) were given, by gavage, endosulfan at dose levels of 0, 11, 22, 36.6 and 55.0 mg/kg/day for 5 days. The vehicle control group received peanut oil. Cochicine (4 mg/kg) was administered to each rat (ip) 4 h prior to being killed. Femur bone marrow and seminiferous tubules were collected, cell suspensions made and slides prepared. The mitotic index and chromosome damage in bone marrow and spermatogonial cells was assessed.

Endosulfan at doses of 36.6 and 55 mg/kg induced mortalities in the rats and showed signs of toxicity at 22 mg/kg/day. However, there were no major chromosomal aberrations in either bone marrow or spermatogonial cells. There was no significant mitotic inhibition in any of the treated groups.

9.2.4 Chromosomal Aberration in Cultured Human lymphocytes

Asquith, JC (1989a). Endosulfan substance technical (Code Hoe 002671 0I ZD95 0005). Metaphase analysis of human lymphocytes. Toxicol Laboratories, UK, Study M/HL/1307, March 1989. Hoechst document A40411. (AgrEvo 11303) and

Asquith, JC (1989c). Human lymphocyte chromosome aberrations. Background data on negative and positive controls. Toxicol Laboratories, UK, February 1989. Hoechst document A40412. (AgrEvo 11303)*[This report consists of historical data for solvent and positive controls, compiled from experiments conducted with human lymphocytes at Toxicol laboratories in 1987/88. The report was prepared as a adjunct to Toxicol study no M/HL/13071]*

Endosulfan technical (Hoechst, 95.4% purity) was tested for its clastogenic potential using human lymphocytes in whole blood cultures, with and without the addition of the liver microsome S9 fraction for metabolic activation, at dose levels of 0, 10, 20, and 40 µg/mL. The test material was dissolved in DMSO at 0.1 µg/mL medium for addition to the cells. The test material was found to precipitate on addition to the culture medium at final concentrations greater than 200 µg/mL. Vehicle control (DMSO) and positive control (methyl methane sulphonate 25 µg/mL without S9; cyclophosphamide 20 µg/mL with S9) materials were also tested. This study was conducted in accordance with the UK GLP Compliance Programme (DHSS1986), the OECD Principles of GLP 1982, The US EPA GLP standards (FIFRA v48, no 230, 1982), and the Japanese MAFF Notification no 3850, 1984.

Positive control materials gave significantly more structural aberrations than the vehicle controls. In the absence of S9, all concentrations of endosulfan resulted in low numbers of alterations, but the incidence of alterations was greater than the solvent controls. There was no dose relationship, and the increase in aberrations was not statistically significantly increased compared with controls at any dose level. In the presence of S9, the incidence of aberrations was greater at all endosulfan dose levels than for solvent controls, and this increase was statistically significant at 10 µg/mL. However, in the absence of any dose relationship, and given the low incidence of aberrations in the endosulfan treated cells, this increase was not considered to be toxicologically significant. As an illustration, the average percentage of cells with aberrations (including gaps) in the presence of S9 was 2, 1.5, 4.5, 4, and 0.5 for untreated controls, solvent controls, 10 µg/mL, 20 µg/mL, and 40 µg/mL endosulfan, respectively. The average percentage of cells with aberrations without S9, for the same groups, was 3, 1, 3.5, 4, and 3, respectively.

Under the conditions of this study, endosulfan was not found to a clastogen to human lymphocytes at doses up to 40 µg/mL, with or without S9 metabolic activation.

9.2.5 Chromosomal Aberration Study in Cultured Human Lymphocytes

Istituto Di Ricerche Biomediche, (1986). Endosulfan, Substance technical. Chromosome aberration in human lymphocytes cultured "in vitro". RBM experiment no M 822, 20 March, 1986. Hoechst document A33127. (AgrEvo 11303).

To test the clastogenic potential of technical endosulfan (Hoechst), the test material was dissolved in dimethyl sulfoxide (DMSO) and diluted to concentrations of 0.05, 0.5, 5, and 10 mg/mL, and applied to cultured human lymphocytes, with and without S-9 metabolic activation. Dose levels of endosulfan, expressed as concentration in the incubation mixture, were 1, 0, 100, and 200 µg/mL. Controls were DMSO (vehicle control), mitomycin C (2 µg/mL, without metabolic activation), and cyclophosphamide (34.5 µg/mL, with metabolic activation). This study was conducted in compliance with the FDA Good Laboratory practice Regulations for Non-clinical Laboratory Studies (21 CFR, Part 58).

Endosulfan was toxic to the cultures at 200 µg/mL, both with and without metabolic activation, while at 100 mg/µg/mL, mitotic expression was 39% (- S9) and 30% (+S9). No statistically significant increase in incidence of chromosome aberrations were observed at any test dose level, with or without metabolic activation. Significant increases in total aberrations (excluding and including gaps) were seen for the positive control substances, with and without metabolic activation.

Under the conditions of this study, endosulfan did not induce statistically significant increases in chromosome aberrations in cultured human lymphocytes up to a concentration of 100 µg/mL, either in the presence or absence of metabolic activation.

9.2.6 *In vivo* chromosomal aberration assay in Syrian Hamsters

Dzwonkowska A & Hübner H (1986). Induction of chromosomal aberrations in the Syrian hamster by insecticides tested *in vivo*. Arch Toxicol (1986) 58: 152-156.

Endosulfan, along with a number of other insecticides, was tested for its potential to induce chromosomal aberrations in the bone marrow cells of Syrian hamsters. The test material, Thiodan 35 (a formulation containing 35% endosulfan)was administered to groups of six females via intraperitoneal injection at doses of 8, 16, 40, and 80 mg/kg body weight, with a dose volume of 1 m/100 g body weight. Bone marrow samples were obtained 22 h after administration of the test material. Bone marrow metaphases from eight animals not treated with any compound constituted the negative control group, while six animals received an injection of 40 mg/kg body weight cyclophosphamide, and served as the positive controls for the study. These doses were selected based upon an assumes LD50 of 80 mg/kg. It should be noted that an intraperitoneal LD50 of 8 mg/kg has been reported in rats, and so the dose range used in this study may be high.

Statistically significant increases in the number of cells with all observed aberrations was seen at all test doses. However, there was not dose dependence for these findings, with a higher percentage of cells with aberrations seen at the low dose of 8 mg/kg (10.5%) than was seen at 16, 40, or 80 mg/kg (5.4, 6.0, and 5.0%, respectively). For the positive control, this figure was 9.0%. Statistically significant increases in the number of aberrations including gaps was seen at 8 (1.7%) and at 80 (1.0%) mg/kg, but not at the intermediate dose levels (0.8% at 16 mg/kg and 0.6% at 40 mg/kg). The positive control figure was 5.3%. Again, no dose response relationship was observed.

The number of analysed cells was reduced with increasing doses of endosulfan, and the test material may have been cytotoxic at a number of the test doses. While evidence of genotoxicity was seen at 8 mg/kg, the incidence of aberrations did not increase with dose. The material tested was a formulation of endosulfan, and any mutagenic activity may have resulted from other ingredients in the formulation other than endosulfan. Under OECD Guidelines, a test substance that does not produce a statistically-significant, dose-related increase in the number of structural chromosomal aberrations is considered non-mutagenic in this system, and both biological and statistical significance should be considered together in the genotoxicity evaluation. Under this criterion, the test material did not induce chromosomal aberrations in the bone marrow of Syrian hamsters under the conditions of this study, possibly due to the dose selection used in the study.

9.3 Other Genotoxic Effects Assay

9.3.1 Unscheduled DNA Synthesis in Rat Hepatocyte

Müller (1985). Evaluation of Endosulfan - substance technical (Code: Hoe 002671 0I ZD95 0005) in the Unscheduled DNA Synthesis Test in Mammalian Cells In Vitro. Pharma Research Toxicology and Pathology project number 87.0042 completed 14 January, 1988. Hoechst report number 88.0106, 2 February 1988, document number A38455. (AgrEvo 11303)

The permanent human cell line A 549 was used to determine the potential for endosulfan technical (Hoechst, 95.5% purity) to cause DNA damage and repair in the unscheduled DNA synthesis (UDS) test. Two independent experiments were conducted with the S9 fraction of rat liver homogenate for metabolic activation, with test material dose ranges of 0.1-100 µg/mL, and three independent experiments were conducted without S9 mix, with test material dose ranges of 1-1000 µg/mL. Vehicle control (DMSO) and positive control substances (4-Nitroquinoline-N-oxide 1 µg/mL, and Benzo(a)pyrene 10 µg/mL) were also tested in this study. The study was conducted in compliance with GLP principles (no further details given), and a QA Statement was provided.

At 100 and 1000 µg/mL endosulfan, microscopic alterations in cell morphology were observed, indicating cytotoxicity at these dose levels. No statistically significant increases in the rate of unscheduled DNA synthesis were observed at any concentration of endosulfan. The positive control substances induced statistically significant increases in unscheduled DNA synthesis.

Under the conditions of this study, endosulfan was negative for DNA damage at doses of up to 1000 µg/mL in cultured mammalian cells.

9.3.2 Unscheduled DNA Synthesis in Rat Hepatocyte

Cifone (1984b) Evaluation of HOE 002671 - substance technical in the rat Primary Hepatocyte Unscheduled DNA Synthesis Assay. Litton Bionetics Inc. Genetics Assay No. 7564. LBI Project No. 20991. Report No: A29800, November 1984.

The activity of endosulfan technical to induce unscheduled DNA synthesis (UDS) was assayed in rats (male, Fischer 344) primary hepatocytes. The doses of endosulfan evaluated were 0, 0.102, 0.255, 0.51, 1.02, 5.10, 10.2, 25.5 and 51.0 ug/mL. Appropriate solvent (DMSO, 1%) and positive (2-acetyl aminofluorene, 0.1 ug/mL) controls were included in the assay.

The highest dose of 51 ug/mL endosulfan was cytotoxic. None of the other endosulfan treated cultures produced net nuclear grain counts that were substantially greater than the solvent control. The positive control produced expected results. Under the conditions of this assay, endosulfan was negative for DNA damage at doses up to 51 ug/mL in cultured rat hepatocytes.

9.3.3 Gene Conversion-DNA Repair Test using *Saccharomyces cerevisiae*

Milone & Hirsch (1984b) Mutagenic activity of the compound Endosulfan - technical with Saccharomyces cerevisiae Gene conversion-DNA repair test. Instituto Di Recherche Biomediche. Experiment No. M 707. 18 June 1984.

The genotoxic potential of endosulfan was tested in the gene conversion-DNA repair assay using *S. cerevisiae* (D4 strain) both in the presence and absence of metabolic activation. The doses of endosulfan used were 0, 100, 500, 1,000 and 5,000 ug/mL. A solvent (DMSO, 27.5 mg/mL) was also used. The positive controls were cyclophosphamide (259 ug/mL) used with metabolic activation and methylmethansulphonate (84.5 ug/mL) used in the absence of metabolic activation.

Endosulfan did not induce significant increases in gene conversion in the *S. cerevisiae* strain tested *in vitro* either in the presence or absence of metabolic activation. Positive controls produced expected results.

Under the conditions of this assay, endosulfan did not show genotoxic potential at doses up to 5,000 ug/mL.

9.3.4 Rec-assay

Shirasu et al (1978) Microbial mutagenicity testing on endosulfan. Institute of Environmental Toxicology, Japan, Report No A21215, 1978.

The rec-assay using two strains of *Bacillus subtilis* (H17 and M45) was carried out to determine the DNA-damaging capabilities of endosulfan, by measuring the inhibitory zone of each streak of solution (0.02 mL) when plated overnight with the bacteria. Kanamycin and mitomycin C were used as negative and positive controls, respectively. Endosulfan was used in doses of 0 (DMSO vehicle control), 20, 100, 200, 500, 1000 and 2000 ug/disk. Controls (kanamycin, 10 µg/disk; mitomycin C, 0.1 µg/plate) produced expected results, with the positive control causing a marked difference in inhibitory zones between the strains, while the negative control induced a similar inhibitory zone to the endosulfan solutions. Endosulfan was negative at all doses used. Under the conditions of this study, endosulfan was negative for genotoxicity at doses up to 2000 µg/disk in a rec assay with *Bacillus subtilis*.

9.3.5 Dominant lethal test

Dzwonkowska A & Hübner H (1991). Studies on commercial insecticides with the dominant lethal mutations test. Polish Journal of Occupational Medicine and Environmental Health Vol 4, No 1, 043-053, 1991.

In a dominant lethal study, endosulfan (Thiodan 35; Hoechst), along with a number of other chemicals, was administered via intraperitoneal injection to adult male Balb/c mice. Animals received either a single administration (0.64 mg/kg; 17 animals) or daily administrations for 5 days (0.64 mg/kg/day; 20 animals), with the dose estimated as 20% of the LD50 value.

Vehicle control animals received distilled water, while positive control animals received cyclophosphamide at 40 mg/kg (25 animals per group). Administration of the test material was followed by twelve 4-day mating periods where each male was mated with untreated virgin females. Females were examined for the number of total implantations, the number of live implantations, and the number of dead implantations.

The administration of endosulfan did not result in statistically significant changes in the parameters measured, and did not induce an increase in the dominant lethal index. Under the conditions of this study, endosulfan was negative for genotoxicity.

10. SPECIAL STUDIES

10.1 Immunotoxicity

10.1.1 6-Week Rat Dietary Study

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

Male Wistar rats (16/group) were fed a diet containing 0, 10, 30 or 50 ppm of endosulfan for six weeks. Animals were immunised subcutaneously (sc) with tetanus toxoid (0.2 mL) and Freund's complete adjuvant after 25 days of pesticide exposure. Liquid parafin was injected intraperitoneally (ip) in these immunised rats 48 h before terminating the exposure. Blood samples were collected after 6 weeks of exposure by cardiac puncture and serum and leucocytes collected for immunoglobulin (Ig) level estimation and for leucocyte migration inhibition (LMI) test. Peritoneal macrophages were collected for the macrophage migration inhibition (MMI) test. The liver, spleen and thymus weights were determined at the end of the treatment period. Serum antibody titre to tetanus toxoid was estimated by indirect haemaglutination technique and quantitation of serum IgM and IgG was carried out by single radial immunodiffusion method.

Treated rats did not show any overt signs of toxicity or symptoms. No significant differences were noted in body, spleen and thymus weights between control and treated rats. A significant increase in liver weight was observed in rats exposed to 50 ppm endosulfan.

A significant decrease in total serum antibody titre to tetanus toxoid occurred at 30 and 50 ppm endosulfan with a slight decrease (not statistically significant) at 10 ppm. The decrease was observed in both IgM and IgG levels at 50 ppm. Measurement of total gamma globulin content of rat serum again indicated suppression at 50 ppm. Rats exposed to endosulfan and subsequently immunised with tetanus toxoid showed a significant decrease in LMI and MMI responses in a dose-dependent pattern, the decrease becoming statistically significant at the 30 and 50 ppm level.

These results indicate that both humoural and cellular immunity was depressed as a result of exposure to endosulfan at doses of 30 and 50 ppm. No suppression of immunity was seen at 10 ppm (0.5 mg/kg/day).

10.1.2 22-Week Rat Dietary Study

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

Endosulfan was administered to male Wistar albino rats (50-60/group) in their diets at concentrations of 0, 5, 10 or 20 ppm for up to 22 weeks. At 20 days before the end of exposure, rats were immunised, sc, with tetanus toxoid in complete Freund's adjuvant. Interim kills (10-12 animals) were carried out at 8, 12, 18 or 22 weeks of study. Total Ig, IgM and IgG levels were estimated. Leucocyte migration inhibition test and macrophage migration inhibition test were also carried out at 8, 12, 18 or 22 weeks of exposure.

No overt signs of toxicity were reported. Mortality rates, body growth rates and food intake rates were comparable between all groups. A slight but significant decrease in spleen weights was noted in the 20 ppm dose group at week 22 of the experiment.

The total Ig levels were increased in all stimulated groups as expected, except in the 20 ppm dose group at weeks 12, 18 and 22 and in the 10 ppm dose group at week 22. A significantly lower increase in serum IgG was seen in rats exposed to 10 or 20 ppm endosulfan at weeks 12, 18 and 22 as compared to antigen-stimulated control. IgM levels were unaffected by the endosulfan treatment. The specific response (serum antibody titre to tetanus toxoid) showed a marked decrease in rats exposed to 10 or 20 ppm endosulfan throughout the experiment in a dose-time dependent pattern. Cellular immunity was assayed by measuring migration inhibition of activated leucocytes and macrophages. Endosulfan treatment diminished migration inhibition responses of both leucocytes and macrophages at the 10 and 20 ppm dose level throughout the study. The endosulfan-related effect on the immune system of the rat did not appear to be secondary to other toxicity since the body weights of the animals were unaffected by the treatment and endosulfan is not known to affect the hormonal system. The effects were dose and time related. The results of the other assays of immune responsiveness although less reliable followed a similar trend. Immune responses were unaffected by endosulfan treatment at 5 ppm (0.25 mg/kg/day).

10.2 Neurobehavioural studies

Three published papers from a single laboratory report neurobehavioural effects with endosulfan in rats.

10.2.1 90-Day Oral Study

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.

Endosulfan (purity 95%) was administered by oral intubation to random groups of immature male rats (10-12 rats/group) at a dose of 2 mg/kg/day for 90 days. A suspension of endosulfan was formulated in distilled water with an equivalent amount of tragacanth powder. A control group of 10-12 rats received only tragacanth suspension. One of the groups was treated intraperitoneally with picrotoxin (4 mg/kg/day) 24 hours after the last endosulfan administration. Myoclonic latency (the time between picrotoxin injection and the appearance of the first clonic movement), the intensity of the convulsions and the number of animals exhibiting tonus and mortality were recorded.

Animals were observed during the treatment period for mortality and any obvious behavioural changes such as tremors and convulsions. Food consumption and body weight were measured prior to commencement of treatment and then every 15 days until completion of treatment. Spontaneous motor activity (in a vibration sensing cage) and motor co-ordination (using a rota-rod apparatus) were recorded every 15 days.

Twenty four hours after the last treatment (either with endosulfan or tragacanth) some animals (the actual number selected not stated) were subject to necroscopy. The brain, heart, liver, spleen, kidneys and adrenals were dissected and weighed. Protein concentrations were estimated in the serum, brain, liver, heart and skeletal muscle (gastrocnemius). The activities of GOT and GPT were determined in the serum and liver of the remaining animals.

No clinical signs of convulsions occurred due to treatment with endosulfan. A significant reduction ($P<0.05$) in food consumption (44% with respect to controls) and bodyweight gain (37%) occurred 16-30 days after the start of treatment, and continued throughout the treatment period. A significant increase ($P<0.05$) in motor activity was noted in animals treated with endosulfan, particularly at the 75th and 90th day of treatment (48% at this time). Motor co-ordination on the rota-rod apparatus was not effected by treatment with endosulfan.

A significant ($P<0.05$) increase in liver weight (9%), liver GOT (64%) and GPT (44%) and increased serum GPT (39%) were found in animals treated with endosulfan. Animals treated with endosulfan showed significantly decreased myoclonus latency (15%), increased tonus (67%), mortality (9/12 animals) and intensity of myoclonus (33%) (measured 1-10 minutes after injection) with respect to control animals. The authors concluded from this study that endosulfan induced changes in the liver and central nervous system effects, but did not impair motor responses in male rats.

This study does not appear to use typical measures for neurobehavioural effects, and it is difficult to interpret the relevance of these results.

10.2.2 90-Day Oral Study

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.

Endosulfan (purity 95%) was administered by oral intubation to six groups of immature male rats (10-12 rats/group) at a single daily dose of 2 mg/kg/day for 90 days. A suspension of endosulfan was formulated in distilled water with an equivalent amount of tragacanth powder. A control group of 10-12 rats received only tragacanth suspension. One of the groups was treated intraperitoneally with p-chlorophenylalanine (PCPA) methylester HCL (100 mg/kg/day) during the last 3 days of the 90 day treatment. PCPA is known to produce an 80% depletion of 5-HT in the brain.

Animals were observed during the treatment period for mortality and any obvious behavioural changes such as tremors and convulsions. Food consumption and body weight were measured prior to commencement of treatment and then every 15 days until completion of treatment. Spontaneous motor activity (in a vibration sensing cage) and motor co-ordination (using a rota-rod apparatus) were recorded every 15 days. Learning and memory processes (by an inhibition of pole-climbing escape response to electric shock (unconditioned) and avoidance response to buzzer (conditioned) were determined 24 hours after the last treatment. The concentration of 5-hydroxytryptamine (5-HT), brain protein, and acetylcholinesterase (AChE) activity in the cerebrum and midbrain were measured, 24 hours after the last treatment.

No clinical signs or deaths occurred due to treatment with endosulfan. A significant reduction ($P<0.05$) in food consumption (44% with respect to controls) and bodyweight gain (37%) occurred 16-30 days after the start of treatment, and continued throughout the treatment period.

A significant increase ($P<0.05$) in motor activity was noted in animals treated with endosulfan, particularly at the 75th and 90th day of treatment (38%). Motor co-ordination on the rota-rod apparatus was not effected by treatment with endosulfan.

A significant ($P<0.05$) inhibition of pole-climbing escape response to electric shock (unconditioned) and avoidance response to buzzer (conditioned) occurred in treated rats compared to controls. The escape response was reinstated by administration of PCPA, whereas the avoidance response was only partially reversed by PCPA. A significant increase in 5-HT concentration in the cerebrum (40%) and midbrain (70%) of treated animals occurred, however, no effect on brain protein nor AChE activity occurred.

The authors suggested that endosulfan may impair learning and memory in rats as evidenced by suppression of escape (learning) and avoidance (memory) acquisition respectively. This was possibly facilitated by a serotonergic involvement (increased 5-HT levels) as PCPA reinstated significantly the escape response but not the avoidance response in endosulfan treated animals. In conclusion this suggested that there was an endosulfan-induced increased serotonergic impairment of learning but that the contribution of this mechanism to memory disruption is negligible. Given the relatively large dose level employed, the effects seen in

this study may reflect the high toxicity of endosulfan and the general physiological status of the animals rather than any learning impairment due to endosulfan administration.

As it is not possible to assess a dose response relationship for the findings reported in this study, and as the single dose used resulted in signs of systemic toxicity (reduced bodyweights), it is difficult to determine the biological relevance of the findings in this study.

10.2.3 30-Day Dietary Study

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.

Male and female Wistar rats (10/group) were fed a diet containing endosulfan (95% purity) at concentrations of 0, 3, and 6 mg/kg/day for 30 days. Mortality and body weight gain was assessed during the study. The neurobehavioural effect of treatment was determined by testing spontaneous motor activity (in a vibration sensing cage), motor co-ordination (using a rota-rod apparatus) and learning and memory processes (by an inhibition of pole-climbing escape response to electric shock (unconditioned) and avoidance response to buzzer (conditioned)). Liver weight and liver and serum concentrations of glutamic oxaloacetic acid (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (AP) and acetylcholinesterase (AChE) were measured to determine any hepatotoxic effects of treatment.

No significant sex-related differences in any of the parameters tested were found in control rats. High dose females showed an increase in mortality rate compared with controls and male and female rats at the other doses. Bodyweight gain, motor co-ordination and AChE activity was unaltered in male and female rats treated with endosulfan. A significant ($P<0.05$) dose-related increase in liver weight and motor activity occurred in both male and female rats, at both low and high dose. Hepatomegaly was found to be greater in females and a greater dose-related increase in motor activity was found in males compared to females.

Dose-related significant increases ($P<0.05$) in serum and liver GOT and GPT levels occurred in females compared to males. The liver AP was significantly increased in females compared to males, however, a sex related difference was not found in serum AP. The learning and memory processes (as evidenced by responses to conditioned and unconditioned stimulations) were considered to be impaired in treated groups of both sexes by the authors, and the authors concluded from this study that treatment with endosulfan found sex-related differences in regard to motor activity, liver toxicity and mortality in rats. Learning and memory processes were equally impaired in both sexes.

The lack of detailed reporting for this study, and the relative insensitivity of the methods used to assess unconditioned and conditioned responses in this study, makes it difficult to interpret these findings in terms of biological relevance.

10.3 Sperm Abnormality Studies

10.3.1 Mouse Germ Cell Study

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

The genotoxicity potential of endosulfan technical (Hoechst, 97.03% purity) in mouse germ cells was assessed *in vivo* in two tests: the dominant lethal and the sperm shape abnormality test. The test material was dissolved in DMSO and administered daily to male Swiss albino mice (20/group; Industrial Toxicology Research Centre, India) via intraperitoneal injection for 5 consecutive days, at dose levels of 9.8, 12.7, and 16.6 mg/kg/day. Vehicle controls received DMSO and positive controls received cyclophosphamide (120 mg/kg/day). After treatment, males were mated with virgin females in 7-day mating periods, for a total of 56 days (8 mating intervals). The uterine contents were examined 11 days after the separation from males, and the frequency of induced dominant lethal mutations was calculated as:

Dominant lethal mutations =1 - (Live implants per female of the test group/Live implants per female of the control group) x 100

The caudae epididymides were excised, weighed, minced, and filtered, and sperm were stained, counted, and examined for morphological abnormalities.

For assessment of sperm morphology, an additional dose group received 21.6 mg/kg/day for 5 days, and positive controls received cyclophosphamide at 20 mg/kg/day.

Results

At the sixth week post treatment (mating interval 36-42 days), reductions in the number of live implants/pregnant females (2.2 versus 9.0 in controls), total implants/pregnant females (4.5 versus 9.0), and corpora lutea/pregnant females (6.7 versus 9.7) were observed at a dose of 16.6 mg/kg/day, along with an increase in the number of dead implants/pregnant females (2.25 versus 0.0 in controls). At this mating interval, a statistically significant ($p < 0.001$) increase in the induced dominant lethal mutation rate was calculated (75.55% increase compared with controls). 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. As expected, statistically significant ($p < 0.01$-0.001) increases in the rate of induced dominant lethal mutations were observed at all mating intervals for the positive control, cyclophosphamide.

However, the induction of dominant lethal effects occurred only at a high dose of endosulfan of 16.6 mg/kg/day, equivalent to a total dose of 83 mg/kg, and no adverse effects were reported in this study at doses below 16.6 mg/kg/day. This dose level represents a significant proportion of the LD50 dose. In rats, a single ip dose LD50 of 8 mg/kg/day has been reported (FAO, 1988). The lack of detail in the reporting of this study makes the significance of the isolated finding questionable. The fact that an increase in dominant lethal mutations was seen only in a single mating interval, with no adverse effects on implants of fertility seen at other

intervals, suggests that the result may be an artifact, and not related to treatment. There is no individual animal data to determine if there was large intra group variation in the sixth mating interval, and if a single outlying result led to a statistically significant outcome for this mating interval. The test was not reproduced, and so it is difficult to determine whether the effects seen from the sixth mating interval were spontaneous, or related to endosulfan administration.

Increases in abnormal sperm were observed at 16.6 and 21.6 mg/kg/day. At these doses, the percentage abnormal sperm was 20.7 and 23%, respectively, compared with 4.7% in vehicle controls, and 4.7% and 8.1% at 9.8 and 12.7 mg/kg/day, respectively. Statistically significant ($p<0.01$) increases in the total number of sperm head abnormalities were also seen at the high doses, with 16.3% and 18.1% seen at 16.6 and 21.6 mg/kg/day, respectively, compared with 3.8% in vehicle controls. The incidence of tail abnormalities was also increased at 16.6 mg/kg/day and above.

Statistically significant ($p<0.001$) decreases in sperm count were observed at 16.6 and 21.6 mg/kg/day, with sperm counts reduced by 34-39% compared with vehicle controls, while sperm motility was not affected by treatment. A statistically significant ($p<0.01$) decrease in testis weight was seen at 21.6 mg/kg/day, with weights reduced by approximately 22% at this dose level. Epididymis weights were also slightly decreased at this dose level, but the magnitude of this effect was not statistically significant. The positive control material resulted in increased sperm abnormalities and decreased testis and epididymis weights, and decreased sperm count and motility, as expected.

These effects on sperm, as with the induction of dominant lethality, was confined to high doses of endosulfan (16.6 mg/kg/day and above), with no adverse effects seen at doses of 9.8 or 12.7 mg/kg/day. 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. Unless the sperm abnormalities were found almost exclusively in the sixth mating interval, it is highly unlikely that there is any causal relationship between the sperm abnormalities and the induction of dominant lethal mutations, even at the high doses of endosulfan administered in this study.

Under the conditions of this study, the intraperitoneal administration of endosulfan to Swiss mice at doses of 16.6 mg/kg/day for five consecutive days, resulted in an increase in the incidence in sperm abnormalities, along with decreased sperm counts and decreased testis weights. It is unclear whether the decrease in sperm count and increase in sperm abnormalities are biologically significant. It is unlikely that this increase in sperm abnormalities is causally related to any adverse effects on fertility or other reproductive parameters 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 administered endosulfan at doses of 12.7 mg/kg/day or lower.

10.3.2 Mouse Sperm Morphology Study

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.

To investigate the effect of pesticides on sperm morphology and sperm count, and to test the ameliorating potential of vitamin C on such effects, a number of compounds, including endosulfan, were administered to male Swiss albino mice (6-8 weeks old; Central Drug Research Institute, India). The endosulfan (35% emulsifiable concentrate) was administered to groups of six animals via oral gavage at a dose of 3 mg/kg/day (estimated to be the maximum tolerated dose) for 35 consecutive days, with a dose volume of 0.2 mL of an aqueous solution of the test material (0.1% v/v). One group of treated animals received the endosulfan only, while three other treatment groups received endosulfan via gavage, plus intravenous administrations of vitamin C at 10, 20, or 40 mg/kg/day. A vehicle control group was also used, but no positive control group was used in this study. Animals were sacrificed 24 h after the final treatment, with sperm collected from the cauda and cauda epididymides of each mouse for counting and morphological analysis.

Results

Statistically significant decreases in sperm count were seen in all groups administered with endosulfan, but the sperm count decrease was ameliorated by treatment with vitamin C in a dose related manner, with sperm counts increasing with the dose of vitamin C. In the absence of vitamin C, the reduction in sperm count was about 80% compared with controls, while at 40 mg/kg/day vitamin C the reduction in sperm count was only about 22% compared with controls. Statistically significant increases in the total abnormal sperm were seen in animals treated with endosulfan alone, with 14% abnormal sperm, compared with about 5% in controls. In the presence of vitamin C, the incidence of sperm abnormalities reduced to about 7-8%, but there was no dose relationship, and this figure was statistically significant both from controls and from endosulfan-only group incidences.

Under the conditions of this study, the administration of endosulfan at a dose of 3 mg/kg/day resulted in an increase in abnormal sperm from 5 to 14%, and this effect was reduced slightly in animals also administered vitamin C. 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 were seen following the administration of endosulfan (80% reduced), and this effect was lessened in animals also administered vitamin C. However, the test material was a 35% emulsifiable concentrate containing solvents, emulsifiers and stabilizers, and it is unclear whether thesefindings are related to endosulfan or these non active constituents.

10.3.3 Rat Biochemistry Study

Sinha N, Narayan R, Shanker R, Saxena DX (1995). Endosulfan-induced biochemical changes in the testis of rats. Vet Human Toxicol 37 (6), December 1995.

Technical grade endosulfan (95.32% purity; Bharat Pulverizing Mills, Bombay) was administered via oral gavage to groups of male Druckrey rats (3 months old; Industrial Toxicology Research Centre, India) at doses of 0, 2.5, 5, and 10 mg/kg/day, on 5 days/week for 70 days.. The test material was administered in 0.2 mL peanut oil. After termination of dosing, blood was collected, and the testes were weighed and kept for biochemistry and intratesticular sperm counts.

At 10 mg/kg/day, 2 animals died during the study, but no mortality was reported at other dose levels. No change 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. The reduction in sperm count at 5 and 10 mg/kg/day was also statistically significant when compared with the reduction seen at 2.5 mg/kg/day. The incidence of sperm abnormalities was statistically significantly increased at 5 and 10 mg/kg/day, but this increase was very slight (increasing from 6% abnormalities in controls to 7% abnormalities at the high dose level), and it is unlikely that such an increase is biologically significant. Statistically significant reductions in spermatid count (about 16%) and sperm production rate (about 22%) were also reported at 5 and 10 mg/kg/day compared with controls, but there is no consistent dependence on endosulfan dose for these effect, with similar reductions seen at both of the higher dose levels. At 2.5 mg/kg/day, these parameters were similar to control values.

The authors postulated that endosulfan impairs testicular functions by altering the enzyme activities responsible for spermatogenesis, thus influencing intratesticular spermatid count, and resulting in low sperm production and increased sperm deformities. The data presented in this report supports the notion that the administration of endosulfan at relatively high doses (2.5 mg/kg/day and above) for several months resulted in an increase in activity of a number of enzymes found in the testes, and that at doses of 5 mg/kg/day and above, there was a marked reduction in sperm count (up to 47%) compared to controls. 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 normal biological ranges for the test animals. The reductions in other parameters (sperm abnormalities, spermatid count, sperm production), while statistically significantly different to concurrent controls, were only slightly reduced, and in the absence of consistent dose response relationships for these effects, it is considered that these effects are not biologically significant.

10.3.4 Rat Androgen Study

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.

This paper evaluated the biochemical toxicity of sub-chronic endosulfan treatments in relation to gonadal hormones, via 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 -hydroxysteroid dehydrogenase (3 -HSD), and 17 -hydroxysteroid dehydrogenase (17 -HSD). The contents/activities in testicular microsomes of microsomal mixed function oxidases (MFOs), namely, cytochrome P-450, cytochrome b5, NADPH cytochrome (P-450) C reductase, and NADH-cytochrome b5 reductase was determined, due to reports of their possible actions in testicular steroidogenesis. Additionally, the cytosolic enzyme, glutathione (GSH)-S-transferase in testes of treated animals was studied to evaluate cellular toxicity of endosulfan treatment.

Forty eight male Wistar rats were divided into three subgroups (A, B and C). Group A was further divided into 3 groups of six rats, the first group receiving vehicle control (ground nut oil), the second group endosulfan at 7.5 mg/kg bw via oral gavage, and the third group 10 mg/kg bw for 15 days. Group B received identical treatment as group A for 30 days. Group C was divided into two subgroups, half of which served as controls and the other half receiving endosulfan at 10 mg/kg b.w. for 30 days followed by a normal dietary regime for 7 days.

Animals were sacrificed on the 16th, 31st and 38th day respectively.

Following sacrifice the testes were removed, subcellular fractions were prepared, and the appropriate enzyme assays performed. Radioimmunassays were perfomed to determine levels of plasma gonadotrophin and testosterone, FSH, and LH.

Treatment with endosulfan did not affect body weight or testicular weights and there was no alteration in testicular cytosolic or microsomal protein contents.

The plasma FSH, LH, and testosterone levels were significantly ($P<0.05$) reduced in rats treated for 15 and 30 days at both dose levels. Plasma testosterone and testicular testosterone levels at the lower dose of 7.5 mg/kg were not significantly reduced after 15 days of treatment.

A significant inhibition of 3 –and 17 -HSDs in the testes of treated animals occurred at 30 days of treatment. A significant decrease in the contents/activities of microsomal cytochrome P-450 and related mixed function oxidases (MFOs) in the testes of treated animals was observed, along with a marked inhibition in the activity of glutathione-S-transferase at both dose levels. These changes were reversed when endosulfan was withdrawn, however, the testicular testosterone levels remained significantly reduced.

10.4 Endocrine Disruption Studies

10.4.1 E-Screen Study

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.

A bioassay (referred to as the E-screen) was used to assess the oestrogenic effects of several pesticides, namely; o,p-DDT, chlordecone, endosulfan, DDT, dieldrin and toxaphene using a human breast cancer oestrogen-sensitive MCF7 cell line. The test compares the cell yield achieved after 6 days of culture in medium supplemented with 5% charcoal-dextran-stripped human serum in the presence (positive control) or absence (negative control), of oestradiol and with varying concentrations of chemicals which are suspected of being oestrogenic. Specifically, the oestrogenic activity of these chemicals was assessed by determining (1) the relative proliferative potency (RPP), ie., the ratio between the minimal concentration of oestradiol needed for maximum cell yield at 6 days and the dose of the test compound to achieve a comparable proliferative effect; and, (2) measuring the relative proliferative effect (RPE), i.e. 100x the ratio between the highest cell yield obtained with the chemical and with oestradiol. The RPE indicates whether the compound being tested induces a proliferative response quantitatively similar to the one obtained with oestradiol, i.e., if it is a full agonist (RPE=100), or a proliferative yield significantly lower than the one obtained with oestradiol; i.e. if it is a partial agonist.

The results revealed that the RPP for all the chemicals tested was 0.0001% of that of oestradiol. This suggested that they were very weakly oestrogenic, however, there was variation in cell yield with RPE's somewhat lower than that of oestradiol (o,p-DDT, RPE=86%; chlordecone, 84%; and endosulfan 81%). The RPE values of dieldrin (55%) and toxaphene (52%) were lower than those of endosulfan. The concentration range for oestrogenic activity was from 10-25 μM, and at higher concentrations cytoxicity was observed, thus excluding the possibility establishing at higher concentrations whether full oestrogenic activity could be obtained.

To further investigate whether mixtures of chemicals could in fact act cumulatively (i.e. when each chemical is present at levels lower than those needed to express oestrogenicity), a mixture of the 10 oestrogenic chemicals was administered to MCF7 cells at concentrations lower than that required to produce an oestrogenic effect when administered alone. While the authors interpreted the the results as “a significant proliferative effect”, there was no evidence of synergy, with the 10-chemical mixture causing a two-fold increase only in cell proliferation above that seen in the hormone-free control cells.

10.5 In vitro Cholinesterase Study

Müllner H (1989) Effects of endosulfan and aldicarb on rat brain acetylcholinesterase. Hoechst report dated 16 June 1989. Reference no: A43395. [Submission; AgrEvo]

To assess the effects of endosulfan on rat brain cholinesterase activity *in vitro*, a Wistar rat (sex and source unstated) was killed, and the brain (without cerebellum) was removed, washed, homogenized and centrifuged to obtain a preparation of acetylcholinesterase-rich membranes. The preparation was incubated with 10 µM solutions of alpha-endosulfan or aldicarb (source and purity not stated) in a 10% ethanol/Tris biffer solution for up to 75 minutes. Acetylcholinesterase activity was determined at 0, 5, 10, 15, 30, 45, 60 and 75 minutes.

Results

Neither the solvent control (10% ethanol/buffer) nor the endosulfan solution inhibited the cholinesterase activity, with mean activity in the range of 95-100% of baseline levels at each sample interval. The aldicarb positive control solution had a marked, time-dependent inhibitory effect on cholinesterase activity, with activity inhibited by about 15% compared with baseline levels after 5 minutes, and by greater than 80% at the 75-minute sample interval.

Under the conditions of this study, endosulfan did not inhibit brain cholinesterase activity *in vitro*.

11. HUMAN STUDIES

11.1 Poisonings

(a) Lehr (1996). Summary of intoxications with endosulfan. Clinical cases and poisoning incidents. AgrEvo report PSR 96/006, 12 March 1996. Hoechst document A56361 (AgrEvo 11303).

In this summary of human intoxications, the lowest reported dose that resulted in death in humans was 35 mg/kg body weight, and deaths have also been reported after ingestions of 295 and 467 mg/kg. Intensive medical treatment within one hour of endosulfan administration has been successful at doses of 100 and 1000 mg/kg, with clinical signs in these patient consistent with those seen in laboratory animals, dominated by tonoclonic spasms. In the case of the 1000 mg/kg dose, neurological symptoms requiring anti-epileptic therapy were still required one year after endosulfan exposure.

(b) Bernadelli & Gennari (1987). Death caused by ingestion of endosulfan. Journal of Forensic Science, Vol 32, July 1987, pp 1109-1112. (AgrEvo 11303)

In a fatal intentional poisoning case, a 55 year old female weighing 75 kg ingested 100 mL of a formulation containing 35% endosulfan. The interval between ingestion of the insecticide and death was approximately 1 h. The lethal dose was estimated to be 467 mg/kg body weight.

(c) Sauer, Jakober & Luft (1989). Suicidal intoxication with the insecticide endosulfan. Intensivmedzin, 26: 35-37 (1989). English abstract only. (AgrEvo 11303)

A 22-year old man was found unconscious thirty minutes after ingesting 7 g endosulfan, and was suffering from tonic-clonic cerebral seizures. High doses of benzodiazepine, methohexital, phenobarbital, and phenytoin did not influence the seizures, but administration with thiopental (4 g in 3 h) decreased the seizures, which stopped after 4 h. Symptomatic treatment resulted in a normal neurological status after decrease of the thiopental effect.

(d) Shemesh et al (1988). Survival after acute endosulfan intoxication. Clinical Toxicology, 26 (3&4), 265-268 (1988). Hoechst document A40161 (AgrEvo 11303).

In a case study of an attempted suicide, a 20-year old man ingested approximately 200 mL of Thionax formulation containing 30% endosulfan. Symptoms upon administration included unconsciousness and convulsions, with foaming from the mouth, skin cyanotic and diaphoretic, and pupils miotic and reactive to light. Intensive medical attention was needed to prevent the patient from dying, including the use of thiopental and carbamazepine to control repeated convulsions. A year after treatment, the patient's mentation was still severely impaired carbamazepine was required to prevent seizures.

The clinical course of the case was divided into several stages: The convulsive and haemodynamic instability stage (lasting 16 h, and consisting of hypoxia, due to alveolar hypoventilation and pulmonary oedema and extreme haemodynamic instability. Episodes of tachycardia, hypertension, mydriasis followed by cardiogenic shock).; The subacute pulmonary and convulsive stage (lasting two weeks, characterised by convulsions, recurrent aspiration pneumonias and need for mechanical ventilation) and; The slow recovery stage.

(e) Demeter J. et al. (1977) Toxicological analysis in a case of endosulfan suicide. Bull Environ. Contam. Toxicol., 18, 110-114.

Demeter J. Heyndrickx A. (1978) Two lethal endosulfan poisonings in man. J. Analyt. Toxicol., 2, 68-74.

Following a fatal intentional poisoning with endosulfan the stomach, small intestine, blood, liver, kidney and urine were analysed for residues of endosulfan. The commercial formulation ingested consisted of 12.4% alpha and 8.1% beta endosulfan with alpha/beta ratio of 1.53. A similar ratio was detected in the gastrointestinal tract while an increase in the ratio occurred in all other samples. The amount found were 2,610 ppm (alpha) and 1,900 ppm (beta) in stomach, 190 ppm (alpha) and 99 ppm (beta) in the small intestine, 12.4 ppm (alpha)

and 5.2 ppm (beta) in the liver, 0.06 ppm (alpha) and 0.015 ppm (beta) in blood, 2.48 ppm (alpha) and 1.8 ppm (beta) in the kidney and 1.78 ppm (alpha) and 0.87 ppm (beta) in urine. The combined effect of alcohol and endosulfan intoxication contributed to the death.

A further two toxicological analyses in lethal endosulfan poisoning cases indicated that, in man, endosulfan sulfate was the metabolite in liver, brain and kidney.

(f) Havaldar PV, Patil VD and Siddibhavi BM (1990) Prophylactic anticonvulsive therapy in endosulfan (pesticide) poisoning. Indian Paediatrics, 27, 1222-1224.

This brief report cited case reports of two female siblings (aged 2 years) who were admitted to hospital following endosulfan consumption (quantity unknown). Clinical signs of drowsiness, constricted pupils, secretions in the chest, and tonic seizures developed, but these symptoms were variable in intensity and not all of the above symptoms were seen in both children. Treatment was commenced with a loading dose of intravenous (iv) phenytoin (15 mg/kg), with iv gluconate (dose not stated) as an additional measure. Repeat doses of phenytoin (7 mg/kg) was administered every 8 hours. Both the children recovered after 24 hours.

(g) Grimmet WG, Dzendolet I and Whyte I (1996) Intravenous thiodan (30% Endosulfan in Xylene). Clinical Toxicology, 34, 447-452.

A case report of a 28 year old female admitted to an emergency department in an advanced state of epilepticus was reported. The patient had self administered by the iv route approximately 1 mL of Thiodan in a xylene solvent.

Seizures began 15 minutes after administration, and upon arrival at the hospital grand mal seizures were still in progress. Administration of midazolam (15 mg) and thiopentone (375 mg) given over 10 minutes controlled the seizures. Mechanical ventilation was required for 9 days, and cholestyramine (4 g) was administered by a nasogastric tube twice daily in order to interrupt the enterohepatic circulation of endosulfan.

The patient developed proximal myopathy secondary to rhabdomyolysis, renal failure (as evidenced by peak creatine kinase levels of 117,000 IU/L after 24 hours), and liver dysfunction (ALT and AST levels reaching 3136 U/L and 4622 U/L respectively, 36 hours after admission). The LDH levels had peaked 12 hours previously at 6040 U/L. Pulmonary complications and neurological complications were minimal with the patient making a full recovery over three months.

(h) Lo RSK, Chan JCN, Cockram CS and Lai FMM (1995) Acute tubular necrosis following insecticide poisoning. Clinical Toxicology, 33, 67-69.

A 72 year old man was admitted to hospital due to a suicidal ingestion of an unknown insecticide. On admission he was unconscious, febrile, exhibited tachycardia (110 bpm), muscle fasciculations, episodes of convulsions, and a Glasgow coma scale of 5/15. His pupils were equal and reactive to light, and there were no focal neurological signs. Intubation was performed and atropine 0.6 mg and pralidoxine 1 g were given iv because of suspected anti-cholinesterase poisoning. Blood haematology and chemistry tests were performed, the results of which were unremarkable other than an increased white cell count. The patient

subsequently regained consciousness within 2 hours of admission and was transferred to a medical ward. The bottle containing insecticide was later identified as endosulfan, although the concentration was not listed on the label.

Three days later he developed a fever and a non-productive cough. Intravenous antibiotics were commenced but he developed proteinuria with deteriorating renal function (plasma urea increased from 7.3 mmol/L to 16.7 mmol/L, and plasma creatinine concentration from 121 µmol/L to 189 µmol/L). Liver function was normal. Ten days after admission his plasma creatinine levels had risen to 242 µmol/L, and his renal function continued to deteriorate with oliguria and generalised oedema, and the patient died 10 days after his initial presentation.

Post-mortem findings showed inflammation of stomach and proximal small intestine, focal consolidation in lungs (consistent with reactive tuberculosis), cardiomegaly, congestive heart failure, pulmonary oedema and slight centrovenular congestion and prominence of bile canaliculi. Both kidneys were swollen and enlarged, and on microscopy extensive tubular necrosis was identified in the form of tubular dilatation, focal loss of epithelium, granular casts, and interstitial oedema. However, no inflammatory cells, glomerular or vascular abnormalities were found.

(i) Segasothy M and Pang KS (1992) Acute interstitial nephritis due to endosulfan. Nephron, 62, 118.

A 24 year old male drank half a glass of beer that has been accidentally contaminated with 1 mL of endosulfan. Six hours later he developed nausea, vomiting and dizziness followed by loss of consciousness for 2 hours. No tremors or convulsions occurred and the only physical abnormality appeared to be a rise in blood pressure (180/110 mm), however, the patient had a history of hypertension. Four days later the patient developed facial oedema and loin pain. There was no associated fever, rash, arthralgia, or inophilia.

The serum creatinine increased to a peak of 774 µmol/L and returned to normal levels 2 weeks later. A renal biopsy was performed and showed an acute interstitial nephritis with normal glomeruli. The serum creatine phosphokinase was normal and there was no myoglobinuria thus excluding rhabdomyolysis as a cause of renal failure.

Eight weeks after the patient was discharged the serum creatinine was 100 µmol/L.

(j) Blanco-Coronado JL, Repetto M, Ginestal RJ, Vicente JR, Yelamos F and Lardelli A (1992) Acute intoxication by endosulfan. Clinical Toxicology, 30, 575-583.

Six case reports of patients with endosulfan poisoning were reported. Three men (cases 2,3,5) and three women (cases 1,4,6) average age 35 years (range 20-53 years) ingested endosulfan in unknown quantities. Case 1 had suicidal intent and the other five were family members who had eaten contaminated cake made by one of them (case 6). All six patients had nausea, vomiting, headache, dizziness and tonic-clonic convulsions beginning at 2.7+-0.5 h (range 1-4 h) after ingestion. Following the seizures, all patients had metabolic acidosis and hyperglycaemia.

The patients were treated with gastric lavage with activated charcol and iv sodium bicarbonate and diazepam which rapidly corrected the acidosis and hyperglycaemia. Decreased platelet counts were found at 4 hours post admission. Five patients required mechanical ventilation.
One female patient developed renal failure and had elevated SGOT and SGPT levels. She went on to develop anuria, metabolic acidosis, prolonged prothrombin time with thrombocytopenia. The patient died 8 days after admission. Postmortem findings included lung pathology, areas of renal tubular necrosis in the kidney, dilatation and congestion of hepatic sinusoids.

SUMMARY

Introduction

Endosulfan is a synthetic dioxathiepin cyclodiene compound used in agriculture to control a range of insects and mites on a broad spectrum of crops. Endosulfan has been available in Australia for over 30 years and over this period has been used in the home garden (currently not permitted), commercial food crops, and other crops such as cotton. There are some 18 end use products (EUPs) of endosulfan with over 400 approved (registered) uses Australia wide. Endosulfan has established maximum residue limits (MRLs) in a wide range of food crops.

In Australia, the current Acceptable Daily Intake (ADI) is 0.007 mg/kg/day, based on a no-observed-effect-level (NOEL) of 0.7- 0.75 mg/kg/day, established in a 1-year dog dietary study, a 13-week dietary rat study, and rat reproduction and developmental studies, and using a 100-fold safety factor. Endosulfan is in Schedule 7 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP), for “Poisons which require special precautions in manufacture, handling, storage or use, or special individual regulations regarding labelling or availability”.

Endosulfan is one of some 80 agricultural and veterinary chemicals identified as candidates for priority review under the Existing Chemicals Review Program. Following data call-in processes, a number of additional data submissions on the toxicology of endosulfan have been received from industry and the public. These data, together with all previously submitted data, have been evaluated and are detailed in this report below.

Metabolism and Toxicokinetics

When radiolabelled endosulfan was administered to mice as a single gavage dose (4 mg/kg), single dietary dose (4.7 mg/kg), or 21 day dietary administration (2.4 mg/kg/day), recovery of the radiolabel was predominantly via the faeces, with a smaller amount excreted in the urine. Within three weeks after cessation of treatment, total recovery of the radiolabel was 87% - 100% of the administered dose, and recovery did not differ greatly between the various dosing regimes. Whilst biliary excretion was not studied, oral absorption would appear to be moderate to high. Three weeks after the final test material administration,

tissue residues were greater in those animals on the 21 day feeding study, with the highest residues remaining in the livers (about 2 ppm) and spleen (about 1.4 ppm) at this time. Residues in the kidneys and fat were generally low, even after repeat administration, and endosulfan residues did not accumulate in the tissues following oral or dietary administration of the radiolabelled test material. (Christ and Keller, 1968)

When administered to male Balb/c mice at a single dose level of 0.3 mg/mouse, endosulfan (and its two isomers) was not completely absorbed from the gastrointestinal tract but was excreted, along with the metabolites endosulfan sulfate and diol, in the faeces. Only the diol metabolite was excreted via the urine while the sulfate metabolite was the only form of endosulfan found in tissues, with relatively large amounts were found in liver, small intestine and visceral fat, and trace amounts in muscle and kidney. When fed to Balb/c mice at dietary levels of 10 ppm for up to 49 days, the sulfate metabolite was detected in the liver and visceral fat of all mice tested. Both isomers and the sulfate and diol metabolites of endosulfan were detected in the faeces, while the only endosulfan product detected in the urine of these animals was the diol metabolite. Following a single dose of ^{14}C-labelled endosulfan to Balb/c mice of up to 0.3 mg/mouse, approximately 65% of the radiolabel was recovered; based on the radioactivity/g of tissue and excreta, the faeces accounted for the highest levels followed (in rank order) by visceral fat > urine > small intestine > kidney > brain > respired CO_2 > blood. (Deema et al., 1966)

After completion of a 24-month feeding study in mice (Donaubauer, 1988), the levels of endosulfan and its main metabolites endosulfan-hydroxyether, -sulfate, -lactone, and -diol were investigated in the liver and kidneys of the animals. No parent compound was detected in either liver or kidney samples. In animals administered endosulfan at 18 ppm in the diet, the levels of the hydroxyether, lactone and diol metabolites were at or below the level of detection (0.02 ppm), while the endosulfan-sulfate concentrations were 0.1-0.2 ppm (kidneys) and 0.7-1.1 ppm (liver). At a dietary concentration of 2 ppm the endosulfan-sulfate concentrations were 0.2-0.4 ppm in kidneys and 0.06-0.07 ppm in the livers, and at a dietary concentration of 6 ppm, the kidney residues were 0.04 ppm, and liver residues were 0.12-0.45 ppm. (Leist, 1989)

Following administration of ^{14}C endosulfan via oral or intravenous routes to male and female Wistar rats at doses of 2 or 0.5 mg/kg, respectively, excretion was extensive, with greater than 80% (intravenous) or 90% (oral) of the administered dose eliminated in the urine and faeces within the seven days after dosing. The urinary and faecal elimination half-lives for males and females were biphasic, with the earlier $t_{1/2}$ of less than 14 h, and the latter $t_{1/2}$ ranging from 33 to 67.5 h. However, excretion was relatively rapid, and essentially complete within the first 1-2 days. Urinary elimination was greater in females than males with both routes of administration, with 11-13% excreted in the urine of males compared with 2-24% of radiolabel excreted in the urine of females. Faecal elimination was 65-82% in males, and 60-72% in females (iv-oral). The highest tissue concentrations were found in the kidneys (1.8 ppm) and liver (0.23 ppm in males; 0.48 ppm in females), and retroperitoneal fat in females (0.16 ppm). The endosulfan residues were below 0.1 ppm in all other examined tissues. Based on comparison between intravenous and oral AUC data, the absorption of endosulfan was estimated to be 60-70%; by comparison of elimination of radiolabel, the absorption was estimated to be about 90%. (Kellner& Eckert, 1983; Stumf & Lehr, 1993)

^{14}C-Endosulfan (α - or β - isomers) was rapidly excreted by female rats following single oral administration of 2 mg/kg, or via dietary administration at doses of 5 ppm. After single oral administration, greater than 85% of the administered radiolabel was excreted within 120 h (>70% after 48 h), mainly in the faeces, and to a lesser extent in the urine. After dietary administration for 14 days, followed by a 14 day recovery period, recovery of the radiolabel was > 72% of the administered dose. Biliary excretion of radiolabel in male rats administered 1.2 mg/kg endosulfan as a single dose approached 50% for the α -isomer, and 30% for the β -isomer. There appeared to be little enterohepatic circulation from the bile. Tissue residues were generally greatest in the kidneys and liver, with smaller residues detected in other tissues and fat. After 14 days off the treatment, tissue residues were confined to the kidneys and, to a lesser extent, the liver, with a half life of about 7 days for the kidneys, and 3 days for the liver. Most of the identifiable radiolabelled compounds in the excreta and tissues were very polar, and no bioaccumulation in the fatty tissues was found. (Dorough et al., 1978)

Male Sprague-Dawley rats (24/group) were dermally exposed to ^{14}C labelled endosulfan at 0.10, 0.76 and 10.13 mg/kg without washing. Four animals from each group were sacrificed at 0.5, 1, 2, 4, 10 or 24 h and radioactivity was determined in the collected excreta and various organs and tissues. There was no skin irritation at the application site. Absorption of the dose into the skin was rapid and substantial at all doses but movement through the skin was slow and the 0.10, 0.76 and 10.13 mg/kg groups recorded respectively 73.0%, 73.0% and 88.8% of the absorbed dose still bound to the skin at 24 h. At 10 h each dose group had excreted less than 1% of the applied dose. At 24 h the excretion was ca. 11%, 10%, and 4% of the applied dose for the 0.10, 0.76 and 10.13 mg/kg groups respectively. The percentage absorption of applied dose ranged from 21.5% at a dose of 0.10 mg/kg, to 8.4% at a dose of 10.13 mg/kg. (Craine, 1986)

Female Sprague-Dawley rats (16/group) were treated dermally for 10 h with ^{14}C labelled endosulfan at 0.09, 0.98 and 10.98 mg/kg. Four animals from each group was sacrificed at 24, 48, 72, or 168 h after the dose application and radioactivity was determined in the collected excreta and various organs and tissues. There was no skin irritation at the application site, and no signs of systemic toxicity. Recovery of radiolabel ranged from 84-115%. Movement through the skin was rate limiting but was almost complete by day 7 with little label remaining at the application site. By 168 h only 45%, 46% and 20% of the applied dose had fully penetrated the skin for the 0.09, 0.98 and 10.98 mg/kg dose groups respectively. Excretion peaked between 24-48 h, with faeces accounting for about two thirds of the label. Total residues at 168 h, present mainly in liver and kidney, were 2.5%, 2.3% and 1.3% of the applied dose for the 0.09, 0.98 and 10.98 mg/kg dose groups respectively. (Craine, 1988)

Following oral administration of endosulfan to lactating goats at a dose of 1 mg/kg/day for 28 days, the tissue residues were generally low, with the highest tissue concentrations detected on the first day after cessation of treatment being 0.29 ppm in kidney, 0.2 ppm in the gastrointestinal tract, and 0.12 ppm in the liver. Kidney endosulfan residues were increased one week after treatment, reaching 0.49 ppm on day 8 after treatment, but no tissue residues were detected 21 days after treatment ceased. Endosulfan residues did not accumulate in the fat, with tissue concentrations reaching 0.06 ppm on day one after

treatment ceased, but no residues were detected in the fat by day 8 after treatment. (Indraningsih, McSweeney & Ladds, 1993)

When milk cows were fed a combination of endosulfan isomers (5.0 ppm) and endosulfan sulfate (5.0 ppm) in their diets, daily for 30 days, endosulfan sulfate was the only residue detected in the milk in amounts ranging from 0.01 to 0.16 ppm. The sulfate was also detected in fat (0.89 ppm), liver (0.63 ppm) and kidney (0.07 ppm) tissue samples of cows killed immediately following treatment. (FMC Corporation, 1965)

When a single oral dose of ^{14}C-labelled endosulfan was administered to two lactating East Friesian sheep at a dose level of 0.3 mg/kg, the amount of radiolabel in the blood peaked after 24 h with levels equivalent to 0.07 µg endosulfan/mL. Over a 17 day period the total amount of radiolabel eliminated in milk for each sheep was 0.37% and 1.82%, respectively, of the administered dose. Excretion of radiolabel was mainly via the urine and faeces, with 41% of the radiolabel administered eliminated via the urine and 50% of the administered radiolabel eliminated via the faeces. It was determined that approximately half of this 50% was unmetabolized endosulfan. The organs and tissues of the sheep killed after 40 days revealed concentrations of 0.02-0.03 µg endosulfan/g in fat, kidney and liver. All remaining tissues had considerably lower levels. Total radioactivity found in organs and tissues accounted for less than 1% of the administered label. (Gorbach et al., 1965)

In pigs fed endosulfan (2 ppm) in their diets for up to 81 days, endosulfan was detected in fatty tissue at levels of 0.07, 0.09 and 0.04 ppm after 27, 54 and 81 days of treatment, much less than the residues seen after administration of DDT (7 ppm), where residues in fatty tissues were 8.3, 9.1 and 9.7 ppm DDT after 27, 54 and 81 days treatment, respectively. Liver and muscle contained about 15 fold less DDT residues than found in fat. Thus, while endosulfan was found in fatty tissues it does not appear to bioaccumulate as does DDT. (Maier-Bode, 1966)

The systemic absorption of endosulfan in the 96 h following dermal administration of single doses (2.2-3.0 mg/kg) of aqueous suspensions of ^{14}C-endosulfan (94.6% pure) for a 10-hour exposure period to shaved skin in two Rhesus monkeys was determined to be 22% of the administered dose. An additional 11% of the administered dose remained in the skin, 10.5% was found in the carcasses, and 4.3% and 3.7% of the administered radioactive dose was excreted in faeces and urine, respectively. However, only 50% of the administered dose was recovered in this study, and thus the absorption figures calculated in this study may not be an accurate indication of the extent of dermal absorption of endosulfan. A plateau of blood and plasma levels was reached at 36 h, and there may not have been significant additional dermal absorption after this time. Levels in the liver, kidneys and fat tissue were highest (0.478, 0.083, and 0.233 ppm, respectively), while there are negligible levels in the brain. (Lachmann, 1987)

The penetration of endosulfan through rat and human skin was studied *in vitro*. The test material consisted of radiolabelled endosulfan formulated as an emulsifiable concentrate (containing 353 g/L endosulfan), which had been diluted to concentrations ranging from 0.4 to 4.0 mg/mL in water. The test material was applied at nominal doses of 0.01, 0.1 and 1 mg/cm^2 to rat and human skin mounted in dermal penetration cells, the rate of penetration was determined. The penetration rate for rats was, on average, 4.3 times that of humans.

The percentage of the applied dose varied with concentration with 61% of the lowest dose applied to human skin penetrating (96% in the rat) and 20% of the highest dose penetrating (40% in the rat). When the skin was washed 10 h after application, the amount of endosulfan penetrating decreased to 4% in the human and 9% in the rat. Endosulfan passing through human skin was metabolised or degraded to a greater extent than that passing through rat skin. (Noctor & John, 1995)

Acute Toxicity

Endosulfan has high acute toxicity in experimental animals, with wide variation in the LD50 of endosulfan depending on the route of administration, species, chemical specification of the test material, dosing vehicle and sex of the animal. Females were generally more sensitive to the acute toxicity effects of endosulfan than males, often by one order of magnitude or more. In many older toxicity studies, the chemical identity of the test material, including impurities, stabilisers and metabolites, was often poorly characterised. The oral LD50 for endosulfan in rats ranged from 9.6 to 160 mg/kg, and in mice from 13.5 to 35 mg/kg. However, the lowest oral LD50 for rats, using technical endosulfan known to conform to current FAO specifications, was 22.7 mg/kg. the lowest dermal LD50 in rabbits was 106 mg/kg, and in rats was 290 mg/kg, but the lowest dermal LD50 using current FAO specification endosulfan was 500 mg/kg in female rats, and >4000 mg/kg in male rats In a 4-hour, whole body inhalational study the lowest LC50 was 13 mg/m^3 in female rats. The clinical signs of intoxication include piloerection, salivation, hyperactivity, respiratory distress, diarrhea, tremors, hunching and convulsions. The isomers of endosulfan also show acute oral toxicity profiles similar to that of technical endosulfan.

The acute toxicity of formulations containing endosulfan was dependent upon the concentration of the active ingredient in the end use products, and was similar to that seen following administration of the active ingredient.

Like endosulfan, the toxicity of the metabolites varied depending upon vehicle and species used. In general the toxicity of the metabolites were similar to or lower than the parent compound, except for endosulfan diol which has low acute oral toxicity in the mouse. The clinical signs of intoxication were similar to that of the parent compound and included piloerection, salivation, hyperactivity respiratory distress, diarrhea, tremors, hunching and convulsions.

Endosulfan was not an eye irritant in rabbits, while the 33.7% emulsifiable concentrate formulation of endosulfan was a severe eye irritant to rabbits. Endosulfan was a slight skin irritant in rabbits, while the emulsifiable concentrate formulation containing 33.7% endosulfan was a moderate skin irritant to rabbits. Endosulfan was not a skin sensitiser to guinea pigs.

Phenobarbital administration proved to be an effective therapeutic measure against an absolute lethal dose of endosulfan in rats, with reductions in the clinical signs of intoxication and in the mortality rate. Diazepam did not have a therapeutic effect against endosulfan intoxication in rats.

Short Term, Repeat Dose Toxicity

Technical endosulfan was applied to the shaved nape skin of Wistar rats 21 times over a 30 day period (5 days/week) in solutions in sesame oil, for 6 h periods under an occlusive bandage (Ebert, Weigand & Kramer, 1985). For males, the dose levels were 0, 12, 48, 96, and 192 mg/kg/day, and for females the doses were 0, 3, 6, 12, and 48 mg/kg/day. In males, the no observed effect level was 96 mg/kg/day, based on mortality and clinical signs of intoxication (tremors and/or hypersalivation) at 192 mg/kg/day. In females, clinical signs of intoxication were observed at doses of 12 mg/kg/day and above, while mortality was mainly confined to the group receiving 48 mg/kg/day. However, at 3, 6, and 12 mg/kg/day, single animals also died, possibly due to poor application technique. As such, the sponsors followed this study immediately with another study using a modified method of test material application, to determine whether the mortality seen at the lower doses in females was related to treatment, and the report of that study is summarised immediately below.

Technical endosulfan was applied to the shaved nape skin of Wistar rats 21 times over a 30 day period (5 days/week) as solutions in sesame oil, for 6 h periods under an occlusive bandage, and the dose levels were 0, 1, 3, 9, 27, and 81 mg/kg/day, with 6 animals/sex/group (males only at 81 mg/kg/day). The males that died at 9 mg/kg/day had reduced or immature testes and/or sex organs, and the livers of these animals has accentuated lobular markings. The study investigators reason that these effects resulted from a non-substance-related developmental disturbance already present prior to treatment. No mechanism has been proposed as to the cause of such an effect. The no observed effect level (NOEL) for this study is 9 mg/kg/day based on mortality in females at 27 mg/kg/day (Ebert, Leist, Kramer, 1985)

Endosulfan was administered to male albino rats by gavage at a dose level of 11 mg/kg/day for 30 days. A vehicle control group received peanut oil over the same treatment period. In addition, the possible interaction between endosulfan and the chemosterilant, metepa, was investigated with further groups of animals receiving either metepa alone (30 mg/kg/day for 30 days) or in combination with endosulfan at the above mentioned dose levels. There were 3 deaths in the endosulfan-treated group, one of which showed signs of endosulfan induced toxicity. Endosulfan administration produced no significant changes in organ weights or body weight, did not alter clinicochemical parameters and was without histopathological effects. When administered in combination with metepa, no potentiation of toxicity was seen.(Nath et al.,1978)

Rats were exposed to endosulfan technical for a 29-day inhalation toxicity study for 6 h/day, 5 days/week with a total of 21 exposures. The concentrations tested were 0.5, 1.0 and 2.0 mg/m^3 air. Except for one male rat from the high dose group, which showed clinical signs of emaciation, pale skin and high-legged position, no other clinical signs were seen in any of the treated animals. No neurological disturbances, impairment of dental growth or changes in the oral mucosa were seen within the treated groups. Body weight gain tended to be depressed in the high dose males from day 20 of exposure period until the end of the recovery period. No other changes in body weight gain or food consumption were seen. Transient, non-dose related increases in RBC and haemoglobin levels were seen; levels were reported to be within normal ranges for this strain of rat. Apart from a transient non-dose related increase in creatinine and decrease in SGOT levels in high dose females, no treatment-related changes in clinicochemical parameters was noted. No

pathomorphological changes were seen in any of the test animals. No treatment related toxicity was observed at 2.0 mg/m^3. (Hollander et al.,1984)

Sub Chronic Toxicity

In a 13-week dietary study in CD-1 mice, animals received endosulfan at 0, 2, 6, 18 or 54 ppm for 3 months, equivalent to doses of 0, 0.24/0.27, 0.74/0.80, 2.13/2.39, or 7.3/7.5 mg/kg/day for males/females, respectively. Clinical signs attributable to treatment consisting of convulsions and salivation were seen in one male and one female from the high dose group. There was a marked treatment-related decrease in the survival rate of male and female animals from the high dose group. The mean food intake of males and females receiving the highest dose was significantly reduced for the first 2 weeks of the study, and mean bodyweight gain was reduced in high dose males during the first week of treatment only. The NOEL was 18 ppm (2.13 mg/kg/day for males and 2.39 mg/kg/day for females), based on clinical signs (convulsions, salivation), decreased survival, and increased serum lipids seen at 54 ppm (7.3 to 7.5 mg/kg/day). (Barnard et al.,1984)

In a 12-month interim report of a lifetime dietary study in mice, endosulfan technical was administered daily at dietary levels of 0, 10, 30, 100 and 300 ppm, equivalent to 0, 1.17, 4.08, 15.2 and 41.7 mg/kg/day in males and 0, 1.41, 4.74, 13.5 and 42 mg/kg/day in females. There were no apparent treatment related clinical signs or deaths. In the high dose males, a small but significant decrease in mean corpuscular volume was noted, and some transient, non dose related increases in haemoglobin, haematocrit and eosinophils were seen in males at 30 ppm. Clinical chemistry changes consisted of a significant decrease in SGOT in males at 100 and 300 ppm and a decrease in bilirubin in high dose males. Organ weight changes were confined to a dose related increase in the relative adrenal weights in females; this was significant at 300 ppm. There were no treatment related effects on gross pathology, and histopathological effects consisted of dose related granulomatous changes in the liver and lymph nodes. In the liver, granuloma, giant cell infiltration and/or large histocytic cells filled with brown pigment were found in treated mice; these effects were significant in the high dose groups (100 and 300 ppm). In lymph nodes, giant cell infiltration and/or reticuloendothelial cell proliferation were found in the 100 and 300 ppm groups but not at lower dose levels. The NOEL is 30 ppm (4.1 mg/kg/day in males; 4.7 mg/kg/day in females), based on histological findings in the liver and lymphatic system at 100 ppm (13.5 mg/kg/day in females; 15.2 mg/kg/day in males) and 300 ppm (42 mg/kg/day, males and females). (Arai et al.,1981).

In a 13-week dietary study in rats (Barnard et al.,1985) endosulfan technical was administered via the diet at 0, 10, 30, 60 or 360 ppm for 3 months, with some animals maintained for an additional 4 week recovery period. Slight, but statistically significant, dose related reductions in RBC and Hb count were seen in both sexes (at 30 ppm and above in males, and at 60 ppm and above n females), and an increase in MCV was seen in males at 30 ppm and above, and at 60 ppm and above in females. These haematological parameters were within normal historical control ranges. In males at 360 ppm, a number of reversible findings were reported following urinalysis examination, including increased urine volume and urinary protein levels, and decreased specific gravity. Pathologic examination revealed enlargement of the liver at 360 ppm and of kidneys at 60 ppm and 360 ppm, in males only, and increases in absolute liver, kidney and epididymides weights of males, and liver and kidneys in females. The kidney weights remained significantly elevated in the male rats at

the end of the withdrawal period at 360 ppm. Histopathological examination revealed traces of brown pigment in scattered hepatocytes in male rats, and minimal centrilobular enlargement of hepatocytes in females at 360 ppm. These changes were not observed in rats at the end of the withdrawal period. Yellowish discolouration of the kidney proximal tubule cells was seen in males at 10 ppm to 360 ppm, and at 30 to 360 ppm in females, with the degree of pigmentation increasing in a dose-related manner, but no cell death was associated with this finding. In addition, granular pigmentation was seen in proximal tubular cells in males at 60 and 360 ppm. The discolouration of the kidney tubules in male rats decreased at the end of the withdrawal period, but trace and minimal pigmentation were still seen at doses at and above 30 ppm. In females, traces of pigmentation persisted at and above 60 ppm. In addition, males at 360 ppm had yellow coloured protein aggregation in the proximal convoluted tubules and intracytoplasmic eosinophilic droplets in the tubules. The increase in incidence and severity of the yellowish discolouration of proximal tubular cells appears to be treatment related, with no control animals displaying these effects, but this effect alone does not appear to be toxicologically significant, with no adverse effects associated with these findings alone. At 30 ppm, all animals displayed either trace or minimal discolouration, and at this dose level, signs of granular/clumped pigment were not observed during the treatment period, but traces of pigment were seen at a low incidence during the recovery period, in males only. At doses of 60 and/or 360 ppm, when the pigmentation was present, other treatment related effects were also seen, including enlarged kidneys and centrilobular hepatocyte enlargement. No treatment related increase in the incidence of other kidney related effects were reported at 10 ppm. The NOEL was 30 ppm (1.92 mg/kg/day) based on increased kidney weights and granular pigment formation in kidney proximal tubule cells at 60 ppm (3.85 mg/kg/day) and above.

Chronic Toxicity

Male and female B6C3FI mice were administered endosulfan technical via the diet for 78 weeks, with the low and high time-weighted average concentrations being 3.5 and 6.9 ppm for the males, and 2 and 3.9 ppm for the females. There were no definite compound-related effects on appearance or behaviour in any of the treated groups, and body weights in both males and females were unaffected by treatment**.** There was an increase in the mortality rate with high dose males early in treatment. No treatment related neoplastic lesions were seen in the study. The NOEL for female mice was 3.9 ppm (0.58 mg/kg/day). Due to the high early mortality, no conclusion as to the oncogenic potential of endosulfan in males could be drawn. No non-neoplastic changes in the kidneys or sex organs of male and female mice could be atrributed to treatment with endosulfan. (Powers et al., 1978)

In a combined chronic toxicity/carcinogenicity study in NMRI mice, technical endosulfan was incorporated in the diet at concentrations of 0, 2, 6, and 18 ppm for up to 24 months, with 60 animals/sex/dose. The intake of endosulfan for males was calculated to be 0.28, 0.84, and 2.51 mg/kg/day, and in females was 0.32, 0.97, and 2.86 mg/kg/day, at dietary concentrations of 2, 6, and 18 ppm, respectively. Satellite groups of animals (10/sex/dose) were killed after 12 and 18 months. The behaviour and general health conditions of animals in this study were not affected by treatment with endosulfan. In male mice, reductions in body weight were seen throughout the study, and a statistically significant increase in mortality was observed in females, both at 18 ppm. 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 with endosulfan. At

the terminal sacrifice, no statistically significant changes in organ weights were seen in treated animals. On occasion, slight but statistically significant changes in organ weights were observed at the 12 or 18 month interim sacrifices (decreased lung and ovary weights in females at 12 months; decreased liver weights in males and ovary weights in females at 18 months) at the high dose level. Histopathological examination did not reveal any effects that were related to the administration of endosulfan. The no-observed-effect-level (NOEL) for this study is 6 ppm endosulfan (approximately 0.84 mg/kg/day in males and 0.97 mg/kg/day in females), based on decreased body weights in males (24-month sacrifice) and decrease organ weights (liver, ovaries, lung) in males and females at the 12 and /or 18 month interim sacrifices at 18 ppm (approximately 2.51 mg/kg/day in males and 2.86 mg/kg/day in females). No increase in the incidence of neoplastic or non-neoplastic lesions was observed in mice administered endosulfan at dietary levels of up to 18 ppm for 24 months. (Donaubauer, 1988, 1989)

Male and female Osborne-Mendel rats were administered endosulfan technical via the diet, with time-weighted average doses of 0 ppm (20/sex), 223 ppm (50 females), 408 ppm (50 males), 445 ppm (50 females) and 952 ppm (50 males) for 78 weeks with a return to control diets for a further 4 weeks. A highly significant morbidity rate was seen in male rats, and by week 54, 52% of the high dose rats had died. Due to the high mortality rates no conclusion could be drawn on analysis of tumour rates in male rats. A dose related reduction in body weights was found at all treatment concentrations in male rats. Histopathological examination revealed a high incidence of toxic nephropathy (>90%) in low dose and high dose males and females, but none of the control animals exhibited nephropathy. Chronic renal inflammation was observed in 40% of male controls, and the incidence of this effect doubled in treated males. 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. Some tubules had hyalin casts, and infrequent enlarged dark-staining regenerative tubular epithelial cells were observed. 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. The NOEL for male or female rats could not be established due to the renal effects observed at low dose in both sexes. Due to the high early mortality, no conclusion as to the oncogenic potential of endosulfan in male rats could be drawn. There were no treatment related neoplastic lesions seen in female rats and it may be concluded that endosulfan lacks oncogenic potential in this sex. Treatment related non-neoplastic changes in the kidneys of male and female rats and the testes of males were observed at the low doses of 408 ppm for male rats (approximately 20 mg/kg/day), and 223 ppm for female rats (approximately 10 mg/kg/day). (Powers et al., 1978)

In Sprague-Dawley rats administered endosulfan in the diet at up to 75 ppm (2.9-3.8 mg/kg/day) for two years, there was no evidence of increased carcinogenicity findings at any dose tested. Reductions in body weights and body weight gains were observed in males and females at 75 ppm, but no clinical signs of intoxication were observed at any treatment dose. No increase in mortality was observed in treated groups. 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. The increase severity of PGN suggests that the kidney is the target organ for endosulfan toxicity, although this is complicated by the fact that PGN is a common lesion in aging laboratory rats and occurs at a high incidence in control animals. The no-observed-effect-level (NOEL) for this study was 15 ppm (approximately 0.6 mg/kg/day), based upon the reduced body weights and pathological findings at 75 ppm (2.9 mg/kg/day). (Ruckman et al,.1989)

Endosulfan technical was fed to Wistar rats (25/sex/group) in their diets at dose levels of 0, 10, 30 or 100 ppm for 2 years. There were no treatment related clinical signs, and body weights were unaffected except for a non significant decrease in body weights and food consumption in the high dose males. Survival of treated female rats was reduced, with the deaths predominantly associated with respiratory infections. Upon necropsy, the testes weights of males from the 10 ppm group only were reduced by 7% with respect to controls at 104 weeks ($p<0.05$) and kidney weights were significantly ($p<0.001$) elevated by 16% in the high dose males at 104 weeks. 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. Microscopic cellular alteration, namely focal areas of hydropic cells,were seen in 50% of high dose males only at week 104. These hydropic cells were pale and swollen with the nuclei surrounded by a clear zone, and a few cells appeared to have eosinophilic cytoplasmic inclusions. No tumours occurred consistently or in a dose related manner , and under the conditions of this study, endosulfan lacked carcinogenic potential at doses up to and including 100 ppm. The NOEL was 30 ppm (1.5 mg/kg/day), based on kidney effects at 100 ppm (5 mg/kg/day). (Hazelton Laboratories, 1959a)

Technical endosulfan was administered in the diet to groups of Beagle dogs at dietary concentrations of 0, 3, 10, or 30 ppm for one year. These dietary concentrations were estimated to be 0, 0.23, 0.77, and 2.3 mg/kg/day, respectively. In addition, a group of animals (6/sex) was administered diets containing 30-60 ppm endosulfan, increasing in stages from 30 ppm (54 days) to 45 ppm (52 days) and 60 ppm (19-40 days) as the study progressed. Dogs that were administered 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. Some animals that were administered endosulfan at 30 ppm throughout the 12 month study were observed with violent muscular contractions of the abdominal muscles, and males at this dose level had reduced body weight gains throughout the study, and slightly reduced body weights in the latter stages of the study, compared with control animals. No other effects related to treatment were observed, and no increase in incidence of neoplastic or non-neoplastic lesions were observed in treated animals. Based on these clinical signs and reductions in body weights, the NOEL for this study is 10 ppm (calculated by the study facility to be equivalent to 0.65 mg/kg/day for males, and 0.57 mg/kg/day for females). (Brunk 1989, 1990)

Endosulfan technical was administered orally, via gelatin capsules, to adult mongrel dogs (2/sex/group) at dose levels of 0, 3, 10 and 30 ppm (0, 0.075, 0.25 and 0.75 mg/kg/day) on 6 days/week for one year. The group receiving 3 ppm was originally treated at 100 ppm for the first 3 days of treatment, but clinical signs of vomiting, tremors, convulsions, rapid respiration and mydriasis, salivation, tonic-clonic convulsions and rapid respiration in one male and both female dogs led to the dose being reduced to 3ppm for the remainder of the study. 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. The NOEL for this study was 30 ppm (0.75 mg/kg/day), based on clinical signs of intoxication observed at the initial high dose of 100 ppm (2.5 mg/kg/day). (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 for two mating generations, with two mating phases in each. These dietary concentrations were calculated to be 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, at 3, 15, and 75 ppm, respectively. Group sizes were 32/sex/group for F0, and 28/sex/group for F1B. No clinical signs or mortality related to endosulfan administration were observed during the study. Single mortalities occurred in the F0 females at 0, 3, and 15 ppm, and in F1B control females. Mating performance and pregnancy rates were not affected by treatment during the study. Statistically significant decreases in litter weight were seen on occasion, but there was no effect on the mean pup weights during the study, nor on the litter sizes. No treatment related effect on sex ratios was seen at any dose tested. Statistically significant increases in relative kidney weights were seen at 75 ppm in F0 and F1B males, and statistically significant increases in relative liver weights were observed in F0 males and females at 75 ppm, and in F1B females at 15 and 75 ppm. The effect at 15 ppm in F1B was not seen at this dose level in any other matings during the study, and is considered incidental to treatment. Yellowish discolouration of cells in the proximal convoluted tubules were observed in male F1B rats at 3, 5, and 75 ppm, and in female F1B rats at 75 ppm. The incidence and extent of this effect was dose related, with traces of discolouration seen at all treatment doses, and minimal discolouration seen in male rats at 15 ppm and 75 ppm only. Granular/clumped pigment were seen in proximal convoluted tubular cells in high dose males only. These findings were not associated with any histopathological evidence of renal damage at any dose level tested. While the increase in incidence of the cellular discolouration is related to the administration of endosulfan, these findings were not considered to be toxicologically significant, as they were not associated with any adverse effects on the cells, and the yellow pigment was considered likely to be endosulfan and metabolites being stored and metabolised in lysosomes prior to excretion (JMPR, 1989). The presence of the pigment is an indication of endosulfan exposure, rather than an index of toxicity. The no-observed-effect-level (NOEL) for this study was 15 ppm (approximately 1.0 mg/kg/day), based on the increase in liver and kidney weights at 75 ppm (approximately 6 mg/kg/day). 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 administered endosulfan from days 6-14 of gestation at doses of 0, 5, and 10 mg/kg body weight/day. No marked changes in behaviour or appearance were reported in females administered endosulfan, and body weights in treated animals were similar to those seen in controls. 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/day, and 22.8% at 10 mg/kg/day), and an increase in fetal mortality, though this effect was slight and was not dose related. Slight increases in the incidence of cerebral hypoplasia and enlargement of the renal pelvis were observed during visceral examination, but these effects were not considered to be related to treatment, as the magnitude of these increases was small, the effects were also seen in control animals, and the effects were not dose dependent. No other increases in visceral abnormalities were reported. Skeletal examination revealed a statistically significant increase in incidence of absent 5 th sternebrae, and in the incidence of fetuses with incomplete ossification. A slight increase in the incidence of absent 5th metacarpus, though not statistically significant, was also noted in treated animals compared with controls. 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. Under the conditions of this study, the administration of endosulfan to female rats at doses up to 10 mg/kg/day during organogenesis did not result in an increase in developmental effects in offspring. No maternotoxicity was evident at any dose level. The level of reporting in this published paper is not adequate for the purposes of defining an NOEL for developmental toxicity. (Gupta et al., 1978)

Technical endosulfan was dissolved in sesame oil and administered daily via oral gavage to female Wistar rats on days 7-16 of gestation, at doses of 0 (vehicle control), 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. At 6 mg/kg/day, four dams died, after 6-10 doses of endosulfan, and 3/4 of these animals displayed tonoclonic convulsions for several days prior to death. In the surviving animals, 13 had tonoclonic convulsions for a number of days, generally around day 10 of gestation. A number of these animals also displayed hypersalivation on a number of days during treatment. Statistically significant decreases in body weight and bodyweight gain were observed 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 fetal sex ratio was relatively balanced. No statistically significant increase in the incidence of abnormalities was observed in fetuses during examination. A single oedematous, retarded fetus in the 6 mg/kg group presented with brachygnathia superior with a relatively small alveolar cavity in the upper jaw combined with cleft palate, bending of both hind feet in the tarsal joint, wavy clavicles, and bent and shortened scapula. These findings were considered to be spontaneous in nature, given that no other limb or head defects were observed in any pup in any of the litters at this dose level. Skeletal examination revealed a statistically significant increase in fragmented thoracic vertebral centra at 6 mg/kg. This effect was considered to be treatment related, and reflects the frank maternotoxicity of endosulfan seen at the high dose level. No other significant skeletal abnormalities were seen at 6 mg/kg in this study. The NOEL for maternotoxicity in this study was 2 mg/kg/day, based on mortality and clinical signs (tonoclonic convulsions and hypersalivation) and decreased bodyweights seen at 6 mg/kg/day. The NOEL for developmental toxicity was 2 mg/kg/day based on increased incidence of fragmented thoracic vertebral centra seen at 6 mg/kg/day. No treatment related major malformations were observed in this study. (Albrecht & Baeder, 1993)

Mated CD Sprague Dawley rats (25/group) were administered endosulfan technical (in corn oil), 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 clinical signs including placidity, rough coat, alopecia and hyperactivity being observed. A dose-related decrease in maternal body weight gain was seen at 2 and 6 mg/kg/day. The number of implantations and litter size were unaffected by endosulfan treatment. There was a slight reduction in fetal weight and length in the high dose group. A non dose related reduction in the percent of live fetuses and an increase in the number of resorbed fetuses were seen at 2 mg/kg/day. No statistically significant treatment related effect on sex ratios was observed. No external variations or malformations were seen at 0.66 or 2 mg/kg/day. At the high dose, 5/405 fetuses exhibited lordosis and 6 fetuses had oedema. All five of the fetuses with lordosis (anteroposterior curvature of the spine), and 5/6 of the fetuses with oedema were from a single litter from the one dam (animal 109). One fetus (also from the same litter) had the skin of the upper forelimb webbed to the chest. No significant treatment-related effects were seen on soft tissue development. Common minor skeletal variations were present in all groups. The incidence of poorly ossified sternebrae (6th) in the high dose group was significantly greater than for the control group. Two fetuses had clubbed left hind limbs in the high dose group. The five fetuses from dam no 109 which had oedema and lordosis also had wide and thickened vertebral arches, ribs, and clavicles, and the clavicles were also shortened, curved, and twisted. Four of these fetuses had shortened pubes and two had an unossified hyoid bone. However, the incidence of these effects was generally under 1%, the effects were largely related to delayed development, and mainly confined to a single litter from a single dam that displayed numerous signs of intoxication related to endosulfan administration, including face rubbing, alopecia, flaccidity and hyperactivity, and the developmental effects are probably related to the maternotoxicity of endosulfan at the high dose level. The NOEL for maternotoxicity was 0.66 mg/kg/day based on decreases in body weight gain at 2 mg/kg/day and decreased body weight gain and clinical signs at 6 mg/kg/day. Evidence of delayed development and isolated low incidence of skeletal variations were seen at the maternotoxic dose of 6.0 mg/kg/day. Based on these effects, the NOEL for developmental toxicity was 2 mg/kg/day. (MacKenzie, 1980)

Mated New Zealand White rabbits (20-26/group) were administered endosulfan technical, 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 fetal weight and length and the number of live and resorbed fetuses were unaffected by endosulfan treatment. There were no dead fetuses in any of the treated or control groups. No gross external observations were reported. The only soft tissue anomaly occurred in 6/167 high dose fetuses and consisted of the left carotid arising from the innominate; 1/141 control fetuses also showed this abnormality. Common skeletal variations and minor anomalies occurred with a similar incidence in control and treated fetuses. Endosulfan did not produce any teratogenic or developmental effects even at the maternotoxic dose of 1.8 mg/kg/day. The NOEL of maternotoxicity was 0.7 mg/kg/day based on clinical signs seen at 1.8 mg/kg/day. (MacKenzie, 1981)

Genotoxicity

Endosulfan was negative for genotoxicity in a wide range of assays, both *in vitro* (with and without metabolic activation) and *in vivo*. Included were the following assays:

. Microsomal reverse mutation (Ames) test in *Salmonella typhimurium*, at doses up to 5000 µg/plate, with and without S9 (Shirasu et al., 1978);
. *In vitro* forward mutation assay in *Schizosaccharomyces pombe* at doses up to 500 µg/mL, with and without metabolic activation (Milone & Hirsch, 1984a);
. Mouse lymphoma forward mutation assay at doses up to 75 µg/mL without metabolic activation, and 100 µg/mL with metabolic activation (Cifone, 1984a);
. Mouse micronucleus test *in vivo* at doses up to 5 or up to 10 mg/kg (Jung, 1983; Müller, 1988);
. *In vivo* cytogenetics assay in rats at doses up to 55 mg/kg/day for 5 days (Dikshith & Datta, 1978);
. Induction of chromosomal aberrations in the Syrian hamster *in vivo* at doses up to 80 mg/kg (Dzwonkowska & Hübner, 1986);
. Chromosomal aberration assay in cultured human lymphocytes at doses up to 40 or up to 100 µg/mL with and without metabolic activation (Asquith, 1989a and 1989b; Instituto Di Ricerche Biomediche, 1986);
. Unscheduled DNA synthesis in rat hepatocytes at doses up to 100 µg/mL with metabolic activation, and up to 1000 µg/mL without metabolic activation (Müller, 1985), or up to 51 µg/mL (Cifone, 1984b);
. *Saccharomyces cerevisciae* gene conversion-DNA repair test at doses up to 5000 µg/mL, with and without metabolic activation (Milone & Hirsch, 1984b);
. Rec-assay using *Bacillus subtilis* at doses up to 2000 µg/disc (Shirasu et al., 1978).
. Dominant lethal test in Balb/c mice at a dose of 0.64 mg/kg/day for five days (Dzwonkowska & Hübner H, 1991).

Other studies

Immunotoxicology

In two published immunotoxicity studies, endosulfan was administered to male Wistar rats at dietary doses of up to 50 ppm for six weeks (Banarjee & Hussain, 1987), or up to 20 ppm for 22 weeks (Banarjee & Hussain, 1986) to evaluate the humoral and cell-mediated immune responses.

In the six-week study, a significant decrease in total serum antibody titre to tetanus toxoid occurred at 30 and 50 ppm endosulfan with a slight decrease (not statistically significant) at 10 ppm. The decrease was observed in both IgM and IgG levels at 50 ppm. Measurement of total gamma globulin content of rat serum again indicated suppression at 50 ppm. Rats exposed to endosulfan and subsequently immunised with tetanus toxoid showed a significant decrease in LMI and MMI responses in a dose-dependent pattern, the decrease becoming statistically significant at the 30 and 50 ppm level. These results indicate that both humoural and cellular immunity was depressed as a result of exposure to endosulfan at doses of 30 and 50 ppm. These effects were not seen at 10 ppm (0.5 mg/kg/day).

In the 22-week study, the specific response (serum antibody titre to tetanus toxoid) showed a marked decrease in rats exposed to 10 or 20 ppm endosulfan throughout the experiment in a dose-time dependent pattern. Cellular immunity was assayed by measuring migration

inhibition of activated leucocytes and macrophages. Endosulfan treatment diminished migration inhibition responses of both leucocytes and macrophages at the 10 and 20 ppm dose level throughout the study. The endosulfan-related effect on the immune system of the rat did not appear to be secondary to other toxicity since the body weights of the animals were unaffected by the treatment and endosulfan is not known to affect the hormonal system. The effects were dose and time related. The results of the other assays of immune responsiveness although less reliable followed a similar trend. Immune responses were unaffected by endosulfan treatment at 5 ppm (0.25 mg/kg/day).

It should be noted that the methodology used to assess cellular immunity in these studies is far from ideal as it is flawed by large inherent errors, lack of objectivity and, except in very experienced hands, lack of accuracy. Less subjective tests of cellular immunity eg. cytotoxic T cell response to a virus would have provided more reliable results.

Neurobehavioural studies

In a number of studies conducted by the same group of investigators, endosulfan was administered to rats at doses of 2 mg/kg/day for 90 days (Paul et al., 1993; Paul et al., 1994) or up to 6 mg/kg/day for 30 days (Paul et al., 1995), and behavioural and biochemical changes were determined. At doses which resulted in signs of frank toxicity (reduced body weights, reduced food consumption, mortality, increased intensity of tremors, increased liver enzyme activity), some changes in behaviour were noted, including increased motor activity, and inhibition of conditioned and unconditioned escape and avoidance responses.

Sperm abnormality studies

In studies on the genotoxicity of endosulfan in mammalian germ cells (Pandey et al., 1990), the genotoxicity potential of endosulfan technical was assessed *in vivo* using the dominant lethal and the sperm shape abnormality tests. Under the conditions of this study, the intraperitoneal administration of endosulfan to Swiss mice at doses of 16.6 mg/kg/day for five consecutive days resulted in an increase in the incidence in sperm abnormalities, along with decreased sperm counts and decreased testis weights. It is unclear whether the decrease in sperm count and increase in sperm abnormalities are biologically significant. It is unlikely that this increase in sperm abnormalities is causally related to any adverse effects on fertility or other reproductive parameters 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 administered endosulfan at doses of 12.7 mg/kg/day or lower. The lack of detail in this report is also noted.
To investigate the effect of pesticides on sperm morphology and sperm count, and to test the ameliorating potential of vitamin C on such effects, a number of compounds, including endosulfan, were administered to male Swiss albino mice (Khan & Sinha, 1996). Under the conditions of this study, the administration of endosulfan at a dose of 3 mg/kg/day resulted in an increase in abnormal sperm from 5 to 14%, and this effect was reduced slightly in animals also administered vitamin C. 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 were seen following the administration of endosulfan (80% reduced), and this effect was lessened in animals also administered vitamin

C. However, the test material was a 35% emulsifiable concentrate containing solvents, emulsifiers and stabilizers, and it is unclear whether these effects were due to endosulfan or to these non active constituents in the formulation.

In a study investigating endosulfan-induced biochemical changes in the testis of rats (Sinha et al., 1995), the authors postulated that endosulfan impairs testicular functions by altering the enzyme activities responsible for spermatogenesis, thus influencing intratesticular spermatid count, and resulting in low sperm production and increased sperm deformities. The data presented in this report supports the notion that the administration of endosulfan at relatively high doses (2.5 mg/kg/day and above) for several months resulted in an increase in activity of a number of enzymes found in the testes, and that at doses of 5 mg/kg/day and above, there was a marked reduction in sperm count (up to 47%) compared to controls. In the absence of historical control data, it is unclear whether the decrease in sperm count seen at 2.5 mg/kg/day (22%) was within normal biological ranges for the test animals. The reductions in other parameters (sperm abnormalities, spermatid count, sperm production), while statistically significantly different to concurrent controls, were only slightly reduced, and in the absence of consistent dose response relationships for these effects, it is considered that these effects are not biologically significant.

The biochemical toxicity of sub-chronic endosulfan treatments in relation to gonadal hormones was investigated, via plasma and testicular testosterone, plasma gonadotrophins (follicle stimulating hormone (FSH), and luteinising hormone (LH)), and 3B-hydroxysteroid dehydrogenase (3B-HSD), and 17B-hydroxysteroid dehydrogenase (17B-HSD). Animals received doses of endosulfan up to 10 mg/kg/day for up to 30 days. A significant inhibition of 3B-and17B-HSDs in the testes of treated animals occurred at 30 days of treatment. The plasma FSH, LH, and testosterone levels were significantly ($P<0.05$) reduced in rats treated for 15 and 30 days at both dose levels. Plasma testosterone and testicular testosterone levels at the lower dose of 7.5 mg/kg were not significantly reduced after 15 days of treatment. A significant decrease in the contents/activities of microsomal cytochrome P-450 and related mixed function oxidases (MFOs) in the testes of treated animals was observed, along with a marked inhibition in the activity of glutathione-S-transferase at both dose levels. These changes were reversed when endosulfan was withdrawn, however, the testicular testosterone levels remained significantly reduced. (Singh & Pandey, 1990)

Human studies

In general, characterisation of the dose of endosulfan in poisoning cases has been poor. In a summary of case reports of human intoxications (Lehr, 1996), the lowest reported dose that resulted in death in humans was 35 mg/kg body weight, and deaths have also been reported after ingestions of approximately 295 and 467 mg/kg, with death occurring within 1 h of administration in some cases. Intensive medical treatment within one hour of endosulfan administration was reportedly successful at doses of 100 and 1000 mg/kg, with clinical signs in these patient consistent with those seen in laboratory animals, dominated by tonoclonic spasms. In a case where the dose was 1000 mg/kg, neurological symptoms requiring anti-epileptic therapy were still reportedly required one year after endosulfan exposure.

DISCUSSION

Hazard

A comprehensive data package on endosulfan has been assessed in this review. Endosulfan 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, diarrhea, tremors, hunching and convulsions.

Long-term dietary studies in rodents indicated that endosulfan was without carcinogenic or genotoxicity potential in a range of tests. No adverse effect on reproductive parameters resulted from endosulfan administration. While evidence of delayed development was seen in rats, this was associated with maternotoxicity, and no treatment related teratogenicity was observed in any studies.

Persistence

Endosulfan is rapidly and comprehensively excreted after oral administration, and endosulfan does not bioaccumulate in the fatty tissues. Even after long term dietary exposure to endosulfan, very low residues were detected in body tissues, with the highest residues found in the liver (in several species), while in rats the kidneys had the highest residues. It seems that endosulfan would not be expected to significantly bioaccumulate in humans.

Renal Toxicity

In rats, the kidney appears to be the main target for endosulfan toxicity in a number of studies. Renal effects seen include increases in kidney weights and granular pigment formation in shorter-term administration, and progressive chronic glomerulonephrosis or toxic nephropathy after long term exposure to endosulfan. Granular pigmentation was not observed in rats following long term exposure, and it is possible that this is indicative of an adaptive changes in rats, with the pigment deposition considered likely to be endosulfan and metabolites being stored and metabolised in lysosomes prior to excretion. As such, the presence of the pigment is an indication of endosulfan exposure, rather than an index of toxicity, and in longer term studies in rats, where the induction of lysosomal enzymes is complete, the lysosomal degradation of endosulfan becomes effective and the test substance is fully eliminated, and thus this discolouration is not observed in the kidneys. In the longer term studies, an increase in severity of progressive chronic glomerulonephrosis also suggests that the kidney is the target organ for endosulfan toxicity, although this is complicated by the fact that PGN is a common lesion in aging laboratory rats and occurs at a high incidence in control animals. It is not clear whether the granular pigmentation is related to the longer term renal effects seen in the lifetime studies.

Testicular Effects

In a long term rat study, exposure to endosulfan at a high dose (20 mg/kg/day) resulted in testicular atrophy, characterised by degeneration and necrosis of the germinal cells lining the seminiferous tubules. In addition, decreases in sperm count, along with increases in the incidence of sperm abnormalities, have been reported in the open literature,again at high doses of endosulfan in rodents. These effects may be related to the frank toxicity of

endosulfan, and the functional significance of these findings is unclear, as reproductive and developmental studies in a number of species did not reveal any effect on reproduction indices (such as fertility) nor any increase in the incidence of defects or abnormalities in offspring. Given the high doses at which these effects were reported, it would appear that these effects are of limited significance to humans.

Immunotoxicity

The immunotoxicity potential of endosulfan was originally investigated in the mid-1980s, when it was noted that the tests used for estimation of immune status, with the exception of the specific antibody response to tetanus toxoid, were less than ideal. Whilst the effects of endosulfan on the rat immune system were reasonably convincing, it must be borne in mind that the studies were only generated by one group and used outdated methodology in some of the experiments. Despite these objections to methodology, the two reliable assays in the studies (ie. albumin/globulin (Ig) ratio and the specific Ig response to tetanus toxoid) clearly indicated reduced ability to mount an immune response to an antigen following subchronic exposure to endosulfan. These findings continue to be isolated, with no additional reports on the immunotoxicity potential of endosulfan available. In addition, the absence of other immunotoxicological findings in the large number of bioassays conducted with endosulfan suggests that endosulfan does not have an adverse functional effect on the immune status of laboratory animals.

Endocrine Effects

Several recent studies have reported that endosulfan, alone or in combination with other pesticides, may have some oestrogenic binding capability and therefore might be capable of disturbing the normal balance of the endocrine hormone system. To date, all available studies show only very weak binding to hormone receptors *in vitro*, and there is no evidence for any adverse physiological effects *in vivo*.

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 the endocrine hormone system.

NOEL considerations

To establish the lowest NOEL for endosulfan, a summary of the NOELs determined in those studies **deemed suitable** for regulatory decision making is shown below.

SPECIES/STUDY TYPE	NOEL	LOEL and toxic effect
Rat : 30 day dermal	9.0	Mortality in females at 27 mg/kg/day.
Rat (males): 30 day gavage	11.0	only dose tested (11 mg/kg/day) without effect.
Rat: 29 day inhalation	2.0*	highest dose tested without effect

Mouse: 13 week dietary	2.13 (males) 2.39 (females) (18 ppm)	Clinical signs (convulsions, salivation), decreased survival and increased serum lipids at 54 ppm (7.3-7.5 mg/kg/day)
Mouse: 12 month dietary (interim report)	4.1 (males) 4.7 (females) (30 ppm)	Liver and lymphatic system effects at 100 and 300ppm
Mouse 78 week dietary	0.58 (females) (3.9 ppm)	No effects at highest dose tested. No NOEL for males due to high early mortalities.
Mouse: 24 month dietary	0.84 (males) 0.97 (females) (6 ppm)	Decreased bodyweight in males, and decrease liver, ovary and lung weights at 2.5-2.8 mg/kg/day (18 ppm).
Rat: 13 week dietary	1.92 (30 ppm)	Changes in kidney weight and granular pigment formation in renal proximal tubules at 30 ppm (1.92 mg/kg/day).
Rat: 78 week dietary	none established	Kidney and testes effects seen at lowest dose tested of 408 ppm (20 mg/kg/day, for males) and 223 ppm (10 mg/kg/day for females)
Rat: 2 year dietary	0.6 (15 ppm)	Reduced body weights and kidney pathology at 2.9 mg/kg/day (75 ppm)
Rat: 2 year dietary	1.5 (30 ppm)	Kidney effects at 5 mg/kg/day (100 ppm)
Dog (beagle): 1 year dietary	0.65 (males) 0.57 (females) (10 ppm)	Clinical signs and reduced body weights at 30 ppm.
Dog (mongrel): 1 year PO	0.75 (30 ppm)	Clinical signs at 2.5 mg/kg/day (100 ppm)
Rat: 2 generation dietary	1.0 (15 ppm) 6.0 (75 ppm)	Increased kidney & liver weights at 6 mg/kg/day (75 ppm) No reproductive toxicity seen at highest dose tested.
Rat: PO teratology	2 (maternotox) 2 (fetotoxicity) 6 (teratogenic)	Clincial signs and body weight decreases seen at 6 mg/kg/day Increased incidence of fragmented thoracic vertebral centra at 6 mg/kg/day No teratogenic effects at the highest dose
Rat: PO teratology	0.66 (maternotox) 2 (fetotoxicity) 6 (teratology)	Decreased body weight at 2 mg/kg/day and clinical signs at 6 mg/kg/day Delayed devlopment and isolated skeletal variations at 6 mg/kg/day No teratogenic effects at the highest dose
Rabbit: PO teratology	0.7 (maternotox) 1.8 (teratology)	Clinical signs seen at 1.8 mg/kg/day No teratogenic effects at the highest dose

* LC50 value in mg/m^3.

The NOELs are of a similar magnitude in a range of species, with LOELs based upon body weight changes, clinical signs, and some kidney toxicity. The lowest NOEL is approximately 0.6 mg/kg/day.

. 0.58 mg/kg/day in female mice in a 78 week dietary study, with no effects seen at 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.

Determination of Public Health Standards

Acceptable Daily Intake

The current acceptable daily intake (ADI) is 0.007 mg/kg/day, based upon an NOEL of 0.7-0.75 mg/kg/day (established in a 1-year dog dietary study and rat reproduction and developmental studies), and using a 100-fold safety factor.

It is proposed that the ADI for endosulfan be 0.006 mg/kg/day, based on an NOEL of 0.6 mg/kg/day, and using a 100-fold safety factor.

Public exposure

Endosulfan is not currently registered for domestic use, and so the greatest potential for public exposure is via residues in food, or via incidental exposure (including spraydrift) or intentional poisoning.

Market Basket Survey

The 1994 Australian Market Basket Survey (AMBS) reports that endosulfan residues were detected in a range of foods, including apples, beans, blueberries, cabbage, capsicum, celery, peaches, pears, prunes, and pumpkin, all at levels well below the MRLs for these foods. In addition, endosulfan was detected in lettuce with a residue level of 2.5 mg/kg, while the MRL for endosulfan in vegetables is 2 mg/kg. In addition, there have been isolated reports of exceedence of MRLs in open leaf lettuce. Given the very short withholding period for some of these crops, such MRL exceedence may have an impact on the potential for public exposure via residues in food.

The Market Basket Survey estimated the daily intake of a range of pesticides based on the average energy intake. The highest exposure for endosulfan seen in the groups studied was in infants aged nine months, and was estimated at 0.1 µg/kg body weight.

Dietary Exposure Considerations

The 1994 Australia Market Basket Survey (AMBS) found mean endosulfan consumption values in the group with the highest consumption (infants aged nine months) to be 0.1 µg/kg/day, or 0.0001 mg/kg. Thus, based on actual Australian food consumption patterns (albeit now dated), the highest group mean dietary intake is approximately 60-fold lower than the ADI (or conversely, accounts for approximately 1.7% of the proposed ADI). Based on

these estimations, the potential for public exposure to endosulfan via residues in food would not appear to be of concern.

In estimating dietary exposures, the "Guidelines for Predicting Dietary Intake of Pesticide Residues (Revised)" circulated by the Codex Alimentarius Commission in November 1996, recommends the use of National Theoretical Maximum Daily Intakes (NTMDI) as an initial estimate, while admitting that these can produce a gross overestimation of the exposure for a number of reasons. The calculation involves the use of the MRL as an estimate of the amount of pesticide in the food, and national estimates of consumption for the quantity of food consumed. When using a default MRL value of 2 mg/kg for vegetables, following the NTMDI procedure for endosulfan, and using the Australian 1983 survey of average food consumption, it is calculated that a 70 kg individual would consume 0.012 mg/kg/day of endosulfan. This NTMDI is twice the proposed ADI of 0.006 mg/kg/day. This modelling estimate is significantly higher than the estimates produced by the Dietary Survey approach, and is not considered to give an accurate estimation of the dietary consumption of endosulfan.

Incidental Exposure Considerations

Following a report of a cluster of childhood leukemias in the region in 1986, an air monitoring study was conducted in 1986/87 which detected minute quantities of agricultural chemicals applied by air drift, but the levels were not considered to pose a risk to public health. Due to continued community concern about exposure to locally applied agricultural chemicals, the Queensland Government conducted an air-sampling study in 1990/91 in and around Emerald, a town in the Central Highlands of Queensland with a population of over 7000, and a population in the local authority area of approximately 10, 500. The township is surrounded closely on three sides by cotton farms.

Air-sampling equipment was established at four sites, and 24-hour samples were obtained during the spraying season, a period of 23 weeks. The results obtained were derived using default values that generally overestimate exposure. For example, a bioavailability of 100% was assumed; the exposure estimates assumed year-round spraying, when aerial application does not occur in this region for 6 months of the year; and, all sampling was conducted in the external environment. The standard values used in calculating daily intake were: 25 m^3 /day for adults (60 kg body weight), and 5 m^3 /day for infants (10 kg body weight). For endosulfan, a total area of 77 022 hectares were sprayed with 224 888 litres of spray. About 29% of samples obtained contained pesticide residues, of which 85% were endosulfan samples.

The maximum daily exposure in the Emerald urban community was 0.208 µg/kg body weight for adults, and 0.250 µg/kg body weight for infants. For the Emerald environs community, the maximum daily exposure was 0.375 µg/kg body weight for adults, and 0.450 µg/kg body weight for infants.

The highest figure of 0.450 µg/kg body weight for infants (0.00045 mg/kg/day), represents approximately 7.5% of the proposed ADI. Based on these exposure data, the highest estimated total incidental exposure to endosulfan does not pose a significant public health risk.

Safety Directions

In the Handbook of First Aid Instructions and Safety Directions, the current Safety Directions are: EC LD ULV all strengths 130 131 132 133 210 211 220 223 279 280 281 282 290 292 294 301 340 342 350 360 361 364 366

First Aid Instructions

In the Handbook of First Aid Instructions and Safety Directions, the current First Aid Instructions are: a, b, d, f T-value 2

It is proposed that the T-value be changed to 1, as the lowest oral LD50 in rats is 9.5 mg/kg. No changes are proposed to the current First Aid Instructions.

Committee Considerations

Note: Comments from the Advisory Committee on Pesticides and Health and/or the National Drugs and Poisons Scheduling Committee will be included here for completeness.

CONCLUSIONS

1. Acceptable Daily Intake

The proposed acceptable daily intake (ADI) is 0.006 mg/kg/day, based on the lowest NOEL estimated in animal studies of approximately 0.6 mg/kg/day (78-week dietary study in mice, 1-year dietary study in dogs, developmental study in rats), and using a 100-fold safety factor.

2. Poisons Scheduling

No change is recommended to the current restrictive schedule of Schedule 7 of the SUSDP is proposed for endosulfan.

3. First Aid and Safety Directions

The proposed Safety Directions based on hazard are:

100 130 131 132 133 207 162 161 164 210 211 220 222 223 340 341 343 340 342 350

No change is proposed to the existing First Aid Instructions

It is recommended that the T-value be changed to 1, based on an LD50 of 9.5 mg/kg in rats.

Note: Recommendations on Safety Directions relating to the use of personal protective equipment are to be provided by Worksafe Australia.

4. Clearance Status

No change is recommended to the clearance status of endosulfan.

SUMMARY OF ACUTE TOXICOLOGY HAZARD

Date of Preparation:	April 1997
Chemical name:	Endosulfan
Worst oral LD50 in rats:	9.5 mg/kg
Worst oral LD50 in other species:	13.5 mg/kg in mice
Worst dermal LD50:	106 mg/kg in rabbits
Worst inhalation LC50:	13 mg/m^3 in rats
Skin irritation:	Slightly irritating to the skin of rabbits
Eye irritation:	Not irritating to the eyes of rabbits
Skin sensitisation:	Not a skin sensitiser to guinea pigs
Remarks:	
T-value:	1
NOEL:	0.6 mg/kg/day

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