ADDENDUM TO ANNEX B

ENDOSULFAN

B - 6 : TOXICOLOGY AND METABOLISM

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This addendum corresponding to Mammalian Toxicology (Section 6) has been prepared after the ECCO 102 meeting. As the monograph, it has been divided in different points. Each point includes a summary with overall conclusions of concern and in some cases, an evaluation of additional studies not included at the original monograph (new data) or a re-evaluation of studies included at the original monograph (corrigenda data).

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B 6 : TOXICOLOGY AND METABOLISM

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (IIA, 5.1)

Summary

The pharmacokinetics and metabolism of endosulfan were investigated in rats, mice, goats, sheep and cattle. Additional data are available for dogs, rabbits and pigs.

Absorption

In rats, the intestinal absorption after oral dosing was estimated to be 60% (males) and 70% (females). However when comparing urinary excretion after oral and i.v. application, absorption was estimated to be 87% and 92% (Kellner and Eckert, 1983, AII 5.1.1).

In base to the reported data, the rate of absorption was estimated to be 60%, within 24 h in rats, by estimation on the basis of the areas below the blood level curves (60-70%).

Main References for Absorption Determination		
REFERENCE	**DESCRIPTION**	**DATA**
Kellner and Eckert, 1983, AII 5.1.1	Rat oral and i.v. single dose toxicokinetic studies Animals dosed at 2mg/kg b.w (oral) and 0.5 mg/kg b.w. (i.v.). The animals were sacrificed 7 days after treatment (killed by exanguination). Blood in increasing intervals (5 min. to once a day), urine and faeces (6 h after treatment and daily) and wide variety of organs and tissues were collected after sacrifice. The samples were analysed by LSC.	**Absorption:** Estimation on the basis of the areas below the blood level curves yielded an absorption between 60 and 70 % and comparison of the elimination of radioactivity after i.v. and oral administration and absorption of 90 %. **Excretion (24 h after dosing):** Oral: urine: 11.9% (males), 22.3% (females). faeces: 82.2 % (males), 71.8 % (females). I.V.: urine: 13.3% (males), 24.1% (females). faeces: 65.7 % (males), 59.9% (females). **Distribution:** 7 days after application revealed that the highest concentrations were to be found in the kidneys.

Distribution

In rat oral single dose toxicokinetic studies, endosulfan levels in blood peaked after 7 hours in male rats (0.25 µg/ml) and after 18 hr in female rats (0.2 µg/ml). Elimination from blood in male rats was biphasic with biological half lives of 8 hr and, from the second day, 110 hr and monophasic in female rats with a biological half life of 75 hr. After seven days endosulfan levels in blood had dropped down to 15% (males) and 23% (females) of the peak values. Five minutes after the i.v. injection of the radio-labelled endosulfan, the concentration in blood was 0.18 mg/ml. Elimination of endosulfan and its metabolites was triphasic in male rats with biological half lives initially of 0.8 hours, then between 6 hours and 3 days post application of 12.5 hours and from day 3 onwards of 157 hours. In the female rats elimination was biphasic with biological half lives of 1.2 and after 6 hours of 47 hours. After six days endosulfan levels in blood had dropped to the limit of detection of 0.014 µg/ml (Kellner and Eckert, 1983, AII 5.1.1).

In rat 30-day feeding study with tissue analysis, pharmacokinetics of endosulfan and the mechanism of kidney pigmentation and subsequent yellow discoloration of kidney tubules as seen in the oral rat 90-day

study and in the rat multigeneration study. Samples of liver, kidneys and blood of the rats were analysed for α-endosulfan, β-endosulfan, endosulfan-sulphate, endosulfan-hydroxy-ether, endosulfan-lactone and endosulfan-diol. α-endosulfan is stored only temporarily in the kidneys and that β-endosulfan is hardly if at all stored (α/β in kidneys was 230/1, whereas α/β in administered test substance was 2/1). Endosulfan-hydroxyether and endosulfan-diol were only present as traces or not at all. Presence of the metabolites endosulfan-sulphate and endosulfan-lactone in liver and kidneys indicates active metabolism. Storage in the kidneys proved to be temporary. Reversibility is also shown by levels found after 30-day recovery period. Only traces of α-endosulfan and two metabolites could still be found in the kidneys at that time point (Leist and Mayer, 1987; AII 5.1.1). The lower concentration of endosulfan in kidneys after feeding 360 ppm for 30 days as compared to that after feeding 25 ppm for 14 days (see above Dorough *et al*., 1978; AII 5.1.1) points at a steadily accelerating enzyme induction. This was confirmed in the long term study on rats, where essentially no residues were found in the kidneys.

Main References for Distribution Determination		
REFERENCE	**DESCRIPTION**	**DATA**
Kellner and Eckert, 1983, AII 5.1.1	Rat oral and i.v. single dose toxicokinetic studies Animals dosed at 2mg/kg b.w (oral) and 0.5 mg/kg b.w. (i.v.). The animals were sacrificed 7 days after treatment (killed by exanguination). Blood in increasing intervals (5 min. to once a day), urine and faeces (6 h after treatment and daily) and wide variety of organs and tissues were collected after sacrifice. The samples were analysed by LSC.	**Absorption:** Estimation on the basis of the areas below the blood level curves yielded an absorption between 60 and 70 % and comparison of the elimination of radioactivity after i.v. and oral administration and absorption of 90 %. **Excretion (24 h after dosing):** Oral: urine: 11.9% (males), 22.3% (females). faeces: 82.2 % (males), 71.8 % (females). I.V.: urine: 13.3% (males), 24.1% (females). faeces: 65.7 % (males), 59.9% (females). **Distribution:** 7 days after application revealed that the highest concentrations were to be found in the kidneys.
Leist and Mayer, 1987; AII 5.1.1	Rat 30-day feeding study with tissue analysis The animals were ultiple oral dosed (4 weeks) at 360 and 720 mg/kg b.w. Histological examinations, including electron microscopy, were performed on tissues of liver, kidneys and brain.	**Distribution:** Residue analysis revealed treatment related residues of endosulfan (mainly α-endosulfan) and its metabolites in the kidneys. The liver contained mainly endosulfan-sulphate and -lactone, though at much lower concentrations. Residues in the blood were still lower.
Dorough *et al*., 1978; AII 5.1.1	Rat oral single and multiple dose toxicokinetic studies, and *in vitro* liver enzyme assays Animals were dosed at 1.2-2 mg/kg b.w. (single) or 5 mg/kg b.w. (multiple, 14 days). Urine and faeces were collected daily, bile hourly and tissues(kidney, liver, visceral and subcutaneous fat, muscle and brain) at sacrifice. The samples were analysed by two-dimensional TLC. For *in vitro* enzyme assays, livers used for cytochrome P-450 and epoxidase enzyme assays were from female albino rats maintained on a normal diet or one containing 50 ppm of α- or β-endosulfan for 28 days.	**Excretion (120 h after dosing):** α-endosulfan: 13.2% (urine), 74.8% (faeces) β-endosulfan: 18.5% (urine), 68.3% (faeces) At the end of a 14 day feeding period 60-65 % were eliminated irrespective of dose, and about another 8 % were eliminated during the 14 day recovery period. Only 15-18 % of the applied dose were eliminated unchanged in the faeces. **Distribution:** The only organs with significant residues right after treatment were kidney and liver and, to a much less extenct , the visceral and subcutaneous fat.

Accumulation

^{14}C-Residues in organs and tissues, seven days after application, accounted for 3.7% of the dose in the males and 4.7% in the females. Approximately half of this amount (1.5% of the dose) was found in kidneys and liver. Levels found after 7 days were in blood 0.05 µg/ml, kidneys 1.8 µg/g, liver 0.2 µg/g (males) and 0.5 µg/g (females) and in peritoneal fat below detection in males and 0.16 µg/g in females. In all other organs the levels were below the detection limit of 0.1 µg/g (Kellner and Eckert, 1983, AII 5.1.1).

In rat oral multiple dose toxicokinetic study at single dose level, 1.5% of the total residue after 14 days was present in kidneys and liver. Levels after 14 days of feeding 5 ppm endosulfan were 3 µg/g in kidneys, 1 µg/g in liver, 0.5 µg/g in visceral fat, 0.2 µg/g in subcutaneous fat, 0.05 µg/g in muscle and 0.07 µg/g in brain. In the rats fed 25 ppm ^{14}C-α-endosulfan in the diet for fourteen days, the kidneys contained 20 µg/g and the liver 6 µg/g of ^{14}C-endosulfan equivalents. Analysis of subcutaneous and visceral fat samples showed that endosulfan did not accumulate to any great extent in fatty tissue. Major metabolites were endosulfan-sulphate, endosulfan-diol, endosulfan-ether, endosulfan-hydroxy-ether and endosulfan-lactone (Dorough et al., 1978, AII 5.1.1).

In mouse oral single dose, retention of the administered dose went down from 50% 24 h after dosing, to 6% after 5 days and 0.4% after 24 days. Highest ^{14}C-residues were found in liver and intestinal tract. These residues were very low after 24 days (Christ and Kellner, 1968, AII 5.1.2).

In mouse oral multiple dose, from analysis in various organs and tissues, it was clear that endosulfan does not preferentially accumulate in fat. Highest concentration was found in the liver (7.0 µg/g). Some residue in this organ (0.86 µg/g) was still demonstrable 35 days after treatment had stopped (Christ and Kellner, 1968, AII 5.1.2).

In goat oral multiple dose (1 mg/kg/day endosulfan for 28 days), total residues were detected in kidneys (0.29 mg/kg), gastrointestinal tract (0.20 mg/kg), liver (0.12 mg/kg) brain (0.06 mg/kg) muscle and spleen (0.04 mg/kg), lung and heart (0.01 mg/kg) and milk (0.02 mg/kg) on the first day after dosing. Within 15 days concentrations had dropped to below (0.01 mg/kg) except in the kidneys (0.02 mg/kg). Twenty one days after dosing endosulfan could not be detected any more (Indraningsih *et al*., 1993, AII 5.1.4).

In cow oral multiple dose (0, 0.3, 3 or 30 ppm, 30 days), highest residues were measured in the liver. Analysis in blood showed a gradual rise reaching a plateau after 21 days. In the recovery period of 14 days the residue levels came down significantly, though in most cases not yet below detection limit.

Main References for Accumulation Determination		
REFERENCE	**DESCRIPTION**	**DATA**
Kellner and Eckert, 1983, AII 5.1.1	Rat oral and i.v. single dose toxicokinetic studies Animals dosed at 2mg/kg b.w (oral) and 0.5 mg/kg b.w. (i.v.). The animals were sacrificed 7 days after treatment (killed by exanguination). Blood in increasing intervals (5 min. to once a day), urine and faeces (6 h after treatment and daily) and wide variety of organs and tissues were collected after sacrifice. The samples were analysed by LSC.	**Absorption:** Estimation on the basis of the areas below the blood level curves yielded an absorption between 60 and 70 % and comparison of the elimination of radioactivity after i.v. and oral administration and absorption of 90 %. **Excretion (24 h after dosing):** Oral: urine: 11.9% (males), 22.3% (females). faeces: 82.2 % (males), 71.8 % (females). I.V.: urine: 13.3% (males), 24.1% (females). faeces: 65.7 % (males), 59.9% (females). **Distribution and accumulation:** 7 days after application revealed that the highest concentrations were to be found in the kidneys.
Dorough *et al*., 1978; AII 5.1.1	Rat oral single and multiple dose toxicokinetic studies, and *in vitro* liver enzyme assays Animals were dosed at 1.2-2 mg/kg b.w. (single) or 5 mg/kg b.w. (multiple, 14 days). Urine and faeces were collected daily, bile hourly and tissues(kidney, liver, visceral and subcutaneous fat, muscle and brain) at sacrifice. The samples were analysed by two-dimensional TLC. For *in vitro* enzyme assays, livers used for cytochrome P-450 and epoxidase enzyme assays were from female albino rats maintained on a normal diet or one containing 50 ppm of α- or β-endosulfan for 28 days.	**Excretion (120 h after dosing):** α-endosulfan: 13.2% (urine), 74.8% (faeces) β-endosulfan: 18.5% (urine), 68.3% (faeces) At the end of a 14 day feeding period 60-65 % were eliminated irrespective of dose, and about another 8 % were eliminated during the 14 day recovery period. Only 15-18 % of the applied dose were eliminated unchanged in the faeces. **Distribution:** The only organs with significant residues right after treatment were kidney and liver and, to a much less extenct , the visceral and subcutaneous fat.
Christ and Kellner, 1968, AII 5.1.2	Mouse oral single and multiple dose toxicokinetic studies Animals were dosed at 4 mg/kg b.w. (single) or 2.4 mg/kg b.w. (multiple, 24 days). Urine and faeces were collected daily except on weekends (combined sample of 3 days). Major organs and tissues were collected after sacrifice. All radioactivity measurements were performed by LSC.	**Excretion (120 h after dosing):** 13.8% (urine), 77% (faeces) **Distribution and accumulation:** Residues in organs are generally low. However, highest residues are found in the liver (30 nMol/g at 24 h) shortly after treatment and in the spleen after some weeks. Some residues in organs still exist 35 days after the last treatment.
Indraningsih *et al*., 1993, AII 5.1.4	Goat oral multiple dose toxicokinetic study Animals were dosed orally once daily with 1 mg/kg b.w. for 28 days. On days 1, 8, 15 and 21 after last treatment, 1 group was killed. Samples of milk and venous blood were taken from each animal before being killed. Samples of major organs and muscle were removed at necropsy and were analysed for α- and β-endosulfan and endosulfan-sulphate. The samples were analysed by GC, and plasma was analysed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamil transferase (GGT), alkaline phosphatase (AP) and total bilirrubine.	**Distribution and accumulation:** Kidney: 0.47 mg/kg b.w. 8 days after dosing. Other organs and tissues: <0.01 mg/kg b.w. 8 days after dosing.

Excretion

In rats, following oral single dose, excretion of endosulfan was rapid after single oral administration of 2 mg/kg body weight. 24 h later, 80-90 % of the dose had been eliminated. The major portion of this was found in the faeces (Kellner and Eckert, 1983, **AII 5.1.1**).

Administration of a single oral dose of the α- or β-isomer of ^{14}C-endosulfan to rats revealed some quantitative differences in their route of elimination. In this experiment female rats were gavaged with 2 mg/kg of the ^{14}C-labelled α- or β-isomer, while male rats were given a single oral dose of 1.2 mg/kg of the ^{14}C-labelled α- or β-isomer after a bile cannula was implanted. Faecal and urinary excretion of the first five days accounted for 88% of the dose. Of the α-isomer 75% was excreted with faeces and 13% with urine. More of the α-isomer was excreted in urine (19%) and less in the faeces (68%). With bile 47% of the α-isomer and 29% of the β-isomer was excreted within 48 hr. ^{14}C-Residues in faeces of bile-cannulated rats amounted to 20% of the administered dose and consisted entirely of the administered compound. In faeces of intact animals the administered compound amounted only to 4% suggesting that presence of bile facilitates absorption of endosulfan. Faecal excretion of endosulfan, but not urinary excretion, was reduced by bile collection, suggesting that liver metabolites after elimination from the liver into the intestine of intact rats are not reabsorbed and eliminated via the kidney (Dorough et al., 1978; **AII 5.1.1**).

In rat oral multiple dose toxicokinetic study at single dose level, no significant difference was observed in elimination between the α- and β- sample given at 5 ppm for up to 14 days. In both cases total elimination via urine and faeces amounted to 65%, while another 8% was eliminated in the next two weeks on normal diet. Half-life of the residue was approximately seven days Kellner and Eckert (1983, **AII 5.1.1**)

In mouse oral single dose, 24 h after dosing 44% had been excreted in urine and faeces, after 5 days this amount had increased to 91% and after 24 days to 94%. When administered in the diet, more than 90% of the endosulfan had been eliminated within two days (Christ and Kellner, 1968, **AII 5.1.2**).

In mouse oral multiple dose, excretion occurred predominantly in the faeces. Urinary excretion averaged only 10% of total excretion. This relative proportion increased slowly to a maximum of 25% after dosing was stopped (Christ and Kellner, 1968, **AII 5.1.2**).

Main References for Excretion Determination		
REFERENCE	**DESCRIPTION**	**DATA**
Kellner and Eckert, 1983, AII 5.1.1	Rat oral and i.v. single dose toxicokinetic studies Animals dosed at 2mg/kg b.w (oral) and 0.5 mg/kg b.w. (i.v.). The animals were sacrificed 7 days after treatment (killed by exanguination). Blood in increasing intervals (5 min. to once a day), urine and faeces (6 h after treatment and daily) and wide variety of organs and tissues were collected after sacrifice. The samples were analysed by LSC.	**Absorption:** Estimation on the basis of the areas below the blood level curves yielded an absorption between 60 and 70 % and comparison of the elimination of radioactivity after i.v. and oral administration and absorption of 90 %. **Excretion (24 h after dosing):** Oral: urine: 11.9% (males), 22.3% (females). faeces: 82.2 % (males), 71.8 % (females). I.V.: urine: 13.3% (males), 24.1% (females).

		faeces: 65.7 % (males), 59.9% (females). **Distribution:** 7 days after application revealed that the highest concentrations were to be found in the kidneys.
Dorough *et al*., 1978; AII 5.1.1	Rat oral single and multiple dose toxicokinetic studies, and *in vitro* liver enzyme assays Animals were dosed at 1.2-2 mg/kg b.w. (single) or 5 mg/kg b.w. (multiple, 14 days). Urine and faeces were collected daily, bile hourly and tissues(kidney, liver, visceral and subcutaneous fat, muscle and brain) at sacrifice. The samples were analysed by two-dimensional TLC. For *in vitro* enzyme assays, livers used for cytochrome P-450 and epoxidase enzyme assays were from female albino rats maintained on a normal diet or one containing 50 ppm of α- or β-endosulfan for 28 days.	**Excretion (120 h after dosing):** α-endosulfan: 13.2% (urine), 74.8% (faeces) β-endosulfan: 18.5% (urine), 68.3% (faeces) At the end of a 14 day feeding period 60-65 % were eliminated irrespective of dose, and about another 8 % were eliminated during the 14 day recovery period. Only 15-18 % of the applied dose were eliminated unchanged in the faeces. **Distribution:** The only organs with significant residues right after treatment were kidney and liver and, to a much less extenct , the visceral and subcutaneous fat.
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Christ and Kellner, 1968, AII 5.1.2	Mouse oral single and multiple dose toxicokinetic studies Animals were dosed at 4 mg/kg b.w. (single) or 2.4 mg/kg b.w. (multiple, 24 days). Urine and faeces were collected daily except on weekends (combined sample of 3 days). Major organs and tissues were collected after sacrifice. All radioactivity measurements were performed by LSC.	**Excretion (120 h after dosing):** 13.8% (urine), 77% (faeces) **Distribution and accumulation:** Residues in organs are generally low. However, highest residues are found in the liver (30 nMol/g at 24 h) shortly after treatment and in the spleen after some weeks. Some residues in organs still exist 35 days after the last treatment.

Metabolism

Endosulfan is converted in the animal organism to the following metabolites: endosulfan-sulphate, endosulfan-diol, endosulfan-ether, endosulfan-hydroxyether and endosulfan-lactone. A number of unidentified polar metabolites are probably the conjugates of the metabolites (Dorough et al., 1978, **AII 5.1.1**).

The cytochrome-P450 group of enzymes was not significantly activated by endosulfan. This was the outcome of an experiment where 5 mice were dosed with 5 mg/kg for three days and their livers

examined on day 4 (Robacker *et al.,* 1981, **AII 5.1.2**). A similar outcome with experiments on rats has been published by Dorough *et al.* (1978; **AII 5.1.1**).

In a metabolism experiment Schuphan *et. al.* (1968, **AII 5.1.1** and **AII 5.1.2**) applied the following substances by oral and intraperitoneal route to Sprague-Dawley rats (sex not specified) and were able to determine semi-quantitatively metabolism and excretion in faeces, urine and bile. The majority of orally applied endosulfan (α-E and β-E) was excreted unchanged with faeces in the first 48 hr. In addition, the lactone (EL), the hydroxy-ether (HE) and some sulphate (ES) were found. The ratio found was α-E or β-E/ES/HE/EL as 10/0.3/0.3/1.

The applied metabolites were excreted in faeces unchanged as the lactone, as an unidentified metabolite (M_2) and as the hydroxyether. In urine less unchanged endosulfan isomers, but more lactone (EL), unidentified metabolite (M_1) as well as some sulphate (ES) was present.

The ratio α-E /ES/HE/EL found was 3/1/1/2, while the ratio β-E/ES/HE/EL was 2/1/6/20. On the third day only both endosulfan-isomers and the lactone were present in urine.

In bile only the lactone and the unidentified substance (M_1) could be found from α-E in a ratio of EL/ M_1 is 5/1 and from β-E in a ratio of EL/ M_1 is 1/30. Metabolism of β-endosulfan was different, as shown by analysis in urine and bile, and also faster than metabolism of α-endosulfan. In the first 24 hours the 1/1 ratio in a mixed sample of α/β-endosulfan did not change in faeces, whereas in urine this ratio had become 5:1. Therefore β-endosulfan is more quickly metabolised (Schuphan et al., 1968, **AII 5.1.1** and **AII 5.1.2**).

Main References for Metabolism Determination		
REFERENCE	**DESCRIPTION**	**DATA**
Dorough *et al*., 1978; **AII 5.1.1**	**Rat oral single and multiple dose toxicokinetic studies, and *in vitro* liver enzyme assays** Animals were dosed at 1.2-2 mg/kg b.w. (single) or 5 mg/kg b.w. (multiple, 14 days). Urine and faeces were collected daily, bile hourly and tissues(kidney, liver, visceral and subcutaneous fat, muscle and brain) at sacrifice. The samples were analysed by two-dimensional TLC. For *in vitro* enzyme assays, livers used for cytochrome P-450 and epoxidase enzyme assays were from female albino rats maintained on a normal diet or one containing 50 ppm of α- or β-endosulfan for 28 days.	**Excretion (120 h after dosing):** α-endosulfan: 13.2% (urine), 74.8% (faeces) β-endosulfan: 18.5% (urine), 68.3% (faeces) At the end of a 14 day feeding period 60-65 % were eliminated irrespective of dose, and about another 8 % were eliminated during the 14 day recovery period. Only 15-18 % of the applied dose were eliminated unchanged in the faeces. **Distribution:** The only organs with significant residues right after treatment were kidney and liver and, to a much less extenct , the visceral and subcutaneous fat.
Robacker *et al.,* 1981, **AII 5.1.2**	**Oral Administration in mice/multiple dose. Mammalian metabolism** Animals were dosed with 5 mg/kg/day (3 days). Livers were removed and freshly prepared microcosms were used for all assays. Samples of stored microsomal preparations were examined by electrophoresis.	**Metabolism:** Investigations on the induction of cytochrome P-450 and monooxygenase activity by endosulfan resulted in data showing that the cytochrome P-450 group of enzymes is not significantly activated by endosulfan.

Schuphan et al., 1968, **AII 5.1.1** and **AII 5.1.2**	**Oral and i.p. Administration in the rat and mouse /single dose** Animals were dosed at 0.8-16 mg/kg b.w. (oral) or 4 mg/kg b.w. (i.p.). Samples of urine and faeces from all animals were collected, bile only from those dosed by via duodenum-fistula.	**Metabolism:** Endosulfan is rapidly metabolised after application to rats and mice via different routes. Most of the metabolites could be identified. The metabolisation of the β -isomer was much faster compared to that of the α-isomer.

Reevaluation of studies included at the original monograph

B.6.1.1 Rat

Oral and i.p. Administration in the rat and mouse /single dose.			
Autor(s):	Schuphan I, Ballschmiter K & Tölg G.	**Study design:**	Animals were kept in cages that permitted separate collection of faeces and urine. Sampling: urine and faeces from all animals , bile only from those dosed by via duodenum fistula
Study Title:	**Zum metabolismus des endosulfans in ratten und Maeusen** 5.1.3.2/01		
Testing facility:	[redacted]		
Report Number:	A16758		
Study duration:	Not provided in the report	**Dose:**	orally : 0.8, 4, 8, 16 mg/kg b.w. i.p.: 4 mg/kg b.w.
Date of report:	1968	**Vehicle/Solvent:**	Starch mucilage and 'oil'
Test Substance:	^{14}C labelled and unlabelled endosulfan sulphate, endosulfan diol, endosulfan ether, endosulfan hydroxy ether, and endosulfan lactone	**Route:**	Oral (by gavage) and i.p.
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:	Not included in the report		
Test Animals:	Sprague-Dawley rats and mice	**GLP:**	Prior to GLP regulations
Origin:	Not provided in the report	**Guideline:**	
Bodyweight:	Rat: 110-280 g	**Deviation:**	
Groups:	Not included in the report	**Acceptability:**	The study is acceptable

Results

After application of αE and βE, mainly the applied isomers but also ES and HE and especially EL were detected in faeces. After application of any of the metabolites, the dominant excretion products in faeces were the applied molecule, HE and EL. In urine the amount of isomers was rather low after their oral application. EL was the major metabolite for renal excretion irrespective of the substance applied. In addition an unidentified metabolite was found after application of the isomers, especially βE. This unidentified metabolite together with EL were the only substances found in the bile after treatment with the isomers via duodenum fistula. The metabolization of the β-isomer was much faster compared to that of the α-isomer.

Conclusions

Endosulfan is rapidly metabolised after application to rats and mice via different routes. Most of the metabolites could be identified.

Rat oral single and multiple dose toxicokinetic studies, and *in vitro* liver enzyme assays			
Autor(s):	Dorough HW, Huhtanen K, Marshall TC & Bryant HE.	**Study design:**	Animals were kept in cages that permitted separate collection of faeces and urine. Samples: urine and faeces separately and daily, bile hourly, kidney, liver, visceral and subcutaneous fat, muscle, and brain after sacrifice
Study Title:	**Fate of endosulfan in rats and toxicological considerations of apolar metabolites**. 5.1.1.1/01		
Testing facility:	[redacted]		
Report Number:	A14276 (HOE) END/L0034 (CALLIOPE)		
Study duration:	Not provided in the report	**Dose:**	Single oral dose: 2 mg/kg Single dose orally in bile collection: 1.2 mg/kg Multiple oral dose (14 days): 5 mg/kg
Date of report:	1978	**Vehicle/Solvent:**	Corn oil for single oral dosing, acetone for mixing with feed
Test Substance:	^{14}C labelled and unlabelled endosulfan sulphate, endosulfan diol, endosulfan ether, endosulfan hydroxy ether, and endosulfan lactone	**Route:**	Oral (by gavage)
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:	Not included in the report		
Test Animals:	Male and female albino rats	**GLP:**	Prior to GLP regulations
Origin:	[redacted]	**Guideline:**	
Bodyweight:	Rat: 200-400 g	**Deviation:**	
Groups:	Not included in the report	**Acceptability:**	The study is partially acceptable

Results

Elimination of ^{14}C-endosulfan as a single oral dose or as a dietary supplement are summarised in Table 6.1.1-1. Residues in Tissues or female rats fed 5 ppm of α- or β-radiolabel isomers in diet are summarised in Table 6.1.1-2. Extraction characteristics of residues in excreta and tissues are summarised in Table 6.1.1-3. Analysis of apolar endosulfan ^{14}C equivalents are summarised in Table 6.1.1-4. The main route of elimination was via faeces. After a single oral dose almost 90 % were eliminated within 5 days. At the end of a 14 day feeding period 60-65 % were eliminated irrespective of dose, and about another 8 % were eliminated during the 14 day recovery period. Only 15-18% of the applied dose were eliminated unchanged in the faeces. Major metabolites were endosulfan-sulphate, endosulfan-diol, endosulfan-ether, endosulfan-hydroxy-ether and endosulfan-lactone. 50 ppm in the diet for 28 days not increase the levels of cytochrome P-450 or epoxidase activity in the liver microcosms.

Table 6.1.1-1: Elimination of radiocarbon from rats treated with ^{14}C-endosulfan as a single oral dose or as dietary supplement.

Treatment and time	**CUMULATIVE PERCENTAGE OF DOSE(S)**		
	faeces	**urine[a]**	**total**
Single dose, 2 mg/kg			
α-endosulfan			
24 h	11.0	7.7	18.7
48 h	61.6 (21.9)	11.1 (12.5)	72.7
96 h	73.0	12.5	85.5
120 h	74.8	13.2	88.0
β-endosulfan			
24 h	12.5	12.3	24.8
48 h	55.1 (15.2)	16.0 (10.4)	71.1
96 h	66.5	17.7	84.2
120 h	68.3	18.5	86.8
Dietary supplement			
α-endosulfan 5 ppm			
14 days on	56.5	7.8	64.3
+ 14 days off	63.1	9.2	72.3
β-endosulfan 5 ppm			
14 days on	57.0	8.0	65.0
+ 14 days off	63.5	9.3	72.8
α-endosulfan 25 ppm			
14 days on	56.0	8.7	64.7
7:3 α,β-endosulfan 25 ppm			
14 days on	54.0	6.8	60.8

[a]: Values in parenthesis are for animals having the bile duct cannulated; amounts in the bile collected for 48 h were 47.2 and 28.9 % for α- and β-endosulfan, respectively.

Table 6.1.1-2: Residues in Tissues of female rats fed 5 ppm of α- or β-^{14}C-endosulfan in the diet.

Days	**ppm of ^{14}C-endosulfan equivalents per isomer in diet[a]**									
	kidney		**liver**		**visc. fat**		**subcut. fat**		**muscle[b]**	**brain[b]**
	α	**β**	**α**	**β**	**α**	**β**	**α**	**β**	**α/β**	**α/β**
on treatment										
1	0.38	0.47	0.26	0.32	0.34	0.24	0.32	0.30	0.02	0.03
2	1.26	1.21	1.02	0.79	0.85	1.02	0.23	0.34	0.02	0.03
7	1.77	1.87	0.96	0.75	0.74	0.53	0.51	0.30	0.02	0.04
10	2.28	2.08	1.11	0.94	0.94	0.55	0.15	0.28	0.03	0.04
14	3.00	3.26	1.08	1.06	0.62	0.50	0.15	0.32	0.05	0.07
off treatment										
1	2.75	3.34	1.00	0.87	0.45	0.42	0.02	0.08	0.05	0.05
3	1.89	2.21	0.49	0.57	0.15	0.28	0	0	0.02	0.06
7	1.53	1.66	0.28	0.36	0	0	0	0	0	0.04
10	0.94	0.92	0.11	0.19	0	0	0	0	0	0.02

[a]: zero indicates residues were less than 0.02 ppm, the limit of detectability.
[b]: these low residues are representative of both α and β treatments.

Table 6.1.1-3: Extraction characteristics of residue in excreta and tissues of rats treated with ^{14}C endosulfan.

Sample, treatment and fraction	% total ^{14}C in sample/compound	
	α	β
FAECES		
Single dose, 0-48 h		
Chloroform extractables	15.2 (98.5)[a]	17.8 (100)
Methanol extractables	23.0	21.0
Water extractables	30.0	26.8
Total extractables	68.2	65.6
Unextracted	31.8	34.4
5 ppm in diet, 0-14 days		
Chloroform extractables	16.0	16.5
Methanol extractables	35.0	36.0
Water extractables	21.0	14.0
Total extractables	72.0	67.5
Unextracted	28.0	32.5
URINE		
Single dose, 0-24 h		
Ether extractables	42.0	32.0
Water solubles	58.0	68.0
5 ppm in diet, 0-14 days		
Ether extractables	26.5	22.4
Water solubles	73.5	77.6
BILE		
Single dose, 0-48 h		
Ether extratables	42.0	33.5
Water solubles	58.0	66.5
TISSUE	**liver (α)**	**kidney (α)**
25 ppm of α in diet, 14 days		
Ethyl acetate extractables	3.9	2.8
Water solubles	25.1	33.2
Unextracted	71.0	65.0

[a]: values in parenthesis are for animals with the bile duct cannulated.

Table 6.1.1-4: Thin-layer chromatography analysis of apolar endosulfan ^{14}C equivalents extracted from faeces, urine and bile.

	% total ^{14}C in sample as indicated material per endosulfan isomer administered															
	1		2		3		4		5		6		7		8	
sample, fraction and treatment	α	β	α	β	α	β	α	β	α	β	α	β	α	β	α	β
Faeces-chlorof.[a]	1.7	1.9	5.3	4.1	4.5	2.1	1.1	1.1	0.3	1.2	0.1	7.0	0.1	0.4	2.1	0
single dose	6.2	6.4	2.0	2.0	2.9	6.1	1.2	0.5	0.5	0.3	0	1.3	0	0	3.2	0
5 ppm in diet																
Urine-ether																
single dose	19.4	16.5	7.1	6.4	9.1	5.6	5.8	3.4	0	0	0	0	0	0	0.1	0
5 ppm in diet	13.4	14.6	4.2	2.7	3.7	2.2	4.1	2.9	0	0	0	0	0	0	1.2	0
Bile-ether																
single dose	32.3	18.8	1.3	1.0	3.4	4.0	5.0	9.7	0	0	0	0	0	0	0	0

[a]: Polar metabolites indicated as origin materials were removed by column cleanup prior to the analysis.

Conclusions

Endosulfan is rapidly eliminated, mainly in metabolised form, by treated rats, independent of dose and duration of application. The only organs with significant residues right after treatment were kidney and liver and, to a much less extenct , the visceral and subcutaneous fat.

Review			
Autor(s):	Gupta, P. K., Gupta, R. C.		
Date of report:	1979		

Conclusions

Metabolism studies in rats, after an i.p. injection of 20 mg/kg of technical endosulfan in an oil solution reveals the presence of endosulfandiol and an unknown compound in the urine as a water soluble conjugation metabolite. Endosulfan was not excreted in rat urine as endosulfan-diol, but as endosulfan-α-hidroxy ether (Ballschmiter & Tolg, 1966). After either an oral or intraperitoneal dose of α-endosulfan to rats (4-8 mg/kg), the unchanged compound (¡.e. endosulfan), endosulfan-sulphate, endosulfan-α-hydroxy ether and endosulfan-lactone were excreted in the faeces in the ratio of 10:3:3:1. No endosulfan-diol or endosulfan-ether were detected in urine, and the ratio of endosulfan, endosulfan-sulphate, endosulfan-lactone and unidentified metabolites were in the ratio of 3:1:1:2. After the administration of a mixture of isomers, the ratio of unchanged β-endosulfan, endosulfan-sulphate and an unidentified metabolite was 2:1:6:20. The excretion of a-endosulfan through bile via duodenal fistula has been in the form of large amounts of endoketone and traces of an unidentified metabolite while after β-endosulfan large amounts of an unidentical product and traces of endolactone were observed. The distribution pattern of endosulfan in plasma and brain tissues was studied when rats were fed daily doses of endosulfan (5 to 10 mg/kg) for 15 days, 24 h later the animals were killed. The concentration of α-isomers was in the order of cerebrum>remaining parts of the brain>cerebellum. The β-isomers was not detected in the remaining part of the brain. On a per unit lipid basis, the concentration of endosulfan in cerebrum was one and a half times that of the whole brain tissue and might be due to the higher lipid content in the cerebrum than in the rest of the brain. Endosulfan-sulphate was the only metabolite detected in the rat brain (Gupta, 1978)

Rat oral and i.v. single dose toxicokinetic studies			
Autor(s):	Kellner, H. M. & Eckert	**Study design:**	Assessment of helath condition. Acclimatisation period: 3 days. Rats housed single in a metabolism cage. The animals were sacrificed 7 days after treatment (killed by exanguination). Blood in increasing intervals (5 min. to once a day), urine and faeces (6 h after treatment and daily) and wide variety of organs and tissues were collected after sacrifice. The samples were analysed by LSC.
Study Title:	**Hoe 02671-14C Pharmacokinetics and residue determinations after oral and intravenous administration to rats**. 5.1.1.1/02 and 5.1.2.1/01		
Testing facility:	[redacted]		
Report Number:	A49475		
Study duration:	From November 1981 to September 1982	**Dose:**	orally by gavage: 2 mg/kg b.w. i.v.: 0.5 mg/kg b.w.
Date of report:	1983a	**Vehicle/Solvent:**	Oral: cooking oil i.v.: 1,2-propanediol
Test Substance:	^{14}C labelled endosulfan	**Route:**	Oral (by gavage) and i.v.
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical Purity:	98 %		

Test Animals:	Male and female SPF-Wistar rats	**GLP:**	Prior to GLP regulations
Origin:	[redacted]	**Guideline:**	
Bodyweight:	180-200 g	**Deviation:**	
Groups:	groups, 6 males and 5-6 females each	**Acceptability:**	The study is acceptable.

Results

After oral administration, the highest measured concentration in blood were 0.25 ± 0.06 µg/ml in the males and 0.18 ± 0.05 µg/ml in the females. In the males, the maximum values were reached at an earlier time point than in females, with 6.8 ± 2.0 h and 21.3 ± 6.5 h respectively. After i.v. injection, there are a half-lives of 0.77 ± 0.20 h, 12.5 ± 2.9 h and 157 ± 57 h in the males, and 1.2 h and 47.0 ± 12.5 h in the females. The percentage of applied radioactivity collected in the urine and faeces are summarised in Table 5.1.1-5. The t½ for byphasic excretion in the urine was 6-8 h or 30-70 h respectively and the faeces between 8-14 h or 30-40 h respectively. In the distribution 7-days after application, the highest values were in kidneys with 1.8 µg/g, followed by the liver (0.23 µg/g males, 0.48 µg/g females).

Table 5.1.1-5: Relation between sex, route of application and type of sample, with percentage of applied radioactivity.

SEX	**MALES**		**FEMALES**	
ROUTE	**p.o.**	**i.v.**	**p.o.**	**i.v.**
SAMPLE	**% OF APPLIED RADIOACTIVITY**			
Urine + cagewash	11.9±1.8	13.3±2.3	22.3±2.7	24.1±3.7
faeces	82.2±6.5	65.7±11.9	71.8±16.2	59.9±5.1

Conclusions

There were sex dependent differences in the development of the blood levels. Byphasic decrease in concentration took place in the males with biological half-lives (t½) of 8.07 ± 1.12 h and 110 ± 21 h and monophasic decrease in the females with 75.4 ± 13.5 h. After i.v. injection, the decrease in concentration (assuming identical levels 5 min after injection) occurred in 3 phases in the males and biexponentially in the females. Regardless of the route of administration, excretion was higher in the faeces than in the urine, and the level of renally eliminated radioactivity depended on the sex of the animals. The examination of distribution 7 days after application revealed that the highest concentrations were to be found in the kidneys. Estimation on the basis of the areas below the blood level curves yielded an absorption between 60 and 70 % and comparison of the elimination of radioactivity after i.v. and oral administration and absorption of 90 %.

Rat oral and i.v. single dose toxicokinetic studies			
Autor(s):	Kellner, H. M. & Eckert	**Study design:**	Assessment of helath condition. Acclimatisation period: 3 days. Rats housed single in a metabolism cage. The animals were sacrificed 7 days after treatment (killed by exanguination). Blood in
Study Title:	**Hoe 02671-14C Pharmacokinetics and residue determinations after oral and intravenous administration to rats** IIA, 5.1.2.1/02		
Testing facility:	Hoechst RCL		

Report Number:	A49475		increasing intervals (5 min. to once a day), urine and faeces (6 h after treatment and daily) and wide variety of organs and tissues were collected after sacrifice. The samples were analysed by LSC.
Study duration:	From November 1981 to September 1982	**Dose:**	orally by gavage: 2 mg/kg b.w. i.v.: 0.5 mg/kg b.w.
Date of report:	1983b	**Vehicle/Solvent:**	Oral: cooking oil i.v.: 1,2-propanediol
Test Substance:	^{14}C labelled endosulfan	**Route:**	Oral (by gavage) and i.v.
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:	98 %		
Test Animals:	Male and female SPF-Wistar rats	**GLP:**	
Origin:	Breeder Winkelmann, Borchen	**Guideline:**	
Bodyweight:	180-200 g	**Deviation:**	
Groups:	6 males and 5-6 females each	**Acceptability:**	The study is acceptable

Results

After oral administration highest concentrations in blood were 0.25 ± 0.06 µg/ml in males and 0.18 ± 0.05 in females. In males the maximum was reached after about 6.8 hours, in females after 21.3 hours. Elimination from the blood was byphasic in males with t« of 8.07 and 110 hours and monophasic in females with t« of 75.4 hours. After intravenous injection decrease in blood levels occurred in 3 phases in males (t« = 0.77, 12.5, 157 h) and byphasic in females (t« = 1.2, 47 h). Excretion was highest in faeces (60 to 80 %) regardless of sex and route of administration. Renal excretion amounted in males to 12 to 13 % and in females to 22 to 24 %. It was byphasic in urine (6 - 8 and 30 - 70 h) and faeces (8 - 14 and 30 - 40 h). After 2 days 80 to 90 % of the applied dose were excreted. Remaining residues after 7 days were correspondingly low with 1.8 µg/g in the kidney, followed by the liver with 0.48 µg/g (females) and 0.23 µg/g (males), and the retroperitoneal fat of the females with 0.16 µg/g. All other samples had no detectable residues (< 0.1 µg/g). Kinetic data on concentrations in blood are summarised in Table 6.1.1-6. Kinetic data on excretion are summarised in Table 6.1.1-7. Absorption after oral administration of 2 mg/kg b.w. are summarised in Table 6.1.1-8.

Table 6.1.1-6: Kinetic data on concentration in blood after oral and intravenous administration of ^{14}C-endosulfan to male and female rats.

	ORAL		**INTRAVENOUS**	
	males (2 mg/kg)	**females (2 mg/kg)**	**males (0.5 mg/kg)**	**females (0.5 mg/kg)**
C_{max} (µg/ml)[1]	0.25±0.06	0.18±0.05	0.18±0.04	0.18±0.04
t_{max} (h after appl.)	6.8±2.0	20.8±7.2	0.083[3]	0.083[3]
PHASE I				
$t_{½}$ (h)	-	-	0.77±0.20	1.20[4]
time (h after appl.)	-	-	~ 0.083 - 4	0.083 - 4
PHASE II				
$t_{½}$ (h)	8.07±1.12	-	12.5±2.9	47.0±12.5
time (h after appl.)	t_{max} - 24	-	~ 6 - 48	6 - 120
PHASE III				
$t_{½}$ (h)	110±21	75.4±13.5	157±57	-
time (h after appl.)	48 - 168	24 - 168	72 - 168	-
AUC (µg x ml^{-1} x h)[2] 120h	11.84±2.22	13.86±3.72	19.72±3.44	19.90±7.67
AUC (µg x ml^{-1} x h)[2] 168h	14.01±2.82	16.49±4.28	24.25±5.26	-

[1]: µg-equivalents endosulfan; [2]: calculated for a dose of 2 mg/kg b.w.; [3]: first measured value; [4]: only one animal.

Table 6.1.1-7: Kinetic data on excretion after oral and intravenous administration of ^{14}C-endosulfan to male and female rats.

	ORAL				INTRAVENOUS			
	males (2 mg/kg)		females (2 mg/kg)		males (0.5 mg/kg)		females (0.5 mg/kg)	
	Urine	faeces	urine	faeces	Urine	faeces	urine	faeces
PHASE I								
$t_{½}$ (h)	6.17±1.43	7.67±1.07	5.59±1.11	11.41±3.71	7.48±0.94	8.57±2.37	7.58±1.48	13.59±4.89
time (h)	0 – 48	0 - 48	6 - 48	0 - 48	0 – 48	0 - 72	0 - 48	0 - 48
PHASE II								
$t_{½}$ (h)	67.54±14.4	34.3±4.02	32.8±3.4	29.5±3.3	59.3±19.3	34.5±8.0	41.6±5.8	40.2±10.3
time(h)	48 - 168	48 -168	48 - 168	48 - 168	48 – 168	72 - 168	48 - 168	48 - 168

Table 6.1.1-8: Absorption after oral administration of 2 mg/kg ^{14}C-endosulfan to rats.

	MALES	FEMALES
$AUC_{120\ h}$	61.4 %	69.7 %
Renal excretion	86.7 %	92.0 %

Conclusions

Orally or intravenously applied endosulfan is eliminated rapidly by rats leaving very low residues after 7 days in kidney, liver, and retroperitoneal fat only.

Rat 30-day feeding study with tissue analysis			
Autor(s):	Leist KH & Mayer D.	**Study design:**	Assessment of helath condition. Acclimatisation period: 7 days. Rats housed single in a metabolism cage. Twice daily for general health and behaviour; weekly control of body weight, feed and water consumption as well as for neurological disturbances, impairment to eyes, oral mucosa or dental growth. Necropsy: After terminal sacrifice all animals were thoroughly examined for external and internal abnormalities; weight of the following organs was determined: brain, kidneys, liver; histological examinations including electron microscopy were performed on tissues of these three organs, taken from a few animals of each group. Livers and kidneys not required for histological examination as well as blood of all animals were analysed for residues.
Study Title:	**Endosulfan - Active ingredient technical (code: Hoe 002671 0I ZD97 0003), 30-day feeding study in adult male Wistar rats. IIA**, 5.1.2.2/01		
Testing facility:	[redacted]		
Report Number:	A37112		
Study duration:	From September 17 1984 to November 15 1984	**Dose:**	Multiple oral dose (4 weeks) at 360 and 720 mg/kg
Date of report:	1987	**Vehicle/Solvent:**	
Test Substance:	^{14}C labelled endosulfan	**Route:**	Oral (in diet)

Batch Nº.:	Hoe 002671 00 ZD97 0003	**Statistics/ Measurements:**	
Radiochemical purity:	97.9 %		
Test Animals:	Male Wistar rats	**GLP:**	Yes
Origin:	[redacted]	**Guideline:**	OECD principles of GLP, annex 2 of OECD guidelines for testing of chemicals, 1981
Bodyweight:	138-168 g	**Deviation:**	
Groups:	3 groups, 20-100 animals/group	**Acceptability:**	The study is acceptable

Results

The study was designed to investigate pigmentation observed in kidneys of treated animals in earlier studies. During the study no deviations between animals in the control and treated groups were found for any of the observed parameters. Mortality was restricted to one animal out of each dose group. Liver weights were increased at the end of the treatment period in animals receiving 360 and 720 mg, kidney and brain weights in the 720 mg group only. These effects had normalised at the end of the recovery period. Histological examinations revealed granular pigmentation and an increase in the number of lysosomes in the cells of the proximal convoluted tubules of the kidneys for both, the 360 and, especially, the 720 mg group. These symptoms were much less marked at the end of the recovery period. By staining technique (Prussian blue reaction) it could be demonstrated, that the pigmentation was not caused by siderin deposition. Residue analysis revealed treatment related residues of endosulfan (mainly α-endosulfan) and its metabolites in the kidneys. The liver contained mainly endosulfan-sulphate and -lactone, though at much lower concentrations. Residues in the blood were still lower. The storage is reversible, as minor amounts of α-endosulfan in the kidney were the only detectable residues at the end of the recovery period. Thus the discoloration in kidneys must be regarded as a symptom of transitory storage of endosulfan in connection with its metabolisation in the lysosomes. There is no indication that this inflicts damage to the cells. Accordingly, this discoloration has to be considered as a symptom of detoxification of endosulfan in treated animals.

Conclusions

Discoloration in kidneys of rats as observed after prolonged administration of sublethal doses of endosulfan is not a toxic effect.

B.5.1.2 Mouse

Mouse oral single and multiple dose toxicokinetic studies			
Autor(s):	Christ OE & Kellner HM.	**Study design:**	Animals housed in groups of 5 in metabolism cages (feed and water *ad libitum*). The animals were treated as: a)About 4 mg/kg b.w. were applied by
Study Title:	**Investigations with ^{14}C-endosulfan in mice.** 5.1.1.2/01 and 5.1.2.3/01		
Testing facility:	[redacted]		

Report Number:	A53842		gavage to 3 groups each of 5 animals (sacrifice: 1 group each after 1, 5 and 24 days) b) Animals were fed standard feed spiked with labelled substance for 1 day, thereby ingesting 4.7± 1 mg/kg b.w. (sacrifice: 1 group each on day 2, 6, 22 and 46); and c)Animals were fed standard feed spiked with labelled substance for 21 days, thereby ingesting on the average 2.4±0.7 mg/kg b.w./day; after 21 days the animals received unspiked diet until sacrifice (sacrifice: 1 group each on day 1, 5, 23 and 35 after treatment). Urine and faeces were collected daily except on weekends (combined sample of 3 days). Major organs and tissues were collected after sacrifice. All radioactivity measurements were performed by LSC.
Study duration:	Not provided in the report	**Dose:**	Single oral dose: 4 mg/kg Multiple oral dose: 2.4 mg/kg
Date of report:	1968	**Vehicle/Solvent:**	
Test Substance:	^{14}C labelled endosulfan	**Route:**	Oral (in diet)
Batch N°.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:	Not provided in the report		
Test Animals:	Albino mice	**GLP:**	Prior to GLP
Origin:		**Guideline:**	
Bodyweight:	20 g	**Deviation:**	
Groups:	7 groups, 5 animals/group	**Acceptability:**	The study is acceptable

Results

In the experiment a), cumulative elimination of radioactivity are summarised in Table 6.1.2-1. The liver contained the highest residues (30 nMol/g) 1 day after application, and the fat 12 nMol/g. Residues in all other organs were much lower at this date. After 24 days, the liver contained 0.38 nMol/g and the fat 0.15 nMol/g. Higher residues were found at this date in the spleen (0.63 nMol/g) and in the lung (0.45 nMol/g). In the experiment b), cumulative elimination of radioactivity are summarised in Table 6.1.2.-2. Elimination was nearly complete 24 h after withdrawal of spiked feed. The liver contained the highest residues (4.5 nMol/g) 1 day after application, the kidney 1.2 nMol/g, and all other organs still less. After 5 days, residues were below 0.5 nMol/g in all organs, and after 45 days they were 0.15 nMol/g in the spleen (where accumulation started rather late), 0.1 nMol/g in the liver, and below that value in all other organs. In the experiment c), About 90 % of total ingested radioactivity were eliminated 1 day after last treatment. Elimination was mainly via faeces (about 85 to 90 % of total elimination). The liver contained the highest residues (17 nMol/g) one day after application, the kidney was second with 4.3 nMol/g, and the lung third with 4.0 nMol/g. At this date the spleen contained 2.1 nMol/g and all other organs and tissues except muscles between 1 and 2 nMol/g. In the muscle 0.8 nMol/g were found. 5 days after the last treatment concentrations had not changed much in most organs. Exceptions were the liver with a decrease to 12

nMol/g and the spleen with an increase to 3.3 nMol/g. After 35 days all organs contained less than 1 nMol/g except the liver (2.1 nMol/g).

Table 6.1.2-1: Cumulative elimination of radioactivity after single oral gavege dose of 4 mg/kg b.w.)

	% OF APPLIED DOSE	
DAYS AFTER APPLICATION	**Faeces**	**urine**
1	36.0	7.9
5	77.0	13.8
24	85.6	8.5

Table 6.1.2-2: Cumulative elimination of radioactivity after the animals were fed with labelled substance for 1 day (4.7 ± 1 mg/kg b.w.)

	% OF APPLIED DOSE	
DAYS AFTER APPLICATION	**Faeces**	**urine**
1	94.0	9.2
5	88.9	8.7
21	89.6	9.1
35	74.8	9.2
45	92.3	7.6

Conclusions

After a single oral dose endosulfan is eliminated rapidly in mice, mainly via faeces. Residues in organs are generally low. Highest residues are found in the liver shortly after treatment and in the spleen after some weeks. After repeated oral dosing of mice with endosulfan, about 90 % of ingested radioactivity is eliminated rapidly, mainly via faeces. Some residues in organs still exist 35 days after the last treatment.

Schuphan, I., Ballschmiter, K. & Tölg, G.	See B.6.1.1 point.
1968	

Gupta PK & Gupta RC	See B.6.1.1 point.
1979	

Oral Administration in mice/multiple dose. Mammalian metabolism. In vitro studies.			
Autor(s):	Robacker KM, Kulkarni AP & Hodgson E	**Study design:**	Animals were maintained for 1 week before treatment Fed (Lablox small animal Chow) and water *ad libitum*. The animals were killed on the fourth day by decapitation, livers were removed and freshly prepared microcosms were used for all assays. Samples of stored microsomal preparations were examined by electrophoresis.
Study Title:	**Pesticide induced changes in the mouse hepatic microsomal cytochrome P-450-dependent monooxygenase system and other enzymes.** 5.1.3.2/02		
Testing facility:	[redacted]		
Report Number:	A35754		
Study duration:	Not provided in the report	**Dose:**	Multiple oral dose (3 days): 5 mg/kg/day
Date of report:	1981	**Vehicle/Solvent:**	Corn oil
Test Substance:	endosulfan	**Route:**	Oral (by gavage)
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:	Not provided in the report		
Test Animals:	Male mice	**GLP:**	Prior to GLP

Origin:	[redacted]	**Guideline:**	
Bodyweight:		**Deviation:**	
Groups:		**Acceptability:**	The study is acceptable

Results

In comparison with the control, endosulfan did not significantly influence neither liver weight nor cytochrome P-450 content. There was no significant induction of NADPH-dependent enzyme activity using DCPIP as electron acceptor. On the basis of cytochrome c as electron acceptor, endosulfan caused significant induction. Concerning glutathione peroxidase there was no definite evidence that this enzyme is inducible by endosulfan.

Conclusions

Investigations on the induction of cytochrome P-450 and monooxygenase activity by endosulfan resulted in data showing that the cytochrome P-450 group of enzymes is not significantly activated by endosulfan.

B.5.1.3 Rabbit

Gupta PK & Gupta RC	See B.6.1.1 point.
1979	

B.5.1.4 Sheep

Sheep oral multiple dose excretion study			
Autor(s):	Gorbach SG.	**Study design:**	Milk, tissue fat, faeces, urine and animal tissues were collected, and the samples were analysed by chromatography.
Study Title:	**Investigations on Thiodan in the metabolism of milk sheep.** 5.1.1.3/01 and 5.1.2.4/01		
Testing facility:	[redacted]		
Report Number:	A14209		
Study duration:	From June 20th 1964 to July 16th 1964	**Dose:**	Multiple oral dose (26 days): 15 mg/day
Date of report:	1965	**Vehicle/Solvent:**	
Test Substance:	^{14}C labelled endosulfan	**Route:**	Oral (fed by means of gelatine capsules)
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:	Not provided in the report		
Test Animals:	Lactating milk sheep	**GLP:**	Prior to GLP
Origin:		**Guideline:**	
Bodyweight:	40-66.5 kg	**Deviation:**	
Groups:		**Acceptability:**	The study is not acceptable. The number of animals used does not permit obtaining significant results.

Results

After a feeding period of 26 days, endosulfan was found unchanged in the faeces only. About 10 - 20% of the administered endosulfan was excreted with the faeces. No endosulfan is detectable in the various organs (kidneys, liver, muscle, brain tissue, kidney fat and intestinal fat). Degradation products are endosulfan-alcohol and endosulfan-sulphate. Endosulfan-alcohol was largely found in the urine. The highest concentration of endosulfan-alcohol found in urine was 1 mg/kg. Thus with a daily urine quantity of 1.5 l up to 10% of the daily dose is excreted in the form of endosulfan-alcohol. The urine contained a further endosulfan derivative which could only be extracted after weakly alkaline hydrolysis of the urine and detected gaschromatigraphically after acetylation. On the supposition that the concentrations on which the peaks are based are comparable with those of the endosulfan-alcohol, the amount found in the analysed sample was about 3 mg/kg. Consequently, about 50% to 60% of the total amount of endosulfan offered to the animal body is detected.

Conclusions

Investigations on the degradation and metabolism of endosulfan in lactating sheep resulted in the following Findings Unchanged endosulfan was found in the faeces only (about 10 - 20% of the administered dose). No unchanged endosulfan was found in the various organs. Degradation products are endosulfan-alcohol and endosulfan-sulphate. Endosulfan-alcohol was largely found in the urine. In total 50 to 60% of the amount of endosulfan administered to the sheep were detected.

<table>
<tr><th colspan="4">Sheep oral single dose excretion study</th></tr>
<tr><td>Autor(s):</td><td>Gorbach SG, Christ OE, Kellner HM, Kloss G & Boerner E.</td><td>Study design:</td><td rowspan="4">The animals were individually housed in metabolism cages. The radioactivity in blood was determined at 2, 4, 6, 8, 12, 24 and 48 h after administration, then daily to the 21st day after withdrawal from a vein of the neck or leg. The sheep were milked in the morning and evening and the portions were collected daily. Urine and faeces were collected separately throughout the experiment and the excretion over a 24-h period was examined. 40 days after administration one animal was sacrificed, and various organs and tissues were removed for the determination of radioactivity. The samples were analysed by thin-layer chromatography, GC and LSC.</td></tr>
<tr><td>Study Title:</td><td>Metabolism of endosulfan in milk sheep.IIA, 5.1.4</td></tr>
<tr><td>Testing facility:</td><td>[redacted]</td></tr>
<tr><td>Report Number:</td><td>A14216</td></tr>
<tr><td>Study duration:</td><td>Not provided in the report</td><td>Dose:</td><td>Single oral dose: 0.3 and 0.26 mg/kg</td></tr>
<tr><td>Date of report:</td><td>1965</td><td>Vehicle/Solvent:</td><td>acetone</td></tr>
<tr><td>Test Substance:</td><td>^{14}C labelled endosulfan</td><td>Route:</td><td>Oral (in fed)</td></tr>
<tr><td>Batch Nº.:</td><td>Not provided in the report</td><td rowspan="2">Statistics/ Measurements:</td><td rowspan="2"></td></tr>
<tr><td>Radiochemical purity:</td><td>Not provided in the report</td></tr>
<tr><td>Test Animals:</td><td>East Friesian milk sheep</td><td>GLP:</td><td>Prior to GLP</td></tr>
<tr><td>Origin:</td><td></td><td>Guideline:</td><td></td></tr>
<tr><td>Bodyweight:</td><td>49-52 kg</td><td>Deviation:</td><td></td></tr>
</table>

Groups:	3 animals	**Acceptability:**	The study is not acceptable. The number of animals used does not permit obtaining significant results.

Results

In blood the highest activity was measured 24 hours after application (Table 5.1.4-1). It was equivalent to 0.007 µg endosulfan / ml. Until day 21 the concentration fell to 0.0007 µg/ml. In milk the highest concentration (0.15 µg/g) was reached after 24 hours too (Table 5.1.4-2). It diminished to less than one tenth of the peek value within one week. Up to 88 % of the radioactivity remained in the cream after centrifugation of the milk. It could be characterised as endosulfan sulphate. Within 22 days about 40 % of the radioactivity were eliminated with the urine and about 50 % with the faeces, with peak elimination after 24 and 48 hours respectively (Table 5.1.4-3.). With the faeces mainly unchanged endosulfan was excreted, while the urine contained no parent material but mainly endosulfan alcohol and endosulfan hydroxy ether. 40 days after treatment the organs contained very low residues. The highest activity was detected in the liver (equivalent to 0.03 mg/kg).

Table 5.1.4-1: Radioactivity level in blood calculated as µg/g of whole blood (ppm).

Time after administration	**ppm Endosulfan**	
	sheep 1	**sheep 2**
2 hours	0.016	0.022
4	0.025	0.038
6	0.027	0.050
8	0.037	0.054
12	0.061	0.058
24	0.064	0.061
48	0.062	0.059
2 days	0.047	0.050
3	0.038	0.039
7	0.018	0.024
14	0.010	0.010
21	0.006	0.007

Table 5.1.4-2: Radioactivity of milk in % of that administered.

Time after administration (days)	**sheep 1**		**sheep 2**	
	sample vol (ml)	**%**	**sample vol (ml)**	**%**
1	560	0.35	430	0.46
2	460	0.33	500	0.45
3	290	0.13	460	0.44
4-7	200	0.037	3 200	0.35
8-12	490	0.019	2 900	0.088
13-17	200	0.002	3 100	0.036
Total		0.868		1.824

Table 5.1.4-3: Radioactivity of urine and faeces (% of that administered)

Time after administration (days)	Urine, %		Faeces, %	
	sheep 1	**sheep 2**	**sheep 1**	**sheep 2**
1	18.5	18.5	9.8	11.6
2	13.4	3.6	20.8	18.6
3	5.6	13.0	6.0	7.6
4	2.1	2.9	3.6	4.6
5	0.84	1.2	1.5	2.3
6-7	0.67	1.0	1.2	3.4
8-12	0.48	0.82	5.8	3.4
13-17	0.21	0.19	0.28	0.44
18-22	0.11	0.11	0.18	0.20
Total	41.91	41.32	49.16	52.14

Conclusions

Labelled endosulfan fed to sheep was rapidly excreted via faeces and urine. While in the urine only metabolites could be detected, the faeces contained mainly unchanged substance. Residues in milk and organs diminished to very low levels.

Gupta PK & Gupta RC	See B.6.1.1 point.
1979	

B.5.1.5 Goat

Goat oral multiple dose toxicokinetic study			
Autor(s):	Indranignsih, MxSweeney CS & Ladds PW.	**Study design:**	The animals were placed in individual metabolism cages and fed *ad libitum* a diet of summer supplemented with 100 g of cracked corn a day. Drinking water was available *ad libitum*. The adults goats were dosed orally once daily with 1 mg/kg b.w. for 28 days. On days 1, 8, 15 and 21 after last treatment, 1 group was killed. Samples of milk and venous blood were taken from each animal before being killed. Samples of major organs and muscle were removed at necropsy and were analysed for α- and β-endosulfan and endosulfan-sulphate. The samples were analysed by GC, and plasma was analysed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamil transferase (GGT), alkaline phosphatase (AP) and total bilirrubine.
Study Title:	**Residues of endosulfan in the tissues of lactating goats.** 5.1.2.5/01		
Testing facility:	[redacted]		
Report Number:	A51447		
Study duration:	Not provided in the report	**Dose:**	Multiple oral dose (28 days): 1mg/kg/day
Date of report:	1993	**Vehicle/Solvent:**	
Test Substance:	endosulfan	**Route:**	Oral (in gelatin capsules)

Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:			
Test Animals:	Lactating feral goats	**GLP:**	Prior to GLP
Origin:		**Guideline:**	
Bodyweight:	25-40 kg	**Deviation:**	
Groups:	3 adult goats and their respective kids	**Acceptability:**	The study is acceptable

Findings

There were no clinical signs of toxicity in the 12 goats during the dosing period and feed intake during the fourth week of dosing were the same as in the first week. Total residues of α- and ß-endosulfan and endosulfan.sulphate were summarised in Table 5.1.5-1.

Table 5.1.5-1: Total residues of endosulfan isomers and endosulfan-sulphate, in mg/kg b.w.

	DAY OF SAMPLING AFTER TREATMENT ENDED		
	1	**8**	**15**
kidney	0.29	0.47	0.20
gastro-intestinal tract	0.20	<0.01	ND
liver	0.12	<0.01	ND
brain	0.06	<0.01	ND
fat	0.06	<0.01	ND
spleen	0.04	<0.01	ND
muscle	0.04	<0.01	ND
lung	0.006	<0.01	ND
heart	0.006	<0.01	ND
milk	0.02	<0.01	ND

ND: not detected.

On day 21 of sampling after treatment ended, residues were not detected in any tissues

Conclusions

In conclusion, it would appear that endosulfan residues in the fat of meat of lactating goats would not exceed current Australian maximum residue limits unless the animals were acutely intoxicated.

B.5.1.6 Cattle

Cow oral multiple dose toxicokinetic study			
Autor(s):	Bowman JS.	**Study design:**	During the last 7 days of the predosage period, throughout the 30-day feeding period and during the 14-day recovery period, milk and jugular blood samples were collected from each control and test animal. At the termination, 2 cows from each group were sacrificed and gross autopsies performed. Omental fat, liver, kidney, muscle, brain, pancreas, small and large intestine, compound stomach, heart and rib bone were collected. The samples were analyzed by LSC.
Study Title:	**Subacute feeding - dairy cows, preliminary report.**5.1.2.6/01		
Testing facility:	[redacted]		
Report Number:	A14205		

Study duration:	Not provided in the report	**Dose:**	Multiple oral dose (14 days): 0.3, 3 and 30 ppm
Date of report:	1959	**Vehicle/Solvent:**	
Test Substance:	^{14}C-labelled thiodan	**Route:**	Oral (in diet)
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:			
Test Animals:	Lactating Holstein cows	**GLP:**	Prior to GLP
Origin:		**Guideline:**	
Bodyweight:	25-40 kg	**Deviation:**	
Groups:	4 groups, 3 cows each	**Acceptability:**	The study is acceptable

Findings

Food consumption and milk production for each animal were wthin normal limits during a predosage period, a 30-day feeding period, and a 14-day recovery period. Radioanalysis of milk samples taken at intervals during the experimental and recovery periods indicated a rapid secretion of Thiodan C-14 equivalents in the milk, with an average of 3.4, 40 and 462 ppb appearing after 7 days of feeding at the 0.3, 3.0, and 30 ppm levels, respectively. After eliminating Thiodan from the diet, the concentration of residues in the milk rapidly decreased. Blood samples were collected at intervals during the study and samples of various tissues collected at termination of the 30-day feeding period and the 14-day recovery period. These samples are being held frozen for future radioanalysis.

Conclusions

Residue levels in the milk after 7 days of the feeding period were proportional to dose and decreased rapidly after eliminating Thiodan from the diet.

Cow oral multiple dose toxicokinetic study			
Autor(s):	Keller JC.	**Study design:**	During the last 7 days of the predosage period, throughout the 30-day feeding period and during the 14-day recovery period, milk and jugular blood samples were collected from each control and test animal. At the termination, 2 cows from each group were sacrificed and gross autopsies performed. Omental fat, liver, kidney, muscle, brain, pancreas, small and large intestine, compound stomach, heart and rib bone were collected. The samples were analyzed by LSC.
Study Title:	**Subacute feeding study - dairiy cows (supplement to report dated March 20, 1959).**5.1.2.6/02		
Testing facility:	[redacted]		
Report Number:	A14206		
Study duration:	Not provided in the report	**Dose:**	Multiple oral dose (14 days): 0.3, 3 and 30 ppm
Date of report:	1959	**Vehicle/Solvent:**	
Test Substance:	^{14}C-labelled thiodan	**Route:**	Oral (in diet)
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:			
Test Animals:	Holstein dairy cows	**GLP:**	Prior to GLP
Origin:		**Guideline:**	

Bodyweight:	25-40 kg	**Deviation:**	
Groups:	4 groups, 3 cows each	**Acceptability:**	The study is acceptable

Findings

At the end of the 30-day feeding period the average endosulfan C^{14} -derived residues in the liver were 0.35, 2.45, and 25.3 ppm; in the kidney 0.05, 0.35, and 6.29 ppm; and in the omental fat 0.07, 0.71, and 7.08ppm, at the 0.3, 3.0, and 30.0 ppm dosage levels, respectively. During the 14-day recovery period 31.4%, 68.2%, and 63.2% of the residue was lost from the liver; 0.0%, 62.9%, and 78.2% from the kidney; and 71.4%, 83.1%, and 98.2% from the omental fat at the 0.3, 3.0, and 30.0 ppm dosage level, respectively. Radioanalysis of the jugular blood samples collected throughout the study demonstrated a gradual rise in concentration of endosulfan C^{14} equivalents during the first 21 days of the feeding period, with an average of 0.15 and 1.97 ppm at 3.0 and 30.0 ppm dosage levels, respectively. At the 0.3 ppm dosage level the residues in the blood were below the sensitivity of the method (0.06 ppm) throughout the study.

Conclusions

Testing the deposition and disappearance of endosulfan C^{14} in milk and certain body tissues of dairy cows resulted, at the end of the dosing period, in residue levels in all tissues proportional to the doses, indicating absence of bioaccumulation.

Metabolism study in cow			
Autor(s):	Beck EW, Johnson Jr. JC, Woodham DW, Leuck DB, Dawsey LH, Robbins JE & Bowman MC.	**Study design:**	2 steers were given each of the 4 treatments. Also, 2 steers were placed in metabolism stalls and twice daily fed rations that contained 1.10 mg of endosulfan (in acetone solution)/kg b.w. Omental fat samples were taken by biopsy from all steers before and after exposure. Analyses of the fat samples were made by colorimetric alkali-hexane partitioning.
Study Title:			
Testing facility:	Calliope		
Report Number:			
Study duration:	Not provided in the report	**Dose:**	0.15, 1.10, 2.50 and 5.00 mg/kg b.w./day
Date of report:	1966	**Vehicle/Solvent:**	
Test Substance:	endosulfan	**Route:**	Oral (in diet)
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:			
Test Animals:	Hereford steers	**GLP:**	Prior to GLP
Origin:		**Guideline:**	
Bodyweight:	600-800 lb	**Deviation:**	
Groups:		**Acceptability:**	The study is considered as additional information because in a review of the original paper.

Findings

Feeding study in cattle are summarised in Table 5.1.6-1. Residues in omental fat from steers having grazed on treated Coastal Bermuda grass are comparable to those animals grazing on untreated pasture

and no endosulfan sulphate was detectable. In silage feeding study, daily average of 7.1, 7.5, 7.4 and 7.8 kg of silage no treatment related effect. There are not detectable residues in milk.

Table 5.1.6-1: Feeding study in cattle.

dose/effect (mg/kg/day)	**0.15**	**1.10**	**2.50**	**5.00**
Toxicity[1] Muscle convulsions Salivation Incoordination			+[5] + +	+[6] + +
Residues in omental fat (ppm) - Day 0 - 30 days (metaboic study) - 60 days	 0 0	 0 0.5[3] 1.0[4]	 0 -	 0 -
Urinary excretion (mg/day)		18.5/15.9[2]		
Faecal excretion (mg/day)		6.7/5.0[2]		
Total excretion (% of daily dose)		7.4/4.9[2]		

-: Not determined. +: Sign observed. [1]: In 1 animal/treatment group; both pairs were immediately removed from the program and symptoms disappeared within 2 h. [2]: Format: value for steer 1/value for steer 2. [3]: This residue included some endosulfan for ½ animals of the metabolism study. [4]: At a later date re-analysed for endosulfan sulphate, and found negative. According to the paper, no endosulfan was detectable at that time in fat samples from animals fed control ration or 0.5 ppm in the diet. However, in the experiment design there is no mention of a group fed 0.5 ppm. [5]: After 13 days. [6]: After 2 days.

Conclusions

Cattle fed fattening ration at 1 % of their body weight, containing endosulfan at dose levels 0.15, 1.10, 2.50 and 5.00 mg/kg bw/day, showed signs of intoxication at the 2 highest doses; residues in omental fat were present in the 1.10 mg/kg bw group (0.5 and 1.0 ppm after 30 and 60 days, respectively), but not in the 0.15 mg/kg bw group. Total daily excretion in urine and faeces was 7.4 and 7.9 % of the dose (urine and faeces combined) for animals of the 1.10 mg/kg bw group; considering low residues in fat and the small % excretion, endosulfan must be metabolised by bovine. Residues in fat of cattle having grazed on pastures treated with endosulfan for 31 - 36 days (initial residue 10.6 - 102 ppm, final residue 1.53 - 3.05 ppm, on dry matter basis), were comparable to those of animals having grazed on untreated pastures. In a 21-day silage feeding study in lactating cows (0, 0.41, 0.70, and 2.35 ppm), no effect was seen on silage consumption or milk production; residues in milk were not detectable.

Gupta PK & Gupta RC	See B.6.1.1 point.
1979	

B.5.1.7 Dog

Gupta PK & Gupta RC	See B.6.1.1 point.
1979	

B.5.1.8 Pig

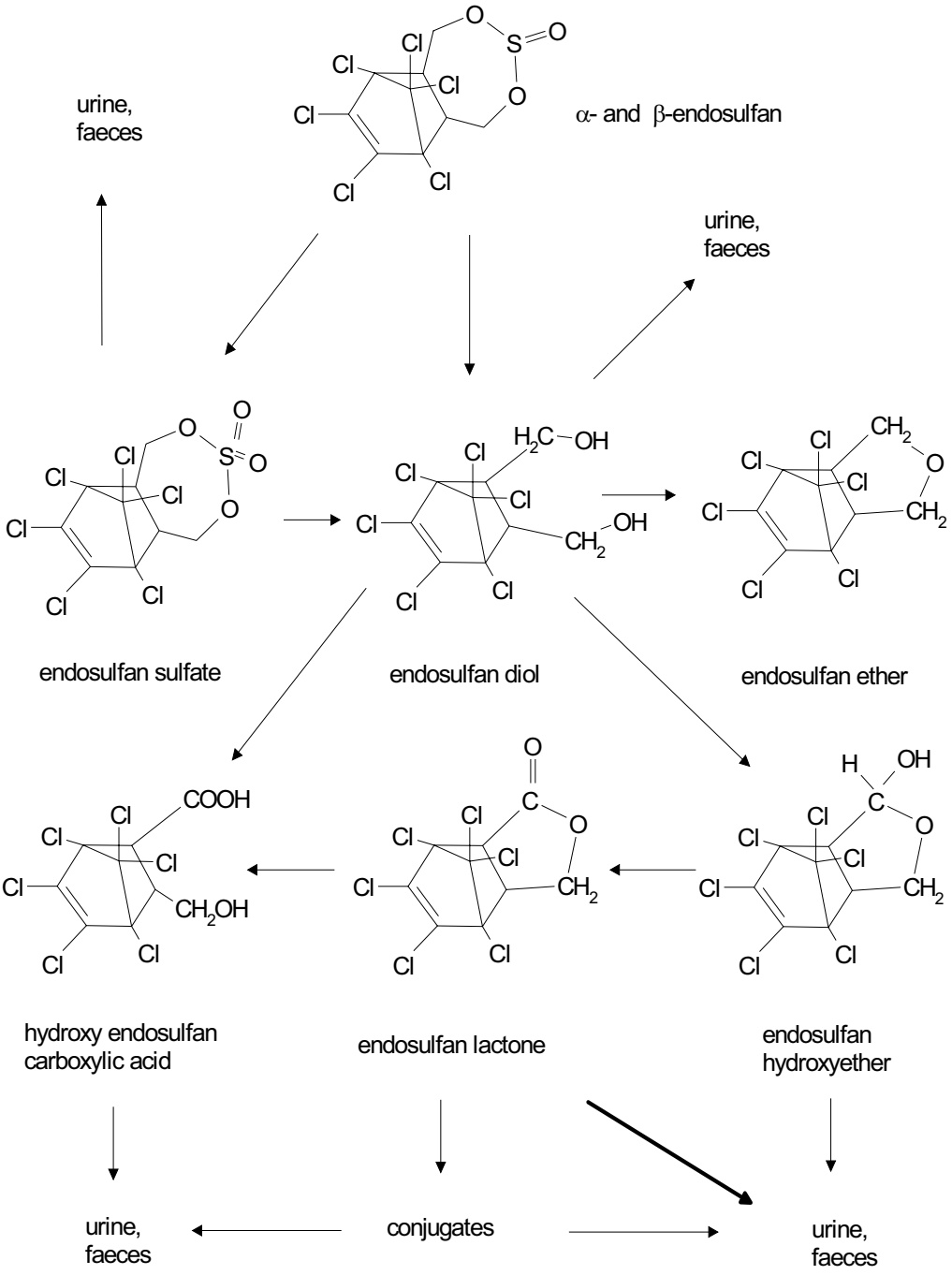
Gupta PK & Gupta RC	See B.6.1.1 point.
1979	

B.5.1.9 Human

Noctor JC & John SA.	See B.6.1.1 point.
1995	

B.5.1.2 Metabolic pathway of endosulfan in mammalians

Endosulfan is converted in the animal organism to the following metabolites: endosulfan-sulphate, endosulfan-diol, endosulfan-ether, endosulfan-hydroxyether and endosulfan-lactone. A number of unidentified polar metabolites are probably the conjugates of the metabolites.



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B.6.2 Acute toxicity including irritancy and skin sensitisation (IIA, 5.2)

Summary

Endosulfan has been thoroughly tested for acute toxicity, primary irritation and sensitisation potential. Three notifiers (Agrevo, Calliope and Excel) have submitted studies. However, the evaluation was confined to studies considered acceptable.

Results obtained in these studies are summarised in Table B.6.2-1.

According to these results:

1. The acute oral median lethal dose LD_{50} of Endosulfan Technical in rats was calculated to be 10 mg/kg bw for female rats. This result would require an EEC classification of “T+” (very toxic) for the technical active ingredient, if based on the more sensitive sex alone.

2. The dermal LD_{50} value for Endosulfan Technical in rats was greater than 4000 mg/kg b.w for males and 500 mg/kg b.w. for females. These results would require an EEC classification of "Xn" (harmful) for the technical active ingredient.

3. For Endosulfan technical an acute inhalation LC_{50} of 0.0345 mg/l air in male Wistar rats, and of 0.0126 mg/l air in females was determined. These results may require an EEC classification of "T+" (very toxic).

4. Endosulfan technical was designated as not irritating to skin. Not classified according to EU criteria.

5. Endosulfan technical was designated as not irritating to eyes. Not classified according to EU criteria.

6. Based on the skin sensitisation studies there is no evidence that Endosulfan is a contact allergen and it is not classified based on EU criteria

In conclusion, based on acute oral toxicity studies in rats, and in accordance with EU criteria for classification, packaging and labelling of dangerous substances, Endosulfan is classified as ‘very toxic’, assigned the symbol “T+” and the risk phrase ‘R28 very Toxic if swallowed’. Based on the dermal LD_{50} value in rats, it also should be classified as “Harmful” and be associated with the risk phrase “R21 harmful in contact with skin”. Based on results of the acute inhalation study in rat, Endosulfan should be classified as ‘very toxic’, assigned the symbol “T+” and the risk phrase ‘R26 very Toxic by inhalation’ in accord with EU Guidelines as additional information.

Table B.6.2-1: Summary of acceptable acute toxicity studies

Route/Species/Sex	Dose range (mg/kg bw)	Vehicle	Result	Reference
Oral				
Rat, Sherman, m	20, 32, 50, 80	ground-nut oil	LD_{50} = 48 mg/kg (m)	Scholz, 1971a AgrEvo IIA, 5.2.1/7
Rat, Sherman, f	6.3, 8.0, 10.0, 12.5	ground-nut oil	LD_{50} = 10 mg/kg (f)	Scholz, 1971b AgrEvo IIA, 5.2.1/8
Rat, Wistar, m/f	50, 100, 160, 250, 315 (m) 12.5, 25, 50 (f)	starch mucilage	LD_{50} = 100-160 mg/kg (m) LD_{50} = 22.7 mg/kg (f)	Diehl 1988b AgrEvo IIA,5.2.1/10
Dermal				
Rat, Wistar, m/f	3150, 4000 (m) 400, 630, 1000 (f)	-	LD_{50} > 4000 mg/kg (m) LD_{50} = 500 mg/kg (f)	Diehl 1988a AgrEvo IIA, 5.2.2/2
Inhalation				
Rat, SPF Wistar m/f	0.0123, 0.0288, 0.040, 0.0658 mg/L (m) 0.0036, 0.0123, 0.0288, 0.040, 0.0658 mg/L (f)	Ethanol-polyethylene 50:50	LC_{50} = 0.0345 mg/L (m) LC_{50} = 0.0126 mg/L (f)	Hollander 1983 AgrEvo IIA, 5.2.3/1
Skin irritation				
Rabbit, New Zealand white,	0.5 g	----	Non irritant	Bremmer1997a* AgrEvo A58442
Eye irritation				
Rabbit, New Zealand white, f	0.1 g	----	Non irritant	Bremmer1997b* AgrEvo A58443
Skin sensitisation				
Guinea pig, SPF Pirbright-White, f (Buehler test)	-	Polyethylene glycol 40%	No Sensitizer	Jung 1983 AgrEvo IIA, 5.2.6/1
Guinea pig, albino, m (M&K)	-	Corn oil	No Sensitizer	Arcelin1996* AgrEvo A58132

*Studies not included at the original monograph.

Evaluation of studies not included at the original monograph

Bremmer, J.N., 1997a (A58442, AgrEvo)

Dates of experimental work: Start of study; December 10, 1996; termination of study December 13, 1996. The method followed the US EPA FIFRA Guideline No 81-5, which is comparable to 92/69/EEC part B4, OECD 1981 Nº 404 and Japan MAFF 1985.

GLP. Yes

The study is acceptable.

Materials and methods

A group of three New Zealand white rabbits, source Charles River, Deutschland GmbH, weighing 2.0 to 2.2 Kg were used. Endosulfan substance technical, lot number Hoe 002671 00 ZD99 0008, purity 98.6% w/w, (0.5 g) was moistened with 0.25 mL deionized water and applied dermally to an approximate 2.5 cm x 2.5 cm cellulase patch on a piece of surgical plaster for the 4-hour exposure period. The plaster was fixed to the prepared skin area and then covered with a semi-occlusive bandage. The remaining untreated skin on each rabbit served as the control.. Dermal scoring and evaluations were conducted at 30 to 60 minutes, and 24, 48 and 72 hours following the 4-hour exposure period using the Draize method. In addition to these scores, any additional dermal observations were recorded, if present.

Findings

The test material did not produce erythema, edema or any other dermal effects in any of the rabbits.

Conclusions

Endosulfan was shown to be a non-irritant to the skin of albino rabbits.

Bremmer, J.N., 1997b (A58443 AgrEvo)

Dates of experimental work: Start of study; December 10, 1996; termination of study December 13, 1996. The method followed the US EPA FIFRA Guideline No 81-4, which is comparable to 92/69/EEC part B5, OECD 1981 Nº 405 and Japan MAFF 1985.

GLP. Yes

The study is acceptable.

Materials and methods

A dose of 0.1 g of the test material Endosulfan substance technical, lot number number Hoe 002671 00 ZD99 0008, purity 98.6% w/w, was placed into the lower conjunctival sac of the left eye of each of 3 female New Zealand White rabbits (source Chemical Pharmaceutical Factory Dr. K. Thomae., weight 2.7 to 3 Kg). The right eye was untreated for control purposes. The treated eyes were washed out 24 hours after application of the test substance. Observations for irritation and ocular lesions were performed at approximately 24, 48 and 72 hours after treatment based on the system of evatuation defined by the EEC. A primary irritation index after one hour was calculated based on the grading scala for ocular lesions employed by the EPA.

Findings

Based on the system of evaluation defined by the EEC, the following individual and group mean scores for ocular lesions after 24, 48 and 72 hours were calculated:

Animal Nº.	**Conjunctiva**		Opacity of cornea	Iris
	Redness	**Chemosis**		
93	0.00	0.00	0.00	0.00
94	1.00	0.00	0.00	0.00
95	1.00	0.00	0.00	0.33
Group mean	0.66	0.00	0.00	0.11

Conclusions

Based on the results obtained in this study Endosulfan is not subject to labelling requirements according to the criteria for classification in Directive 93/21/EEC.

Arcelin, 1996 (A58132 AgrEvo)

Dates of experimental work: Start of study; July 01, 1996; termination of study August 08, 1996.

The method followed the guideline B.6 (Acute toxicity-Skin sensitisation of Directive 92/69 EEC July 31, 1992), which is comparable to, OECD guidelines for testing of chemicals, Nº 406 "Skin Sensitization", adopted by the Council on July 17, 1992.

GLP: Yes

The study is acceptable.

Materials and methods

The cutaneous allergenic potential of endosulfan (purity 98.6%) was examined in 20 treated and 10 control male albino guinea pigs (source: BRL, Biological Research Laboratories Ltd.). The maximal tolerated concentration of endosulfan suitable for the induction phase of the main study and a suitable non-irritancy concentration of topically applied endosulfan were identified for the challenge application in a preliminary study. For intradermal induction, injection of 0.1 ml of 0.5% solution in corn oil emulsified 1:1(v:v) with Freund´s complete adjuvent was selected. One week after these injections, a 6-cm^2 patch of filter paper saturated with about 0.3ml of a 50% solution of endosulfan in corn oil was applied to the shaved skin of each guinea-pig, and the area was occluded with aluminium foil secured by impermeable adhesive tape, which was left in position for 48h. Irritation was assessed 24 and 48h later. On test day 22, the guinea pigs were challenged with the non-irritating 50% endosulfan in corn oil applied as during the induction phase. The dressing was sleft in position for 24 h. The application sites were assessed for erythema and oedema 24 and 48 h later.

Findings

None of the treated guinea-pig developed skin reactions.

ERITHEMATOUS REACTIONS AFTER THE CHALLENGE PROCEDURE

	After 24 hours **Positive/Total** ……	**After 48 hours** **Positive/Total** ……

	% positive of total	% positive of total
CONTROL GROUP		
ENDOSULFAN	0/10	0/10
(Left flank)	……..	……..
	0	0
Corn oil only	0/10	0/10
(Right flank)	……..	……..
	0	0
TEST GROUP		
ENDOSULFAN	0/19*	0/19
(Left flank)	……..	……..
	0	0
Corn oil only	0/19	0/19
(Right flank)	……..	……..
	0	0

One animal of the test group was found death related to the treatment on test day 12 (prior to de 48-hour reading of the epidermal induction) No toxic level was noted in the remaining animals.

No toxic syntoms in the Guinea Pig of the control or test group.

Conclusions

Based on the results obtained in this study Endosulfan is not subject to labelling requirements according to the criteria for classification in Directive 93/21/EEC. Endosulfan was therefore considered to be non-sensitizing for guinea-pig skin.

B.6.3 Short-term toxicity (IIA, 5.3)

Summary

Endosulfan has been thoroughly tested for oral short-term toxicity. Two notifiers (Agrevo and Excel) have submitted studies. Agrevo also submitted data from other routes, three 28-day dermal studies in rat and one nose-only inhalation study in rats.

However, the evaluation was confined to studies considered acceptable.

Results obtained in these studies are summarised in table B.6.3-1.

According to these results:

1. The oral NOAEL in rat was 3.85 mg/kg bw/day for males (90-day study).
2. The oral NOAEL in mouse was 2.3 mg/kg bw/day (90-day study).
3. The oral NOAEL in dog was 0.65 mg/kg bw/day for males and 0.57 mg/kg bw/day for females (one-year study).
4. The dermal NOAEL in rat was 3 mg/kg bw/day males (28-day study).
5. The inhalation NOEL in rat was greater than 0.002 mg/L/day (29-day study).

Table B.6.3-1 Summary of subacute and subchronic acceptable toxicity studies

Study	NOAEL (mg/kg bw/day)	Main adverse effect	LOAEL (mg/kg bw/day)	Reference
Subacute studies				
30-days oral rats. Dose levels: 360 and 720 ppm (equal to 34 and 67.8 mg/kg/day)				Leist & Mayer, 1987 AgrEvo:IIA, 5.1.2.2/1
Subchronic studies				
90-day, diet, rat. Concentrations: 10, 30, 60 and 360 mg/kg feed (equal to 0.64, 1.9, 3.8 and 23 mg/kg/day for males and 0.75, 2.3, 4.6 and 27 mg/kg/day for females)	3.85 (m)	Haematological changes	23.41 (m)	Barnard *et al*., 1985** (AgrEvo IIA, 5.3.2.1/2)
90-day, diet, mouse CD-1 Concentrations: 2, 6, 18, and 54 mg/kg feed (equal to 0.24, 0.74, 2.13 or 7.3 mg/kg/day for males and 0.27, 0.80, 2.39 or 7.5 mg/kg/day for females)	2.3 (m/f)	Lethality and neurological signs	7.4 (m/f)	Barnard *et al*., 1984 (AgrEvo IIA, 5.3.2.4/1)
42 day, diet, mouse NMRKf Dose level:18 ppm				Donaubauer *et al* 1985 AgrEvo IIA, 5.3.2.5/1
1-year, diet, Beagle dog. Dose levels: 3, 10, 30 ppm.(equivalent to 0.23, 0.77 and 2.3 mg/kgbw/day).	0.65 m 0.57 f	LOAEL based on the clinical signs (violent muscular contractions of the abdominal muscles),and reductions in body weights-	2.3	Brunk (1989; 1990)* (AgrEvo: 5.3.2.3/3).
Other routes				
28-day dermal, rat Dose levels: 1, 3, 9, 27 and 81 mg/kg bw/day	3 (m)	Deaths seen at 9 mg/kg/day associated with clinical signs is considered to be typical of endosulfan toxicity. (despite to technical application error).	9 (m)	Ebert *et al* 1985a ** AgrEvo IIA, 5.3.3.1/1
28-day dermal, rat (males) Dose levels: 18.75, 37.50, 62.50 mg/kg bw/day for males and 9.83, 19.66, 32.00 mg/kg for females.		A NOAEL was not determined. Transient clinical symptoms were observed in the treated groups.		Dikshith *et al*. 1988 AgrEvo IIA, 5.3.3.1/4
29-days, nose-only inhalation, rat Dose Levels: 0.0005, 0.0010, 0.0020 mg/L	NOEL>0.002 mg/L	No symptoms up the highest dose tested were observed.		Hollander *et al* 1984 AgrEvo IIA, 5.3.3.2/1
Additional information				
Subacute oral toxicity study in rats. Dose level: 11 mg/kg bw/day	Not identified			Nath et al., (1978) (AgrEvo:ANRA)

*Studies not included at the original monograph.
**Re-evaluated studies.

Evaluation of studies not included at the original monograph and re-evaluation of studies included at the original monograph

Barnard, A.V.; Jones, D.R.; Powell, L.A.J., 1985 (AgrEvo: IIA, 5.3.2.1/2)
Study date: Start: 27 Jul 1983-End: 26 Oct 1983. Date of report: 25 Mar 1985
Test method: US-EPA. FIFRA draft guideline 1982.
GLP: Yes.
The study is acceptable.

Material and methods
5 groups of 25 male and 25 female Sprague-Dawley rats, aged about 4 weeks, source Charles River, received Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) purity 97.2 %, at dose level of 0, 10, 30, 60 and 360 mg substance /kg feed, vehicle acetone plus corn oil for preparation of pre mix, for 13 weeks, 5 each received untreated feed thereafter for a recovery period of 4 weeks.

Husbandry: 5 rats of same dose and sex per wire-mesh cage in air conditioned rooms with feed and water *ad libitum.*

Observations: twice daily for dead or moribund animals; once daily for the first four weeks, thereafter once a week detailed check for symptoms of intoxication; weekly control of weight and feed consumption; during week 6 and 13 (12) haematological and biochemical investigations were performed on the blood of 10 males and 10 females from each group; cholinesterase was investigated at the same time; during week 4 and 12 individual urine samples from 10 males and 10 females of each group were investigated. Identical observations were performed on the remaining animals at the end of the recovery period.

Necropsy: After terminal sacrifice (up to 20 animals from each dose/sex at the end of the treatment period, the remaining 5 at the end of the recovery period) all animals were thoroughly examined for external and internal abnormalities; weight of the following organs was determined: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, spleen, testes, thyroids, uterus;. histological study on a very wide range of tissues. Same exercise for animals that died during the study or were killed *in extremis*. Brain cholinesterase was measured from 10 males and 10 females of each dose at the end of the treatment period and from 5 females of each dose at the end of the recovery period.

Results
No treatment related death occurred.

Clinical signs: The only clinical symptom was an increased loss of dorsal hair in females of the 360 and 60 ppm groups, a finding which regressed by the end of the withdrawal period.

Body weight gain was slightly impaired at 360 ppm. This was partly associated with lower feed consumption and/or inferior feed utilisation. Findings during the withdrawal period were essentially similar between controls and previously treated rats.

The red blood picture was slightly impaired at 30 ppm and higher doses. Similar findings were still apparent at the end of the withdrawal period among males previously treated with 360 ppm.

Blood chemistry showed changes at the highest dose only, reaching similar values between controls and previously treated rats after withdrawal period.

Serum and RBC cholinesterase were lowered in the high dose group, while brain cholinesterase was increased in females at 60 and 360 ppm. The investigation at the end of withdrawal period revealed similar values between controls and previously treated rats.

At 360 ppm kidney and liver weights were increased in both sexes, brain weights in females only. At 60 ppm only males exhibited higher weights of livers and kidneys, while females still had higher brain weights. At the end of the withdrawal period, greater kidney weights were noted among males previously treated with 360 ppm, when compared with the controls.

The urine was dark in colour for the high dose animals and for the males at 60 ppm. It partly contained marginally more proteins and ketones when dark. No similar findings were recorded at the withdrawal period.

Microscopic findings are concentrated on the kidneys, which showed yellowish pigmentation of the cytoplasm in cells of proximal convoluted tubules. A darker granulated pigment was observed at higher doses too. These findings intensified with increasing dose. In male animals kept for a withdrawal period, discolouration decreased whereas granular pigment sill presisted or appeared in the 30 ppm groups in traces/minimal. In females at ≤ 60 ppm, the traces of pigmentation persisted. However, no adverse effects were reported that might be associated with these findings alone.

Conclusion

The NOAEL for oral treatment of male rats with endosulfan technical over 90 days is 60 mg substance /kg feed (ppm). This is equivalent to 3.85 mg/kg bw /day for male. This value of NOAEL is based on the haematological changes observed after recovery period (PCV, Hb and RBC in males $p < 0.01$ in comparison with control value) at 360 ppm dosage level.

Brunk, (1989). (AgrEvo IIA, 5.3.2.3/3) (AgrEvo: ANRA)

Date of report: January 20, 1989.

Brunk (1990) (AgrEvo: ANRA)

Addendum to report (Brunk, 1989).

The objective of this report was testing the toxicity of endosulfan by repeated oral administration (1-year feeding study) to Beagle dogs.

This study was conducted in accordance with OECD 452 and EPA test guidelines.

GLP: Yes

This study is considered as acceptable

Material and Methods

The test substance was Technical Endosulfan (96.5% purity). The test material was received by the testing facility in the form of corn meal premix, then stirred into the mixed food diet (Vipromix) The stability, content and homogeneity of the test substance in corn meal was tested and found suitable for the purposes of this study.

Four groups of Beagle dogs (6 per sex and dose) received at dietary concentrations of 0, 3, 10 and 30 ppm (equivalent to 0, 0.23, 0.77 and 2.3mg/kg bw/day) of endosulfan for one year. The control group received corneal (premix base) in the same proportion as the highest treatment

The dose levels were selected on the basis a preliminary study when endosulfan was fed in dietary concentrations of 10, 30 and 60 ppm In this pre-study a dose level of 3ppm administered orally in the diet to rats was tolerated without any reaction by one male and one female. After 14 treatments, a dose level of 30 ppm led to a disturbance of food consumption (delayed eating, or leftover food), and in the case of the female to vomiting and slightly staggering gait. Another pair of Beagle dogs were treated only twice with 60 ppm, since, after the second application, both of the animals already began to refuse their feed either partly or completely, and the female also vomited. Both animals continued to eat hesitantly during the observation period.

For the present study, toxic effects were expected to be produced by the highest dose level (30 ppm), whereas the lowest dose level (3 ppm) was to be free of substance-related changes. Between both these dose levels there was a logarithmic intermediate dose of 10 ppm.

The study protocol provided for a fifth group in the event that the 30 ppm was tolerated. The dose level for this group was increased in two stages from 30 ppm to 45 ppm to 60 ppm; this group was designated as 30/45/60.

Table B.6.3-2: Treatment groups

Group	**Dietary concentration (ppm)**	**Nº males**	**Nº females**
I	0	6	6
II	3	6	6
III	10	6	6
IV	30	6	6
V	30/45/60	6	6

The animals of group I-IV were killed on the day after the final treatment; the animals of group V were killed day 125 and day 146 of treatment.

Checks were conducted for death and behaviour twice daily, general health and food consumption daily, bodyweight (once weekly), neurological status at the initial of treatment , every 3 months and before the termination of study, ophthalmoscopic, hearing and dental signs. Haematological examinations were

carried out before the start of the study and every 3 months to determine erythrocytes, haemoglobin, haematocrit, leukocytes, thrombocytes, blood count, etc… Clinical chemistry (sodium, potassium glucose, calcium….) was checked 24 after treatment, every 3 months and before the termination of study. At the same time was realised urinalysis examinations.

Hepatic and renal function was also tested. Dissection and macroscopic examination were carried out immediately after the animals were killed by exsanguination to determine the weights of different organs and to realise microscopic examination on organs and specified tissues from all dogs.

The statistical evaluation was carried out comparing individual dose group with the control group. The level of significance was $p < 0.05$.

Results

After administration of 3 and 10 ppm no substance-related reactions or changes were observed.

No spontaneous deaths occurred during the study. At 30/45/60 ppm, one male was killed *in extremis* after 125 doses of endosulfan, and remaining animals in this group were killed on days 146 (6 females) or 147 (5 males) due to marked nervous conditions. One male in the 30 ppm group was killed on day 276 to prevent suffering as the animal was is very poor condition with extensive preputial oedema and oedematous swelling in the knee joints, due also in part to the refusal consumption observed. With the exception of the animals killed during the course of the study, no other animals displayed impairment of physical condition during the study.

On males, at 30 ppm and all dogs in the highest dose group (60 ppm) showed a deleterious of general condition. .No impairment was observed in any of the other animals.

During the first 7 weeks of the study, 3 males and 3 females at 30 ppm showed delayed or marginally reduced food consumption and also in the approximately half of the animals of group 30/45/60 during the first two weeks of the study. A more marked disturbance of food consumption affecting most of the animals became noticeable only during treatment with 60 ppm (in the 16^{th}-21^{st} weeks of the study).

The males treated with 30 ppm showed on average lower body weights gains compared both with the females in the group and with the animals in groups I-III (mean body weights approximately 8% lower than the control. group). In the 30/45/60 ppm group, marginal reductions in mean food consumption were observed at 30 ppm in the early phase of the study.

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 pronounce 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.

All the dogs treated with 3 and 10 ppm and 3 males and 4 females treated with 30 ppm exhibited no sings of abnormalities.

In 1 male and 4 female at 30/45/60 ppm, signs of impairment of the central nervous system were seen at the terminal examination, but no signs were seen in animals at other dose levels during the study. No adverse effects associated with treatment were observed in the opthalmoscopic, hearing or dental inspection.

Haematological and urinalysis examinations does not reveal any effects that were considered to be treatment related.

Clinical chemistry examination revealed a number of statically significant changes in parameter compared with control values but 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 examination during the study; these effects may be related to the administration of endosulfan. However, no gross or histopathological findings associated with these elevations in enzyme levels were observed.

Serum and erythrocyte cholinesterase activity appeared to be similar in control and treated group 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. In conclusion, it is not possible to determine whether treatment with endosulfan significantly affected cholinesterase in dogs.

No treatment related changes in organ weights. A single statistically significant increase in absolute liver weights was reported for males and females at 10 ppm, but in the absence of any effects at the high dose, this effect was not considered to be treatment related.

Conclusions

Dogs that were administered endosulfan in increasing concentrations of 30/45/60 ppm displayed a number of signs of intoxication, including tonic contraction and increased sensitivity to noise and optical stimuli. Some animals at 30 ppm (approximately 2.3 mg/kg/day) throughout the 12 months study were observed with a violent muscular contractions of the abdominal muscle, and males at this dose level reduced body weight gains. No other effects related to treatment were observed.

Based on these clinical signs and reductions in body weights, the NOAEL for this study was 10 ppm (equivalent to 0.65 mg/kg/day for males and 0.57 mg/kg/day for females).

Ebert, E.; Leist, K.-H.; Kramer, M., 1985a (AgrEvo:IIA, 5.3.3.1/1)

Study date: Start: 07 Oct 1983-End: 11 Nov 1983. Date of report: 22 Feb 1985

Test method: US-EPA F, § 82-2, Nov 1982; OECD. No 410, 12 May 1981.

GLP: Yes.

The study is acceptable.

Material and methods

Tests groups of Wistar rat, Hoe WISKf (SPF71), aged 8 - 10 weeks, weighting males 168 - 189 g and females 166 - 192 g, were exposed dermally to endosulfan substance, technical; 97.2 % (Code: Hoe 002671 00 ZD97 0003), in sesame oil, to the shaved nape skin for 6 hours each on 21 days in a 30 days period. 6 males and 6 females each received 0, 1, 3, 9, 27 mg/kg bw. 81 mg/kg were applied to 6 males only. Treated area was covered with occlusive bandages for 6 hours and washed thereafter.

Husbandry: housing individually in wire-mesh cages in air conditioned rooms with free access to feed and water.

Observations: twice daily for general health and behaviour; twice weekly control of body weight and feed consumption; weekly determination of water consumption as well as control for neurological disturbances, impairment to eyes, oral mucosa or dental growth.

Examination of treated skin for macroscopically visible changes prior to each application.

Collection of urine for analysis during one night two days before sacrifice. Necropsy: After terminal sacrifice all animals were examined for organ changes; weight of main organs was determined; histological examinations on a wide range of tissues, including treated skin. Haematology and clinico-chemical analysis were performed.

Results

This short term dermal study with endosulfan technical in Wistar rats yielded no clear cut results. Mortalities were recorded from 9 mg/kg bw up. Prior to death some of the animals exhibited typical symptoms of endosulfan intoxication. The two males in the 9 mg/kg group which died during the study showed very small, immature testes, which certainly resulted from a non-substance-related developmental disturbance already present prior to treatment.

Conclusion

The short term dermal study with endosulfan technical in Wistar rats yielded a NOAEL of 3 mg/kgbw/day based on symptoms of endosulfan intoxication at 9 mg/kgbw/day.

B.6.4 Genotoxicity

Summary

The endosulfan genotoxicity database has been prepared using the documentation submitted by AgrEvo, Excell and Calliope in support of the application. Numerous genotoxicity tests have been conducted with endosulfan. However, evaluation of the mutagenicity is confined to studies carried out with technical endosulfan of stated purity.

Results of these studies are summarised in Table B.6.4-1.

The major features of endosulfan, based in data from these studies are the following:

1. Endosulfan does not induce gene mutation in bacterial or mammalian cells; and it appears to be non-mutagenic for yeast, however, results from the acceptable study cannot be considered conclusive because of its conduct.
2. Endosulfan was not clastogenic in cultured human lymphocytes following a short treatment but a continuous treatment without metabolic activation was not carried out.
3. Endosulfan did not induce DNA damage in bacteria (rec-assay) or in cultured mammalian cell (UDS); however, negative results from the acceptable yeast mitotic gene conversion assay cannot be considered conclusive because of its conduct.
4. Endosulfan is non-clastogenic in mammalian somatic cells *in vivo.*
5. It cannot be concluded that endosulfan is not mutagenic for germ cells.

   The treatment of male mice with technical endosulfan (purity 97.03%) at the highest dose of 16.6 mg/kg for 5 consecutive days induced a single isolated increase in dominant lethal mutations in females from one mating interval (36-42 days) post treatment (Pandey *et. al*, 1990). The mating interval (sixth week) indicates that damage that can result in dominant mutation is induced specifically in spermatogonia. The lack of detail in this published study could make the significance of the isolated finding questionable. However, data from other studies support that endosulfan target is spermatogonial cells. These data, from two published studies on chromosomal aberrations in germ cells, were not included in Table B.6.4-1 because the purity of the test substance was not stated. Negative in rats (Dikshith and Datta, 1978) and positive in mice (Rani and Reddy, 1986) results were reported. It should be taken account all rats dosed at 36.6 and 55 mg/kg died before 24 h and two rats dosed with 22 mg/kg died after 72 h, and no toxicity data were reported for mice treated at similar doses of 22, 32 and 42 mg/kg. Besides, although the treatment period was the same in both studies (5 consecutive days), rats were sacrificed immediately and mice 60 days after treatment, and mouse spermatocytes at meiotic metaphases were presumed to have been spermatogonia at the time of treatment.
6. Endosulfan induced sperm abnormalities in rodents. The postulated mechanism for sperm abnormalities is that endosulfan impairs testicular functions by altering the enzyme activities responsible for spermatogenesis, thereby influencing intratesticular spermatid count and causing low sperm production and sperm deformities, being the young growing animals more susceptible than adult animals to exposure.

In conclusion, it can be said that endosulfan is not mutagenic *in vitro* and *in vivo* for somatic cells. Nevertheless, some positive results obtained in studies *in vivo* with germ cells suggest that endosulfan induces mutations specifically in spermatogonia. Therefore, in order to confirm these results or support the lack of mutagenicity, further testing are required, i. e. a chromosomal aberration assay in rodent germ cells that ensures spermatogonial cells to be exposed to endosulfan.

Table B.6.4-1: Summary of genotoxicity studies

TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
Bacterial gene mutation (plate incorporation) assay	*Salmonella typhimurium* TA1535, TA1537 TA1538, TA98, TA100 *Escherichia coli* WP2 *hcr*	5, 10, 50, 100, 500, 1000 and 5000 µg/plate (±S9)	Negative	No toxicity data. Results should be confirmed	Shirasu, Moriya and Ohta, 1978 (AgrEvo: IIA, 5.4.1.1/1) No published
Bacterial gene mutation assay: (plate incorporation and preincubation tests for non- and toxic concentration, respectively)	*Salmonella typhimurium* TA100, TA98, TA97a Metabolic activation system: CEE (cecal cell-free extract) and S9	41 and 3256 mg/L (±S9) 41 and 3256 mg/L (±CEE)	Negative		Pednekar, Gandhi and Netrawali, 1987 (Calliope: IIA, 5.4.1/02) Published
Yeast gene mutation assay (preincubation).	*Schizosaccharomyces pombe* (SP *ade* 6-60/*rad* 10-198, *h*-)	62.5, 125, 250 500 µg/mL (±S9) The exposure time was 4 h.	Negative	At 500 µg/mL survival rates were greater than 10% (±S9). Results should be confirmed.	Mellano and Millone, 1984a (AgrEvo: IIA, 5.4.1.3/2) No published
In vitro mammalian gene mutation assay	Mouse lymphoma cells (L5178Y $TK^{+/-}$ - 3.7.2C)	6.25, 12.5, 18, 25, 37.5, 50, 75 µg/mL (-S9) 6.25, 12.5, 25, 50, 75, 100 µg/mL (+S9)	Negative	Survival of cells were from 99% to 32.1% (-S9), without taking into account 75 µg/mL because it was lethal; and from 85% to 8.6% (+S9).	Cifone & Myhr, 1984b (AgrEvo: IIA, 5.4.1.3/1) No published
In vitro chromosomal aberration (CA) assay	Human lymphocytes from a healthy male volunteer	1, 10 and 100 µg/mL (±S9). The exposure time was 4 h (±S9)	Negative	200 µg/mL (±S9) was toxic. The reduction in mitotic index was 39% (-S9) and 30% (+S9) at 100 µg/mL. 100 metaphases/ treatment scored	Pirovano & Millone, 1986 (AgrEvo: IIA, 5.4.1.2/1) No published
In vitro CA assay	Human lymphocytes from two healthy volunteers	10, 20 and 40 µg/mL (±S9). The exposure time was 3 h (±S9	Negative	The reduction in mitotic index was 50% at 40 µg/mL (±S9)	Asquith & Baillie, 1989 (AgrEvo: IIA, 5.4.1.2/2) No published
Rec assay	*Bacillus subtilis* H17 Rec^{+}/ M45 Rec^{-}	0.02 ml solution of the sample containing 20, 100, 200, 500, 1000, 2000 µg/mL	Negative		Shirasu, Moriya & Ohta, 1978 (AgrEvo: IIA, 5.4.1.1/1) No published

TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
Yeast mitotic gene conversion assay (preincubation).	*Saccharomyces cerevisiae* D_4 (heteroallelic at *ade 2* and *trp 5)*	100, 500, 1000, 5000 µg/mL (±S9) The exposure time was 4 h.	Negative	At 5000 µg/mL survival rates were greater than 10% (±S9). Results should be confirmed.	Mellano and Millone, 1984b (AgrEvo: IIA, 5.4.2.2/2) No published
In vitro unscheduled DNA synthesis (UDS) assay	Primary cultures of hepatocytes from a single male Fischer 344 rat	0.102, 0.255, 0.51, 1.02, 5.1, 10.2 and 25.5 µg/mL	Negative	51 µg/mL was lethal. Survival at 25.5 g/mL was 31.5%. Results should be confirmed.	Cifone & Myhr, 1984a (AgrEvo: IIA, 5.4.2.2/1) No published
In vitro UDS assay	Human cell line A 549	1st test: 1, 3, 10, 30, 100, 300 and 1000 µg/mL (-S9) 2nd, 3rd tests: 0.1, 0.3, 1, 3, 10, 30, 100 µg/mL (±S9) The exposure time was 3 h.	Negative	Toxicity with alterations of cell morphology was observed at 100, 300 and 1000 µg/mL.	Muller, 1988b (AgrEvo: IIA, 5.4.2.2/3) No published
In vivo micronucleus (MN) assay	Bone marrow cells from male and female NMRI mice strain NMRKf (SPF71).	A single oral gavage dose (2.5, 5 and 10 mg/kg)	Negative	Signs of toxicity were observed in mice treated with 10 mg/kg, but disappeared 6h post-treatment. No toxicity in the bone marrow	Muller, 1988a (AgrEvo: IIA, 5.4.2.1/1) No published
In vivo MN assay	Bone marrow cells from male and female NMRI mice	A single oral dose (0.2, 1 and 5 mg/kg). After 24 h dose was repeated. The sample was collected 6 h after the 2nd dose.	Negative	No toxicity in mice or bone marrow cells.	Jung, Weigand & Kramer, 1983. No published* Submitted by AgrEvo.
In vivo CA assay	Bone marrow cells from male and female Wistar rats	A single oral dose: 2.5, 5 and 10 mg/kg (females) and 6.25, 12.5 and 25 mg/kg (males).	Negative	At 10 mg/kg two females died. Reduction of the spontaneous activity and apathy was observed at 5 mg/kg (females) and at 25 mg/kg (males). Reduction in mitotic index <50%.	Völkner, 2000. No published** Submitted by AgrEvo.

TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
In vivo dominant lethal assay	Swiss albino mice	Males treated i.p. at 9.8, 12.7 and 16.6 mg/kg for 5 consecutive days. A 7-day sequential mating procedure was used (8 mating intervals).	Positive at 16.6 mg/kg during the mating interval of 36-42 days Loss in post-implantation was greater than in pre-implantation.	The only affected mating interval (sixth week) indicates that mutation was specifically induced in spermatogonia. Fertility was also affected during this mating interval.	Pandey *et al,* 1990 (Excel: IIA, 5.4/03) Published
In vivo sperm abnormality assay	Swiss albino mice	Males treated i.p. at 9.8, 12.7, 16.6 and 21.6 mg/kg for 5 consecutive days.	Positive for sperm head abnormalities at 16.6 and 21.6 mg/kg. There was a dose-related response.	Significant decreases in testis weight at 21.6 mg/kg, and in sperm count at 16.6, 21.6 mg/kg. Sperm motility unaffected.	Pandey *et al,* 1990 (Excel: IIA, 5.4/03) Published
In vivo sperm abnormality assay	Druckrey rats (3 months old)	A single oral dose of 2.5, 5, 10 mg/kg/day (5 days/week for 70 days)	At 5 and 10 mg/kg/day a slight positive result was observed (7% abnormalities at the high dose and 6% abnormalities in controls). There was not a dose-related response.	2 rats died at 10 mg/kg/day. In all treated groups, testicular enzyme activities (SDH, LDH, GGT and G6PDH) were increased, and sperm counts were decreased. Spermatid counts were decreased only at the two highest doses.	Sinha *et al.*, 1995 Published* Submitted by AgrEvo
In vivo sperm abnormality assay	Druckrey rats (3 weeks old)	A single oral dose of 2.5, 5, 10 mg/kg/day (5 days/week for 70 days)	Increases in abnormalities in all treated groups. There was a dose-related response	In all treated groups, there were increases in G6PDH, LDH, GGT activities, and decreases in SDH activity, spermatid and sperm counts, and daily sperm production, in a dose dependent manner.	Sinha, Narayan and Saxena, 1997 Published** Submitted by AgrEvo

***** Reports and published papers included at the original monograph, only as summaries.

****** Reports and published papers not included at the original monograph.

Evaluation of studies not included or included only as summaries at the original monograph

The main applicant AgrEvo supplied more information about the genotoxicity of endosulfan. Some studies were already taken into account for preparing the monograph (first group) and other not because they had not been presented by any applicant (second group).

In relation to the first group, studies from Jung, Weigand and Kramer (1983), Sinha *et al* (1995), and Khan and Sinha (1996) are examined below because were included in the monograph as summaries. No comment to the study corresponding to Pandey *et al* (1990) because it was included in monograph just like it is.

With respect to the second group, the study corresponding to Fransson (1990) is a review document where most genotoxicity references were already included in the monograph; therefore, no comment is necessary. Nevertheless, the four remaining studies (Völkner, 2000; Sinha, Narayan and Saxena, 1997; Rupa, Reddy and Reddi, 1989; Rupa, Reddy and Reddi 1991b) are examined below.

Jung, Weigand and Kramer, 1983

Dates of experimental work: The study was performed between May 24, 1983 to May 31, 1983. Date of report: October 3, 1983

The objective of this study was testing endosulfan for its ability to induce micronuclei in mouse bone marrow cells *in vivo*.

Guidelines: No reference to a specific test guideline.

GLP: Yes.

The study is considered acceptable with some reservations derived from the only sample time 6 h after last dose, and the highest dose tested.

Material and methods together with findings

Endosulfan test substance was Code Hoe 002671 OI ZD970003, with purity 97.2%. It was dissolved in sesame oil. The test compound dilutions were freshly prepared each day. The positive control was cyclophosphamide. The study was conducted using male and female NMRI mice, strain NMRKf (SPF71).

Two treatments with 5 mg/kg bw at an interval of 24 hours had been shown in a preliminary study to be the maximum tolerated dose.

The micronucleus test was performed according to the method described by Schmidt (1975). Mice (5/sex/group) were administered endosulfan (at single doses of 0.2, 1 and 5 mg/kg), vehicle and cyclophosphamide, by gavage. 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 polychromatic erythrocytes with micronuclei occurring in 2000 counted polychromatic erythrocytes per animal, and the number of normocytes with micronuclei occurring in 1000 counted normocytes per animal, were evaluated statistically for each test group. The method of binomial increase was used to show any increase as compared with the controls. The ratio of polychromatic to normochromatic erytroytes was also determined.

Both the behaviour of the animals and the ratio of polychromatic to normochromatic erytroytes remained unaffected by treatment with endosulfan.
Endosulfan was without cytogenic activity at all doses tested. Cyclophosphamide induced a marked and significant increase of polychromatic erythrocytes with micronuclei in both sexes.

Conclusion

Endosulfan was considered negative in the mouse bone marrow micronucleus test, under the conditions of this study. OECD Guideline 474 defines the highest dose to be tested as the dose producing signs of toxicity such that higher dose levels, based on the same dosing regime, would be expected to produce lethality. It may also be defined as a dose that produces some indication of toxicity of the bone marrow. Neither signs of toxicity in mice nor indication of toxicity in the bone marrow were observed at the highest dose tested in this study. Besides, samples should be collected between 18 and 24 hours following the final treatment.

Völkner W, 2000

Dates of experimental work: 15 September 1999 to 27 December 1999. Date of report: 6 March 2000.
The objective of this study was to investigate the potential of endosulfan to induce chromosome aberrations in bone marrow cells of the rat.
Guidelines: OECD 475 (1997), EEC B.11 (1992) and EPA 870.5385 (1998). Deviations: The relative humidity was for a short period lower than 30% (lowest value 26%).
GLP: Yes.
The study is considered acceptable.

Material and methods together with findings:

Endosulfan test substance was Code AE F002671 00 1D99 0008, with purity 99.6%. The vehicle was a mixture of DMSO and corn oil (1+9). Endosulfan was found homogeneously distributed in the vehicle and stable over a period of 4 hours at application conditions. The mean concentrations of endosulfan in the vehicle ranged from 85.5% to 121.0% of the nominal concentrations. The positive control was cyclophosphamide. The study was conducted using male and female Wistar rats.

In a series of pre-experiments 2 animals per sex and test group received orally single doses of endosulfan in a mixture of DMSO and corn oil (1+9). Animals were examined for acute toxic symptoms at intervals

of 1, 6, 24 and 48 h after the administration of the test article. On the basis of the results the doses of 10 mg/kg b.w. (females) and 25 mg/kg b.w. (males) were determined as to be close to the maximum tolerated dose and chosen for the mutagenicity assay.

In the chromosome aberration assay, twelve animals, six males and six females, were treated per dose group. Endosulfan was administered orally at three dose levels (6.25, 12.5 and 25 mg/kg b.w. in males and 2.5, 5 and 10 mg/kg b.w. in females). Concurrent negative (vehicle) and positive controls were included. Prior (2.5 hours) to sacrifice, animals were injected i.p. with colcemid, to arrest cells in metaphase. The animals were sacrificed by cervical dislocation 24 h after treatment. For the highest dose level of endosulfan (25 mg/kg b.w. in males and 10 mg/kg b.w. in females) an additional group was included and sacrificed 48 h after treatment. Bone marrow cells were collected and stained. Scoring was made to determine mitotic index and chromosome damage. The mitotic index was calculated as % cells in mitosis after scoring a total of 1000 cells. Gaps, breaks, fragments, deletions, multiple aberrations, exchanges and chromosomal disintegration were recorded as structural chromosome aberrations. At least 100 well spread metaphases per animal were scored for cytogenetic damage. If available 5 animals per sex and group were evaluated as described. A test substance is classified as mutagenic if induces a dose-related increase in the number of structural chromosome aberrations and a statistically significant positive response for at least one of the test points. This can be confirmed by means the non-parametric Mann-Whitney test ($p<0.05$).

After treatment with 10 mg/kg b.w. endosulfan, 2 out of 12 treated females died; 5 mg/kg b.w. proved to be toxic for the treated females (reduction of the spontaneous activity, apathy). After treatment with 25 mg/kg b.w. endosulfan, the males expressed reduction of the spontaneous activity and apathy. The reduction in mitotic index was smaller than 50% in all treated groups when compared with negative control group. No biologically relevant or statistically significant increases in the frequency of aberrant cells were observed after treatment with endosulfan as compared to the frequencies of the vehicle control. The positive control gave a satisfactory response.

Conclusion

Endosulfan did not induce chromosome aberrations in rat bone marrow cells, under the conditions of this study.

Sinha, N., Narayan, R., Shanker, R. and Saxena, D. K., 1995

The study has been published in Vet. Human Toxicol, 37 (6), December.

The objective of this study was to investigate endosulfan-induced biochemical changes in the testis of rats as well as sperm abnormalities among other effects.

Guidelines: There is not one specific test guideline for performing this study.

GLP: No.

The study is considered acceptable.

Material and methods together with findings

The test substance was technical endosulfan (95.32% purity). It was dissolved in peanut oil and administered through oral intubation to groups of 15 male Druckrey rats (3 months old) at doses of 0, 2.5, 5 or 10 mg/kg/day 5 days/week for 70 days. Body weight was monitored twice a week. After termination of dosing, blood was collected for endosulfan estimation. The animals were then sacrificed and the testes were dissected free, weighed and kept for biochemistry and intratesticular sperm counts. The epididymis was dissected out, weighed and its caudal end teased in saline for sperm count and sperm abnormality.

Lactic dehydrogenase (LDH), sorbitol dehydrogenase (SDH), gamma glutamyl transpeptidase (GGT) and glucose-6-phosphate dehydrogenase (G6PDH) activities were measured according to methods of Kornberg (1955), Gerlach (1983), Romi and Goldberg (1981) and Lohr and Waller (1974), respectively. Protein in the samples was measured according to the method of Lowry *et al.* (1951).

For intratesticular spermatids count, the tunica albugenia was gently stripped from the parenchyma. Testicular tissue was then homogenised and divided into different aliquots of appropriate dilutions. A drop of homogenate was placed on a haemocytometer. The number of homogenisation/sonication resistant spermatid nuclei were then calculated and expressed per g of testis. These values were divided by 6.1 to convert them to daily sperm production (Robb *et al.*, 1978).

Both sperm counting and morphological changes in heads and tails observing were done according to the method of Feustan *et al.* (1989).

Data were analysed by one way Analysis of Variance (ANOVA) after ascertaining the homogeneity of variance and normality assumptions of the data. Intergroup comparison was done by computing the least significant difference. The significance levels were ascertained at $p< 0.05$, $p< 0.01$ and $p< 0.001$.

At 10 mg/kg/day, 2 animals died during the study, but no mortality was reported at other dose levels. No change in body or testis weight was seen in treated animals compared with controls.

A significant increase in the activities of the four enzymes (LDH, SDH, GGT and G6PDH) was observed at all treated groups in a dose dependent manner.

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 significant when compared with the reduction seen at 2.5 mg/kg/day.

The incidence of sperm abnormalities was significant 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.

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 effects, with similar reductions seen at both of the higher dose levels. At 2.5 mg/kg/day, these parameters were similar to control values.

Conclusion

The authors postulated that endosulfan impairs testicular functions by altering the enzyme activities responsible for spermatogenesis, thereby influencing intratesticular spermatid count and causing low sperm production and sperm deformities. 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 changes in other parameters (sperm abnormalities, spermatid count, sperm production),

while statistically significant different to concurrent controls, were only slightly changed, and in the absence of consistent dose response relationships for these effects, it is considered that these effects are not biologically significant.

Khan, P. K. and Sinha, S. P., 1996

The study has been published in Mutagenesis, 11 (1): 33-36.

The objective of this study was to investigate the effect of endosulfan on sperm morphology and sperm count, and to test the ameliorating potential of vitamin C on such effects.

Guidelines: There is not one specific test guideline for performing this study.

GLP: No.

The study is considered acceptable only as an additional information because the test substance was a commercial preparation.

Material and methods together with findings

Endosulfan was used as an emulsifiable concentrate containing 35% (w/w) endosulfan technical as active principle, the remaining 65% (w/w) being solvents, emulsifiers and stabilisers (batch nº 397; Excell Industries India Ltd, Bombay). The injectible formulation of vitamin C contained 0.1 g pure synthetic ascorbic acid along with 0.08% (w/v) methylparaben and 0.01% (w/v) propylparaben as preservatives in 1 ml of aqueous preparation (Roche Products India Ltd, Bombay). The study was conducting using male Swiss albino mice (6-8 weeks old, Central Drug Research Institute, India).

Endosulfan was administered to different sets of animals at a dose of 3 mg/kg/day (estimated previously to be the maximum tolerated dose) for 35 consecutive days, by intubating 0.2 mL of an aqueous solution of the test material (0.1% v/v). One group of treated animals received endosulfan only, while three other treatment groups received endosulfan via gavage, plus intraperitoneal administration of vitamin C at 10, 20 or 40 mg/kg b.w./day. A vehicle control group was also used, but no positive control group was included in this study. Animals were sacrificed 24 h after the final treatment, and sperm was collected from the cauda epididymis of each mouse for counting and morphological analysis.

Sperm counts were made in the sperm suspension with the aid of a Neubauer haemocytometer. The significance of differences between the mean sperm counts was estimated by Student's t-test. Variations in the effects of the three doses of vitamin C were initially analysed by one-way ANOVA, to be followed by Duncan's multiple range test (Montgomery, 1976).

About 2000 randomly selected sperm from each experimental group were examined for abnormalities. The gross abnormalities in sperm head shape were described as pin-shaped, bottom-heavy (Soares*et al,* 1979), triangular, amorphous, rudimentary (Rastogi and Levin, 1987; Bhunya and Behera, 1987), hammer-shaped, sickle-shaped and flat-based (Sinha and Prasad, 1990). Some sperm were characterized as having a distinctly larger (twice the area) or smaller (half the area) head size in comparison with normal, as documented by Seuanez *et al,* 1977. Sperm with abnormal head number (twin-headed) were also recorded (soares *et al,* 1979; Sinha and Prasad, 1990). Results were expressed as per cent occurrence of abnormal sperm and were evaluated statistically by the Z-test.

Significant decreases in sperm count with respect to the control 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. 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.

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.

Conclusion

Endosulfan induced an increase in abnormal sperm under the conditions of this study. This effect was reduced slightly in animals also administered vitamin C. No historical control 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. This effect was lessened in animals also administered vitamin C. The test material was a 35% emulsifiable concentrate containing solvents, emulsifiers and stabilizers; therefore it is unclear whether these findings are related to endosulfan or these non-active constituents.

Sinha, N., Narayan, R. and Saxena, D. K., 1997

The study has been published in Bull. Environ. Contam. Toxicol., 58: 79-86.

The objective of this study was to investigate the effects, including sperm abnormalities, of endosulfan on the testis of growing rats.

Guidelines: There is not one specific test guideline for performing this study.

GLP: No.

The study is considered acceptable.

Material and methods together with findings

The test substance was technical endosulfan (95.32% purity). It was dissolved in peanut oil and administered through oral intubation to groups of 5 male Druckrey rats (3 weeks old) at doses of 0, 2.5, 5 or 10 mg/kg/day. The treatment was carried out till 90 days of age, the dose schedule being five days a week. Body weight was monitored twice a week. After the completion of treatment, the animals were sacrificed. Testes were dissected free, weighed and processed for enzymatic analysis and intratesticular spermatids count. The epididymis were dissected out, their caudal end teased in saline for sperm count and sperm abnormality.

Lactic dehydrogenase (LDH), sorbitol dehydrogenase (SDH), gamma glutamyl transpeptidase (GGT) and glucose-6-phosphate dehydrogenase (G6PDH) activities were measured according to methods of Kornberg (1955), Gerlach (1983), Romi and Goldberg (1981) and Lohr and Waller (1974), respectively. Protein in the samples was measured according to the method of Lowry *et al.* (1951).

For intratesticular spermatids count, the tunica albugenia was gently stripped from the parenchyma. The tissue was then homogenised and divided into different aliquots of appropriate dilutions. A drop of homogenate was placed on a haemocytometer and observed under microscope for counting and

calculating the number of homogenisation/sonication resistant spermatid nuclei per ml testis suspension. For calculating daily sperm production, the values obtained were divided by 6.1 (Robb *et al.*, 1978).

Both sperm counting and morphological changes in heads and tails observing were done according to the method of Feustan *et al.* (1989). The criteria chosen for head abnormality were no hook, excessive hook, amorphous, pin and short head. For tail, the abnormalities recorded were coiled flagellum, bent flagellum, and bent flagellum tip.

Data were analysed by one way Analysis of Variance (ANOVA) after ascertaining the homogeneity of variance and normality assumptions of the data (Brunning and Kintz, 1977). Intergroup comparison was done by computing Least Significant Difference. The significance level was ascertained at $p< 0.05$.

No death was recorded in any of the treated group throughout experimentation. No change in body weights or testis weigh was seen in treated animals compared with controls.

A significant reduction in the number of sperm count, dose dependent, was recorded in all treated groups as compared to the control. The intergroup comparison also showed a significant decrease with higher dosed groups being most toxic.

A significant increase in sperm abnormalities, dose dependent, was observed in all treated groups as compared to the control. The changes were less ($p< 0.05$) in lowest dose group but increased with the increase in the dose level at 5 and 10 mg/kg of endosulfan ($p< 0.001$).

Marked depletion in the number of spermatids as well as decrease in daily sperm production was observed in all treated groups as compared to the control. The intercomparison of treated groups showed that the toxicity was in a dose dependent manner.

A significant elevation in LDH, GGT and G6PDH activities was recorded in all treated groups as compared to the control in a dose dependent manner. However, SDH registered a decrease in its activity in a dose dependent manner.

Conclusion

In the present study, endosulfan looks like to impair testicular functions by altering the enzyme activities responsible for spermatogenesis, thus influencing intratesticular spermatid count, and resulting in low sperm production and increased sperm abnormalities. Therefore, it can be concluded that endosulfan exposure during the period of testicular maturation when spermatogenesis is under progress may result in disturbed spermatogenesis at sexual maturity.

Rupa, D. S., Reddy P. P. and Reddi, O. S., 1989

The study has been published in Environmental Research, 49: 1-6.

The objective of this study was to evaluate the frequency of chromosomal aberrations in peripheral lymphocytes of cotton field pesticide sprayers.

Guidelines: There is not one specific test guideline for performing this study.

GLP: No.

The study is considered acceptable only as an additional information. The main reason is that the test substance was a mix of different pesticides (commercial preparations). Besides the source of pesticides was not reported.

Material and methods together with findings

The study was conducted in humans, using 52 male cotton field workers (age range 21-47 years) who were non-smokers, non-alcoholics and exposed to various pesticides. In addition, 25 healthy males (age range 22-44 years) who were non-smokers, non-alcoholics and non-exposed to pesticides were selected for controls. Relevant information on the background of each individual was collected using a standard questionnaire.

The pesticides used by the cotton field workers were: DDT, BHC, endosulfan, malathion, methylparathion, phosphomidon, quinolphos, monocrotophos, dimethoathe, fenvalerate and cypermethrin. The pesticides were sprayed 8 hr per day, 9 months per year, i.e, from July to March. In particular, endosulfan (purity 50%) was used for 25 years during the period 1962-1987. The chemicals were stored in sheds located near the fields and were brought to the fields whenever required. The workers mixed the pesticides in large plastic containers with their bare hands and sprayed them using manual sprayers without any protective measures.

According to the duration of exposure (in years), the worker population was divided in five groups (1-5 years, 6-10 years, 11-15 years, 16-20 years, and 21-25 years). According to the age range (in years), the worker population was divided in six groups (21-25 years, 26-30 years, 31-35 years, 36-40 years, 41-45 years, and 47 years).

Whole blood cultures were set up within 24 h after collection and incubated at 37^{o}C for 72 hr. Each sample was maintained in triplicate. Colchicine was added 2 hr before cells were harvested. Slides were prepared according to the method described by Moorhead *et al.* (1960). For each sample 200 well-spread complete metaphases were scored for structural (gaps, breaks, fragments, deletions, and dicentrics) and numerical (polyploid) chromosome aberrations using the criteria of Evans and O'Riordan, 1975. Gaps and polyploids were excluded from the total number of aberrations. The significance of total chromosomal aberrations was determined by the χ^2 test.

Aberrations like gaps, fragments and deletions increased irrespective of duration of exposure. Breaks and dicentrics increased with the duration of exposure up to 20 years and then remained level. A rise in polyploid was seen with the increased duration of exposure to pesticides. Statistical analysis showed that the total chromosomal aberrations increased significantly for all workers groups. Total chromosomal aberrations were significantly increased irrespective of duration of exposure. Total aberrations increased regardless of the age.

Conclusion

Results from this study indicate that the combination of pesticides (DDT, BHC, endosulfan, malathion, methylparathion, phosphomidon, quinolphos, monocrotophos, dimethoathe, fenvalerate and cypermethrin) caused chromosomal damage in peripheral lymphocytes in the absence of smoking and alcohol consumption in an exposed population of the cotton field workers. However it is impossible to conclude that endosulfan alone caused this damage.

Rupa, D. S., Reddy P. P. and Reddi, O. S., 1991b

The study has been published in Mutation Research, 261: 177-180.

The objective of this study was to evaluate clastogenic effects in peripheral lymphocytes of cotton field workers exposed to different pesticides.

Guidelines: There is not one specific test guideline for performing this study.

GLP: No.

The study is considered acceptable only as an additional information. The main reason is that the test substance was a mix of different pesticides (commercial preparations). Besides, the source and purity of pesticides were not reported.

Material and methods together with findings

The study was conducted in humans, using 26 male cotton field workers (age range 18-50 years) who were non-smokers, teetotallers and exposed to various pesticides. In addition, 26 healthy males who were non-smokers, teetotallers, non-exposed to pesticides and belong to the same age group and socio-economic class were selected for controls. Relevant information on the background of each individual was collected using a standard questionnaire.

The pesticides handled by the cotton field workers until January, 1989 for the past 5 years were: endosulfan, malathion, methylparathion, dimethoathe, phosphamidon, monocrotophos, quinalphos, cypermethrin and fenvalerate. The exposed group worked with pesticides 8 h per day in spring and winter seasons. According to the duration of exposure (in years), the worker population was divided in four groups (2-5 years, 6-10 years, 11-15 years, and 16-18 years).

Whole blood cultures were set up within 24 h after collection and incubated at 37^{o}C for 48 and 72 hr. Duplicates were maintained for each sample and in each culture period. Colchicine was added 2 hr before cells were harvested. Slides were prepared according to the standard method (Moorhead *et al,* 1960; Evans and O'Riordan, 1975). For each sample 300 metaphases were scored for chromosomal aberrations and a minimum of 600 cells were scored for mitotic index. Structural and numerical chromosome aberrations were recorded using the criteria given by WHO, 1985. Statistical analysis was done using the χ^2 test.

The type of chromosomal aberrations observed in the exposed group were gaps, breaks, dicentrics, exchanges, rings and polyploidy. The frequency of total chromosomal aberrations increased significantly irrespective of duration of exposure to pesticides when compared to controls. A significant decrease in mitotic index was observed in the exposed group as compared to the control group and it also decreased as the duration of exposure increased. Although the differences were not significant, the 48-h cultures showed high incidence of chromosomal aberrations and low mitotic index when compared to 72-h cultures.

In exposed group 24 out of 26 individuals showed ill health effects such as severe giddiness and nervous disorders.

Conclusion

Results from this study indicate that the combination of pesticides (endosulfan, malathion, methylparathion, dimethoathe, phosphamidon, monocrotophos, quinalphos, cypermethrin and fenvalerate) caused chromosomal damage in peripheral lymphocytes in the absence of smoking and alcohol consumption in an exposed population of the cotton field workers. However it is impossible to conclude that endosulfan alone caused this damage.

B.6.5 Long- term and carcinogenicity studies

Summary

To assay the long term effect of endosulfan in rats and mice, several chronic, carcinogenic and combined studies have been evaluated. The majority of them were considered as not acceptable owing the nature of the results obtained and the methods employed.

In the following table the only two acceptable studies has been summarised and according with their results, endosulfan did not show any oncogenic potential on rats or mice.

Table B. 6.5: Summary of acceptable long-term toxicity and carcinogenicity studies with ENDOSULFAN

Study	Dose levels	NOAEL		LOAEL		Target organs/main effects	Reference
		ppm	mg/kg b.w./day	ppm	mg/kg b.w./ day		
Combined chronic-carcinogenic study							
Charles River rats Oral.104 weeks	ppm: 0,3,7.5, 15 and 75 mg/kg/day 0, 0.1, 0.3, 0.6 and 2.9 for males and 0, 0.1, 0.4, 0.7 and 3.8 in females	Chronic NOAEL 15(m/f)	Chronic NOAEL 0.6 m 0.7f	Chronic LOAEL 75(m/f)	Chronic LOAEL 2.9m 3.8f	Chronic LOAEL , based on the low body weight gains in both sexes, increase in the incidence of enlarged kidneys in females; increase in the incidence of blood vessel aneurysms mainly in males and increased incidence of enlarged lumbar lymph nodes in males) at 75 ppm No carcinogenic potential	Ruckman SA et al., (1989) AgrEvo: IIA, 5.5.1/4) (AgrEvo: ANRA) Hack et al., (1995) AgrEvo:IIA, 5.5.1/6) (Published)
Carcinogenicity study							
NMRI mice. Oral, 24 months.	ppm: 0, 2, 6, 18 mg/kg/day 0.28, 0.84 and 2.51 for males and 0.32, 0.97 and .2.86 for females	Chronic NOAEL 6	Chronic NOAEL 0.84 (m) 0.97 (f)	Chronic LOAEL 18	Chronic LOAEL 2.51 m 2.86 f	Chronic LOAEL based on an increase mortality in females decreased body weight in males over a period of 24 months ,and significant decrease in the relative lung and ovary weights in female mice after 12 months of treatment No carcinogenic potential	Donaubauer, HH (1989a, 1989b, 1990) (AgrEvo: IIA, 5.5.2/1/2/3) (AgrEvo: ANRA) Hack et al., (1995) (Published) (AgrEvo: IIA, 5.5.1/6)

B.6.5.1Chronic toxicity studies

B.6.5.1.1 Rats

Keller (1959) (AgrEvo: IIA, 5.5.1/1; AgrEvo: ANRA)

Dates of report: May 22, 1959

Guidelines: The study does not claim adherence to a specific test guideline.

The study was performed prior to GLP regulations

The study is not considered to be acceptable. The main reason is that the purity of the technical product was not specified. The number of animals used in haematological examination was 3 per sex and group instead of 10 and there not any indication about urinalysis examination or clinical chemistry . The equivalence in the dose levels(ppm) as mg/kgbw/day should be indicated . At the end of the study, survival in female rats treated at the high dose level was lower than 50%.

Material and Methods

Thiodan Technical was added to the basal laboratory diet on a weight/weight basis to provide the dietary levels and thoroughly mixed in a twin-shell blender. Fresh diet was prepared weekly. The study was conducted using Wistar rats (males /females), weighting 65 - 85 g (males) and 62 -78 g (females).

Four groups (25 males /25 females per group), received 0, 10, 30 ,100 ppm in the diet respectively for 2 years- Groups of 5 of each sex were killed at 52 weeks.

After completion of 104 experimental weeks all surviving control and test animals were sacrificed by exsanguination and autopsies were performed.

The following parameters were checked: gross appearance and behaviour, survival, clinical signs, growth, food and water consumption, organ weights and microscopic pathology. Haematological evaluation was performed on three rats (instead of 10 rats/sex group)of each sex in each control and test group at the following interval, 26, 52, 78 and 104 weeks. No urinalysis or clinical chemistry examination was performed according with the requirement of 452 OCDE guidelines.

Statistical evaluation of data was made using the Chi-square test at the 5% probability level for the survival, and the analysis of variance or F-test at the 5% probability level for all remaining parameters.

Results

The gross appearance and behaviour of the test rats which received the compound at each level were comparable to those of the control rats throughout the course of the study. None of the test rats exhibits gross signs of systemic toxicity or abnormal behaviour. A rather high incidence of respiratory involvement, as evidenced by wheezing, nasal discharge and laboured respiration, was noted during the earlier stages of the study and increased as the animal reached old age, but occurred with approximately equal frequency among the control and test rats. This is a common syndrome among laboratory rodents and not compound-related. Subcutaneous tumour formation (Table B.6.5.1-1), body scores, alopecia and “spinning”, commonly seen in aging rats, were noted in approximately equal numbers of rats in the control and test groups during the last year of the study.

Table B.6.5.1-1: Incidence of subcutaneous masses observed during the second year of the study in rats

Group	males	females
Control	0	5
10ppm	2	4
30ppm	2	3
100ppm	0	2

Survival of the male test rats was not significantly different from the control group. Survival of female rats treated with 10 and 30 ppm was reduced during the second year of treatment, but not significantly different. After the eight experimental week, the mortality rate of the 100 ppm females test group increased as compared to the female control. Statistical analysis at 24 and 104 weeks revealed a significant differences in survival of these two groups

No statistically significant differences in mean body weights, bw gain and food consumption between any treated animals and control groups. No valid comparison was possible between the female test rats in the 100 ppm group and the female controls during the second years of the study due the very small number of survivors in the test group.
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.
No consistent gross pathology which could be attributed to Thiodan administration was noted among animals which died during the later stages of the study. No specific gross pathological findings, other than overwhelming respiratory involvement, reflect the significantly lower survival rate in the 100 ppm female group.

Organ weights and organ-to-body-weight ratios determined from male and female test rats were comparable to the control group. The only exception were in the significantly lower weight of the testes in the 10 ppm and the significantly higher weights of the kidneys and kidneys-to body-weight ratios for rats in the 100 ppm groups as compared to the male controls (Table B.6.5.1.1-2). This finding was correlated by microscopic observations.

Table B.6.5.1.1-2: Organ weight and organ-to-body weight ratios for male rats

Parameter	0ppm	10ppm	30ppm	100ppm
Testes weight (g)				
52 weeks	3.56	3.14	3.58	3.48
104 weeks	3.33	3.10*	3.21	3.32
Testes/bw ratio (%)				
52 weeks	0.711	0.715	0.724	0.775
104 weeks	0.682	0.698	0.690	0.721
Kidney weight (g)				
52 weeks	3.61	3.06	3.47	3.79
104 weeks	3.52	3.54	3.47	4.18**
Kidney/bw ratio (%)				
52 weeks	0.702	0.695	0.702	0.838
104 weeks	0.714	0.812	0.681	0.910**

*Significantly lower than control; ** Significantly higher than control

No consistent gross changes were noted which could be associated with the inclusion of thiodan in the diets of the rats for two years. One of the commonest findings in all animals examined was the lung infection; 0 ppm (11/25), 10 ppm (15/25), 30 ppm (13/25) and 100 ppm (14/25) in males and 0 ppm (11/25), 10 ppm (14/25), 30 ppm (16/25) and 100 ppm (17/25) in females. Incidences of enlarged pituitary glands and adrenals were greater in the female control group than treated animals.

Histopathologic changes were observed in kidney and liver only in male rats treated with 100 ppm and has been summarised in the table B.6.5.1.1-3.

Table B.6.5.1.1-3:Microscopic pathology form male rats which died or were sacrificed at 52 and 104 weeks

Dose group	**0ppm**	**10ppm**	**30ppm**	**100ppm**
Kidney				
Minimal tubule degeneration				
52 weeks	----------	----------	1/5	4/4*
104 weeks	6/14	8/10	4/14	1/12*
Focal lymphocytic infiltration				
52 weeks		1/4		1/4*
104 weeks	2/14	7/10	2/15	7/12*
Tubule dilatation				
52 weeks				1/4*
104 weeks			1/15	12/12*
Irregular albominous cats				
52 weeks	1/5	2/4	3/5	2/4*
104 weeks				10/12*
Liver				
Hydropic hepatic cell areas				
52 weeks				
104 weeks		1/10		7/12**

* $p < 0.05$; ** $p < 0.01$

The tumour incidence is summarised in Table 6.5.1.1-4. None of these tumours noted occurred consistently in any control or test group. However, the incidence of tumours was considered unrelated to the Thiodan administration in the diets and was within normal limits for rats of this age.

Table 6.5.1.1-4: 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

M= males

F= females

Conclusion

On the basis of the date presented, and under the experimental conditions of the study, endosulfan lacked carcinogenic potential at dose levels up to and including 100 ppm. The NOAEL was considered to be 30 ppm (1.5 mg/kg bw /day), on the basis of kidney effects at 100 ppm (5mg/kgbw/day).

Nevertheless, this study is not considered as acceptable because it does not include all the required information by OECD 451.

B.6.5.1.2 mice

Arai *et al.*, (1981) (AgrEvo:ANRA)

Date of report: 28 April 1981

No reference to a specific guideline

No reference to GLPs.

The study is considered as additional information because is a summary of the original report taking account in the ANRA dossier. In addition, it was considered by the Australian authorities as a subchronic study and the methodology employed is more similar to this kind of study (number of animals, observations). Thus this study comprise the majority of the normal life span of the mice (12 months), we are considered this revision as a chronic study with many deviations of the chronic study guidelines (OCDE 452).

Material and Methods

Endosulfan technical (91.4%) was administered daily to 4 weeks old 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. Ophtalmoscopic examinations were carried out on all test mice at 12 months and haematological and clinical chemistry parameters were determined at 0 at 12 months. Gross and histopathological examination were carried out upon termination of the study.

Results

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. Opthalmoscopic examinations revealed a few incidences of granulation on the cornea 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 by 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 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 weights 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 in table 6.5.1.2-1.

Table 6.5.1.2-1: Incidence of histopathological findings in the liver and lymph nodes

Findings	**Sex**	**Doses (ppm)**				
		0	**10**	**30**	**100**	**300**
Limphocytic 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 hiperplasia	M	0/10	0/10	0/9	0/10	1/10
Pigmented histocytic cells	M	0/10	1/10	0/9	1/10	5/10
	F	1/10	2/10	0/10	2/10	8/10
Granuloma	M	1/10	2/10	1/9	5/10	8/10
	F	3710	3/10	3/10	4/10	10/10

In liver, granuloma, giant cell infiltration and/or 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 histopathological findings in the kidney are summarised in Table 6.5.1.2-2

Table 6.5.1.2-2: Incidence of histopathological findings in kidney

Findings	**Sex**	**Doses (ppm)**				
		0	**10**	**30**	**100**	**300**
Interstitial limphocytic infiltration	M	9/10	6/10	610	3/10	5/10
	F	6/10	8/10	7/10	7/10	9/10
Cystic dilation o cortical tubules	M	0/10	0/10	0/9	1/10	1/10
	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
Glomerulonephristis	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 dilation 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 in male mice. Spermatic retention occurred in 10% control, 20% at 10 ppm, 30% at 100 ppm, and 10% at 300 ppm. Due 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 mice.

Conclusion

The Chronic NOAEL was 30 ppm (4.1 mg/kg/day in males and 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 and 15.2mg/kg/day in males).

B.6.5.2 Carcinogenicity studies

B.6.5.2.1 Rats

Thomas, *et al* , (1978) (AgrEvo,: IIA, 5.5.1/2; Calliope: IIA, 5.5/01 and AgrEvo: ANRA)

Date: July 18, 1978

This study has been published in National Cancer Institute. Carcinogenesis, Technical Report Series No 62.

The study does not claim adherence to a specific test guideline

The study was performed prior to **GLP** regulations.

The study is considered not acceptable Due the dose-dependent high mortality in male rats. Only two dose level has been tested instead of three. Not haematology, urinalysis or biochemical analysis were performed.

Material and Methods

Groups of 50 males and 50 females Osborne -Mendel rat were feed diets containing technical-grade Endosulfan, (98.8% purity) at time-weighted average doses of 952 ppm and 408 ppm for males and 445 ppm and 223 ppm for the female rats during 78 weeks. Control group (20 m/20 f) was administered normal diet mixed with corn oil.

Checks were conducted for survival, body weights, food consumption, and clinical signs. Besides, histopathologic examination of major tissues organs or gross lesions was carried out. Not haematology, urinalysis or biochemical analysis was performed

Statistical tests for mortality was performed following several methods: (Kaplan & Meier, 1958; Cox, 1972; Tarone, 1975). Statistical analysis of tumour incidence was developed according to different tests (Cox, 1970; Miller, 1966, Armitage, 1971). The relative risk of each treated group compared to its control was calculated by Gart method (1971). The results were considered significant at $p < 0.05$.

Results

No appreciable differences in mean body weight were observed among female rats A distinct dose-related depressing in mean body weight was evident in male rats as early as week 32. Fluctuations in the growth curve may be due to mortality, as the size of the group diminished, the mean body weight may be subject to wide variations.

No other characteristic clinical signs were observed during the first 6 months of the study with the exception of occasional hunched appearance and reddened or squinted eyes in a few treated rats. As the study progressed, a hunched appearance was noted in increasing numbers of treated rats.

The estimates probabilities of survival for male and female rats are shown in the table B.6.5.2-1. For male rats a positive dose-related trend in mortality was highly significant ($p < 0.001$). By week 54, 52% of the high dose rats had died. For female rats a positive dose-related trend in mortality was not significant. The low dose group had a high early mortality, primarily due to the death of 7 females in week 21. Six of these animals were reported to have cerebral haemorrhage and the seventh to have cerebral angiectasia. These deaths do not appear, therefore to be compound related. Since 50 % of the high dose animals , 62% of the low dose animals and 70% of the controls survived to the termination of the study, adequate numbers of female rats survived from carcinogenic study.

Table B.6.5.2.1: The estimates probabilities of survival for male and female rats

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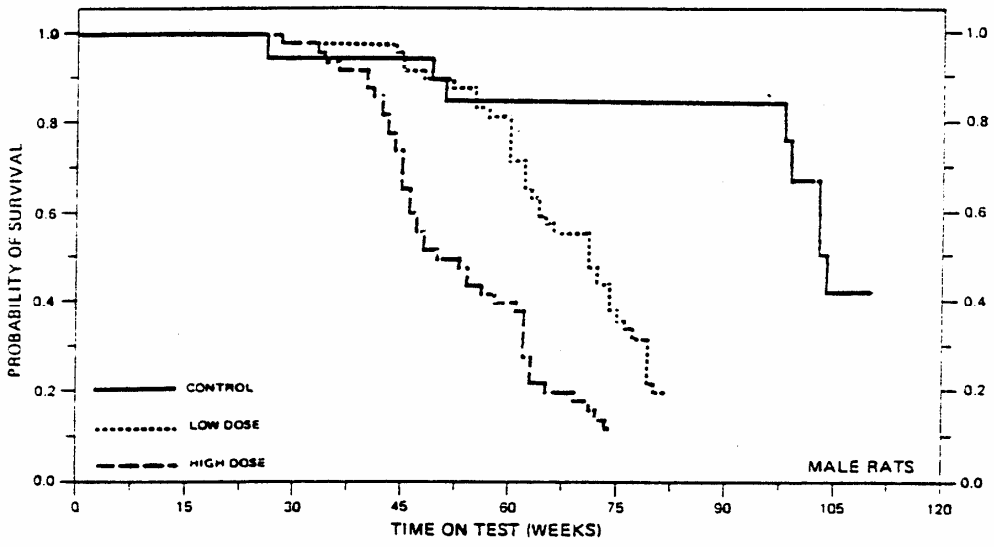
30 (93)



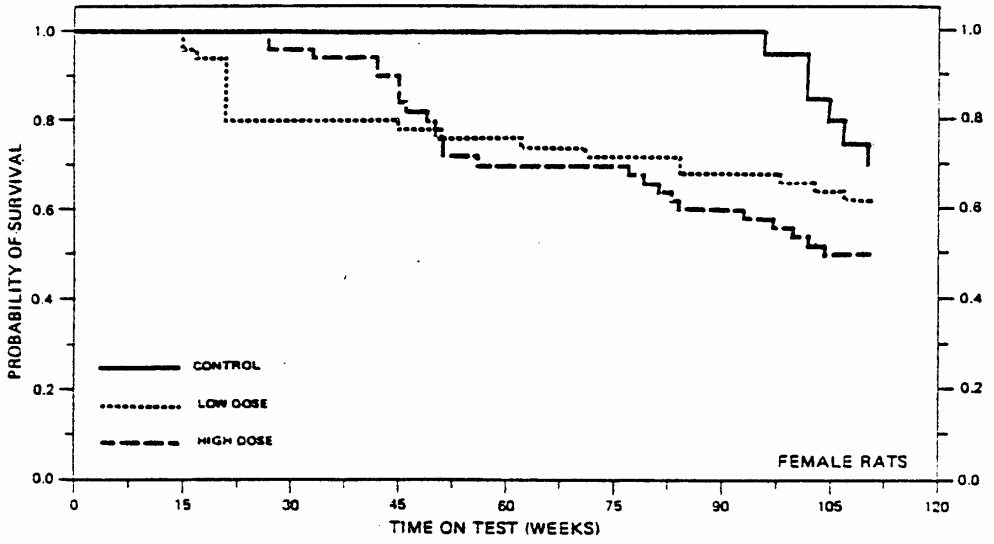


FIGURE 2
SURVIVAL COMPARISONS OF ENDOSULFAN CHRONIC STUDY RATS

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The non-neoplastic lesions are summarised in table B.6.5 2-2. Histopathological examination revealed toxic nephropathy in all treated animals, mainly in male rats, characterised by degenerative changes in the proximal convoluted tubules at the junction of the cortex and medulla with cloudy swelling, fatty degeneration and necrosis of the tubular epithelium 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 exhibits some chronic inflammation and calcium deposits but this did not very from control incidences.

Table B.6.5 2-2: Summary of non-neoplastic lesions in male and female rats

	Males			**Females**		
System	**0ppm**	**408ppm**	**952ppm**	**0ppm**	**223ppm**	**445ppm**
Kidney						
Nephropathy toxic	0/20 (0%)	47/50 (94%)	43/47 (91%)	0/20 (0%)	27/50 (54%)	29/50 (58%)
Chronic inflammation	8/20 (40%)	42/50 (84%)	34/47 (72%)	13/20 (65%)	7/50 (14%)	5%50 (10%)
calcium deposits	1/20 (5%)	29/50 (58%)	22/47 (47%)	1/20 (5%)	3/50 (6%)	1/50 (2%)
Parathyroid						
hiperplasia	0/19 (0%)	21/48 (44%)	18/47 (38%)	0%	1/49 (2%)	0%
Testis						
atrophy	3/19 (16%)	18/47 (38%)	24/47 (51%)			
Respiratory system						
Pneumonia, chronic murin	11/20 (55%)	7/50 (14%)	17/47 (36%)	14/19 (70%)	33/50 (66%)	37/50 (70%)
Calcium deposits	0/20	11/50 (22%)	5/47 (11%)	1/19 (5%)	0/50 (0%)	0/50 (0%)
Circulatory system						
Calcium deposit, heart	1/20 (5%)	9/50 (18%)	10/47 (21%)			
Medial calcification, aorta	1/20 (5%)	29/50 (58%)	22/49 (45%)	1/20 (5%)	1/50 (2%)	0/50 (0%)
Medial calcification, mesenteric artery	1/20 (5%)	28/50 (56%)	23/49 (8%)			
Stomach						
Calcium deposits	1/20 (5%)	31/50 (62%)	21/47 (45%)	0%	2/50 (4%)	1/49 (2%)

Parathyroid hiperplasia 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 calcification and stomach calcium deposits, which did, not very from control incidences.

Testicular atrophy occurred in 3/19 816%) control, 18/47 (38%) low dose, and 24/47 851%) 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 spermatogenesis. No treatment related effects were noted on the reproductive organs in female rats.

Due the high early mortality observed in the male groups no conclusion concerning the carcinogenicity of Endosulfan can be drawn for male rats. Endosulfan did not induced any significant compound-related tumours in female rats.

Conclusion

A Chronic **NOAEL** was not identified, as treatment-related changes occurred in the kidneys and the testis at all doses No treatment-related neoplastic lesions were seen in female rats, owing to the high mortality rate in males, no valid conclusion can be drawn about carcinogenicity.

The study is not acceptable.

B.6.5.2.2 Mice

Thomas, *et al* , (1978) (AgrEvo,: IIA, 5.5.1/2; Calliope: IIA, 5.5/01 and AgrEvo: ANRA)

This study has been published in National Cancer Institute. Carcinogenesis, Technical Report Series No 62.

The study does not claim adherence to a specific test guideline.

The study was performed prior to **GLP** regulations.

The study is not acceptable due that the dose-dependent high mortality in male mice. Only two dose levels has been tested

Material and Methods

The test substance was technical-grade Endosulfan,(98.8% purity). Fresh mixtures of endosulfan in corn oil were prepared each week and stored in the dark. Food and water were available *ad libitum*.

Groups of 50 male and 50 female mice were fed diets containing Endosulfan at time-weighted average concentrations of 3.5 or 6.9 ppm for males and 2.0 ppm and 3.9 for females for 78 weeks. Groups of 20 control received untreated diets.

Animals were observed for clinical signs, mortality, body weights and food consumption. Histopathological examination was carried out on every animal at the end of the study. Not haematology, urinalysis and biochemical chemistry analysis were performed.

Statistical tests for mortality was performed following several methods: (Kaplan & Meier, 1958; Cox, 1972; Tarone, 1975) Statistical analysis of tumour incidence was developed according to different tests (Cox, 1970; Miller, 1966, Armitage, 1971). The relative risk of each treated group compared to its control was calculated by Gart method (1971). The results were considered significant at $P < 0.05$.

Results

Clinical signs and body weight in both male and female mice were unaffected by treatment.

Table B.6.5.2.2-1: Survival rate in male and female mice

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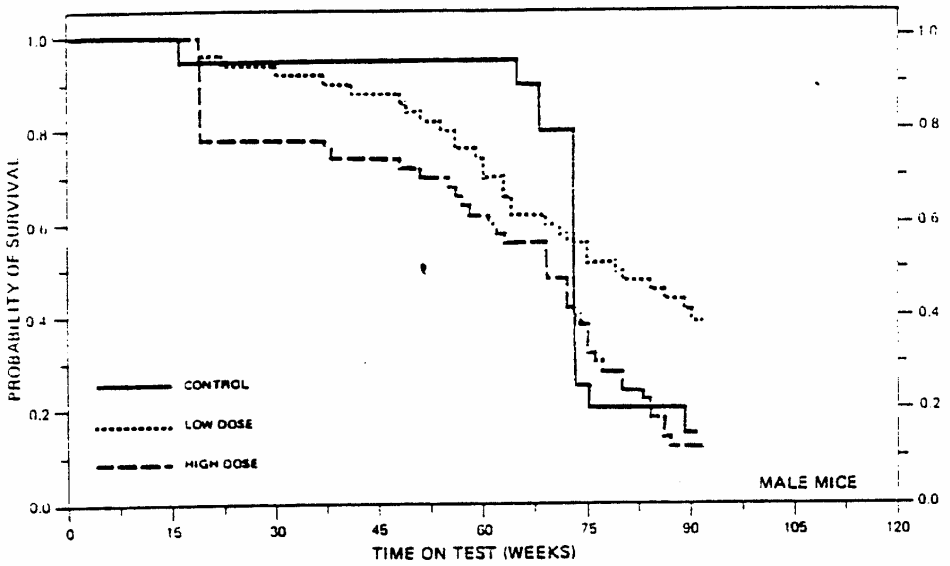
49 (93)



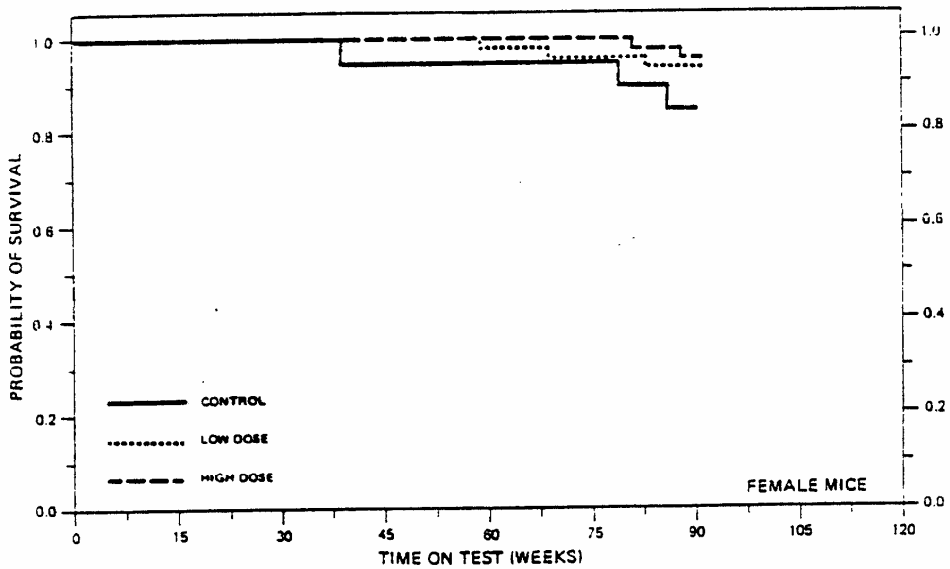


FIGURE 4
SURVIVAL COMPARISONS OF ENDOSULFAN CHRONIC STUDY MICE

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The estimates probabilities of survival for male and female mice are summarised in table B.6.5.2.2-1.Althought high mortality was noted in male test, this was not statistically significant. No common causes of deaths was found for the high dose males and in the control group the death may have been due too fighting among the animals. The mortality to the end of the test was 90% of the high dose, 62% of the low dose and 85% of the control group. Early deaths in the males were not tumours related. In contrast, the survival rate of females was unaffected by treatment.

Histophatological examination revealed non-neoplastic lesions in the kidneys and sex organs of male and female mice treatment–related. No conclusion could be drawn about the carcinogenic potential of Endosulfan in males Owing to the high early mortality rates. In addition, any tumours treatment-related were found in females mice.

Conclusion

Any adverse effect has been observed in female mice at the two dose- level tested. and no conclusion could be drawn about the carcinogenic potential of Endosulfan in male mice.

The study is not acceptable.

Donaubauer, (1988a, b) (AgrEvo: IIA, 5.5.2/1/2)

Date of report:6 April 1988.

Dates of experimental work: the report was performed between 28 January 1985 and 10 February 1987.

The study was conducted to OECD 451 guideline (1981) in compliance with EPA Guidelines, adopted in 1984.

GLP: yes.

The study is acceptable.

Materials and Methods

The test substance was Endosulfan technical (97.9% purity). It was dissolved in sesame oil and mixed with the diet. The stability and homogeneity of the product in food were examined weekly.

The test animals were NMRI-mice (age 4 weeks old and weight of 23 g for males and 22.5 g for females).

In order to test the carcinogenic potential, 60 males and 60 females in each group were fed diet containing endosulfan at concentration of 0, 2, 6 and 18 ppm (equivalent to 0.28, 0.84 and 2.51 mg/kg/day for males and 0.32, 0.97 and 2.86 mg/kg/day for females respectively).for a maximum of 24 months. For evaluating the chronic toxicity, satellite groups of 10 males and 10 females were killed 12 and 18 month after treatment.

Behaviour and general conditions (neurological disturbances, impairment of eyes and teeth, and changes in oral mucosal) were observed twice daily; and body weight, food consumption, survival and laboratory investigations on 10 animals/sex/group (haematology and clinical chemistry in blood) were also carried out. The necropsy: on all killed animals and those died intercurrently, included macroscopic examination, weight of major organs, residue determination in liver and kidney, and histological examination.

For statistical evaluation of the study the parameters (body weight, mortality rate, erythrocyte count, haemoglobin, haematocrit, reticulocyte count, leukocyte count, platelet count, ASAT, ALAT, AP and relative organ weight) were analysed by a parametric method (Dunnett or Sidak) and a distributed-free method (Nemeyi/Dunnett or Nemeyi/Sidak).

Results

Behaviour and was unaffected by administration of endosulfan. Palpation of the skin to detect nodules revealed pathological findings in a number of animals from all groups, including controls.

Mortality at the 24 months treatment period were comparable between Endosulfan-treated and control animals except for females of the 18 ppm group where mortality was statistically significantly increased.

Table B.6.5.2.2-2:. Mortality in the 24 months treatment period in rats (n=60)

	Males				**Females**			
Treatment period (weeks)	**control**	**2ppm**	**6ppm**	**18ppm**	**control**	**2ppm**	**6ppm**	**18ppm**
1-26	3	1	1	2	3	2	0	1
27-52	2	7	9	13	1	0	8	11
53-78	7	6	6	2	7	8	8	11
79-104	15	19	21	18	22	26	22	20
Total mortality	27	33	37	35	33	36	38	43
% Total mortality	45	55	61.7	58.3	55	60	63.3	71.1

Males treated with 18 ppm of Endosulfan showed a slight but frequently statistically significant reduction of bodyweight gains. On the contrary, at the same dose, females showed a slightly increased bodyweight gain compared with the control especially during the first half of the24 months study.

Food consumption, haematology and clinical chemistry did not differ between treated and untreated groups.

Macroscopic examination did not reveal any pathological changes. On occasion, after 12 months of treatment, a significant decrease in the relative lung and ovary weights were seen in the females of the 18 ppm group. After 18 months of treatment all organ weights of the mice killed were unremarkable, except a slight decrease in liver weight in males and ovary weights in of the 18 ppm group, which are considered as incidental findings. All values were within the normal range of the strain of mice nevertheless a compound related effect cannot be excluded.

Histopathological examination did not reveal any effects on neoplastic and non-neoplastic lesions.

Conclusions

The Chronic NOAEL was 6 ppm endosulfan (equivalent to 0.84 mg/kg/day for males and 0.97 mg/kg/day for females), based on decreased body weight in males (24-months sacrifice) and an increased mortality in females at 18 ppm. In addition, minor changes in organ weights (liver, ovaries, lung) were seen in animals treated the thigh dose level.

Endosulfan exhibits no carcinogenic properties in mice.

Leist, (1989b) (AgrEvo, IIA,5.5.2/4) Amendment to report IIA, 5.5.2/1.

Date of report: 27 July 1989.Dates of experimental work: This study was performed between February 1988 and 13 July 1988 .

In support of the study referred to above (IIA, 5.5.2/1), the residues of α-endosulfan, β-endosulfan, endosulfan-hydroxiether, endosulfan-sulphate, endosulfan-lactone and endosulfan-diol, were determined in the liver and kidneys of mice after a chronic (2-year) feeding. study.
The report does not claim adherence to a specific test guideline.
GLP: yes.

This study is acceptable as additional information.

Material and Methods

The test substance was Endosulfan technical (97,9% purity.). For residue determination, pieces of liver and one half kidney (left and right) of each group (10male and 10 female mice) were preserved at terminal sacrifice after 24 month treatment with endosulfan at dietary concentration levels of 0, 2, 6 and 18 ppm. Organs without macroscopic findings were only used.

The residues were determined on the basis of "Instructions for calculating residue levels" (SOP 0781-1/85) and corrected in accordance with the corresponding recovery rates.

Results

The parent compounds, α-and β-endosulfan, were not detected in any animals. The levels of some metabolites, endosulfan-hydroxyether, -lactone and –diol were below or just above the detection limit (0.02mg/kg organ); the endosulfan-sulphate concentrations were the only somewhat higher: in tissues of liver, 0.67/1.10 mg/kg (male/female), and kidneys 0.14/0.23mg/kg (male/female). at the highest dose group. The endosulfan-sulphate levels were marginally higher in females than in males.

Conclusion

Neither the parent compounds, α–and β-endosulfan, nor the metabolites, endosulfan-hydroxyether and–diol , could be definitely detected in liver and kidney.

Endosulfan-lactone was at or just over detection limit in the highest dose group (18 ppm.) In the 2 ppm dose group, the endosulfan-lactone level of 0.11 mg/kg, measured in the kidneys must be seen as an outliner or as due to contamination during analysis because the levels measured in the kidneys from the groups treated with higher doses of 6 and 18 ppm were noticeably lower. Only the endosulfan-sulphate levels reached somewhat higher values, mostly in the highest dose group. A retention and accumulation of the test material in the organism following prolonged exposure can be excluded.

B.6.5.3 Combined chronic/carcinogenic toxicity studies

B.6.5.3.1 Rats

Ruckman *et al*.,1989 (AgrEvo: II A, 5.5.1/4; AgrEvo: ANRA)

Date of report: 1 April 1989.

Test method was designed in accordance with OECD "Short-term and Long-Term toxicology group guideline" (14 August 1981)and EPA FIFRA test guidelines.

GLP :yes

The study is considered to be acceptable

Material and Methods

A pre-mix was prepared each week by first dissolving the test material Endosulfan technical (97.1% purity) in acetone then mixing in corn oil and was administered to five groups of 50 males and 50females Charles River rats at doses of 0, 3, 7.5, 15, or 75 ppm. (equivalent to 0, 0.1, 0.3, 0.6 and 2.9 mg/kg/day for males and 0, 0.1, 0.4, 0.7 and 3.8 mg/kg/day for females respectively) for 104 weeks .Satellite group of 20 male and 20 female rats, were attached to each main group for toxicity evaluation These were intended for blood sampling at intervals and for sacrifice after 104 weeks of treatment and were not included in the tumorogenic evaluation

Clinical signs, mortality, water consumption, food consumption; and bodyweight changes were recorded. Samples of satellite group were obtained to haematological, urinalysis and biochemistry analysis and once a year ophthalmoscopy analysis was carried out. The surviving animals (main and satellite groups) were killed after 104 weeks and necropsy was made. These and all animals that died or had to be killed during the study were checked in detail for any external or internal symptoms of tumour formation. Major organs were weighed and tissues collected for extensive histological examination.

Depending on data, different statistical tests were used for food consumption, water consumption, bodyweight, organ weight and clinical pathology analysis (Fisher, 1959; Mantel, 1963; Bartlett, 1937; Kruskal & Wallis 1952/3; Williams1971/2 and Shirley, 1977) Mortality was analysed using logrank methods (Mantel, 1966). All statistical analysis was carried out separately for males and females.

Results

No clinical signs were attributed to treatment were observed in animals during the study. The percentage of survival rats at the end of 104 weeks of treatment is show below:

B.6.5.3.1-1: Survival rate

Males					**Females**				
0 ppm	3 ppm	7 ppm	15ppm	75ppm	0 ppm	3 ppm	7 ppm	15ppm	75ppm
42%	38%	40%	40%	38%	28%	40%	36%	42%	36%

The overall distribution of decedents and the statistical analysis of mortality gave not indication off a treatment-related effect on mortality.

Overall body weight gain for rats of either sex at 75 ppm was lower than that the controls The effects on the growth of males at 75 ppm was noted apparent until after week 6, whereas the effects of females sat 75 ppm was noted from the start of treatment.

Food consumption and water consumption was generally similar in control and treated groups. Opthalmoscopic 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 control. However, 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 and urinalysis examination also revealed a number of parameters in treated animals that were statistically significantly different to controls, but these changes were not considered to be related to Endosulfan administration.

Macroscopic pathology examination revealed: the following changes which were considered treatment-related :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 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. **(**Table 6.5.3.1-2)

Table 6.5.3.1-2: Incidence of macroscopic lesion observed after administration to endosulfan

Dose ppm	**0**	**3**	**7.5**	**15**	**75**
Aneurysms in blood vessels (males)					
Main	9/50	3/50	10/50	5/50	12/50
Satellite	1/20	2/20	2/20	3/20	6/20
Combined	10/70	5/70	12/70	8/70	18/70
Incidence of bilaterally enlarged kidneys (females)					
Main	8/50	12/50	14/50	13/50	18/50
Satellite	2/20	6/20	5/20	4/20	8/20
Combined	10/70	16/70	19/70	17/70	26/70
Lumbar Lymph nodes (males)					
Satellite	2/20	2/20	3/20	2/20	5/20
Combined	14/70	10/70	8/70	7/70	19/70

Statistically significant ($p<0.01$) decreases in group mean absolutes testes weight were seen in main group at 15 and 75 ppm. As these testis weights were within normal historical control ranges 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 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. Both of them, occur at relatively high frequency in aging laboratory rats and were considered the major factor of death rats (see table 6.5.3.1-3):

Table 6.5.3.1-3: Incidence of tumour s in male and females rats (nº animals with factor/nº animals dead)

Dose	**Males**					**Females**				
Ppm	**0**	**3**	**7.5**	**15**	**75**	**0**	**3**	**7.5**	**15**	**75**
Pituitary tumour										
Main[a]	11/3	10/3	5/31	8/30	11/3	16/3	16/3	13/3	19/2	17/3
Satellite[b]	1/9	5/12	2/15	5/16	2/14	8/16	8/12	6/10	9/14	5/11
Total[c]	12/4	15/4	7/46	13/4	13/4	24/5	24/4	19/4	28/4	22/4
Mammary tumours										
Main[a]						14/3	14/3	14/3	8/29	6/33
Satellite[b]						2/16	4/12	4/10	3/14	2/11
Total[c]						16/5	18/4	18/4	11/4	11/44
Renal lesions										
Main[a]	9/32	7/31	15/3	12/3	14/3	1/37	2/31	1/32	3/29	3/33
Satellite[b]	1/9	5/12	5/15	3/16	9/14	0/16	3/12	1/10	2/14	2/11
Total[c]	10/4	12/4	20/4	15/4	23/4	1/53	5/43	2/42	5/43	5/44

[a)] Main groups consisted of 50 animals/sex/dose

[b)] Satellite groups consisted of 20 animals/sex/dose

[c)] Combined groups of 70 animals/sex/dose

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 75 ppm and above than for control animals, there was no dose dependence for this factor, and overall mortality was not increased in treatment groups.

Progressive glomerulonephrosis (PGN) was a common age-related finding in control and treated animals at histopathological examination. PGN was recorded in 3 grades of severity namely minimal , when the lesions characteristic of PGN affected up to 15% of the nephrons, moderate when 15 to 50 % of the nephrons were affected; and Marked,when majority (greater than 50%) of nephrons was involved with characteristic changes of PGN leaving progressively lesser amounts of normal tissue.

Historical control from six studies indicate an incidence of marked PGN in male 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 was 43% (30/70 animals), and while this incidence is only slightly 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.

Table 6.5.3.1-4: Non-neoplastic findings in kidneys

Dose ppm	Males					Females				
	0	**3**	**7.5**	**15**	**75**	**0**	**3**	**7.5**	**15**	**75**
PGN Main Group (50animals/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
PGN in Satellite Group (20 animals (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	20	18	22	24	30	1	6	6	5	8
PGN Total (70animals/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

Conclusion

The **NOAEL** was 15 ppm, equivalent to about 0.6 and 0.7 mg/kg/day for males and females respectively, based on the low body weight gains in both sexes, increase in the incidence of enlarged kidneys in females; increase in the incidence of blood vessel aneurysms mainly in males and increased incidence of enlarged lumbar lymph nodes (mainly in males) at 75 ppm

Upon the conditions of this study, Sprague-Dawley rats administered Endosulfan in the diet at up 75 ppm (2.9-3.8 mg/kg bw/day) for two years, there was no evidence of increased carcinogenicity findings at any dose tested.

Gopinath & Cannon, (1990) (AgrEvo: IIA, 5.5.1/5)

Addendum to Report IIA, 5.5.1/4**.**

Date of report: 23 November 1990.

In support to report IIA, 5.5.1/4, this study includes 6 photomicrographs of kidneys showing varying grades of progressive glomerulonephrosis, and, 2 photomicrographs of vascular aneurysms.

Leist, (1989[a]) (AgrEvo, IIA,5.5.1/3) Amendment to report IIA, 5.1.1/4.

Date of report: 27 July 1980. Dates of experimental work: the study was performed between 17 October, 1988 and 03 March. 1989.

The objective of this report was to determine the retention and/or accumulation of endosulfan residues and metabolites in the rat liver and kidney tissues from the combined chronic toxicity/carcinogenicity (report IIA, 5.1.1/4).

The study does not claim adherence to a specific test guideline.

GLP: yes

The study is considered acceptable as complementary information to report IIA, 5.1.1/4**.**

Material and Methods

The test substance was endosulfan technical (98,8% purity). A chronic feeding study was conducted with male and female rats using dose levels of 0, 3, 7.5, 15 and 75 ppm. (report IIA, 5.1.1/4.). After two years completion of feeding study, the animals were sacrificed and combined samples of livers and kidneys respectively were prepared from rats of each sex and dose level group. The separate analysis samples were taken from each combined lot in order to determine the levels of endosulfan and its main metabolites (endosulfan-hydroxyether, -sulphate, -lactone and –diol.). There were 70 animals in each dose group.

Results

As no residues above the limits of quantification (LOQ 0.10-0.12) were observed in livers and kidneys from both 15 and 75 ppm groups, the organs from both 7.5 and 3.0 ppm groups were not analysed. No metabolites were observed above the LOQ in the organs from any of the animals, with the exception of the oxidation product, endosulfan-sulphate, which concentration in the livers from 75 ppm group ranged from 0.2 to 0.4 mg/kg tissue.

Conclusion

Based on the results of the analytical examinations of kidneys and liver under, the conditions of this study, it can clearly be stated that no residues of endosulfan or its metabolites were presented in these organs examined with the only exception of endosulfan-sulphate in liver.

B.6.6 Reproduction toxicity (IIA, 5,6)

Summary

A summary of acceptable reproduction studies are presented below.

According with the results from a preliminary study to determine the maximum tolerated dosage of endosulfan suitable for use in multigenerational study, concentrations of 0, 3, 15 and 75 ppm were selected for a subsequent reproduction study in albino rats. No effects on reproduction parameters were noted at any dose tested, only a minimal reduction in litter weight was observed at 75 ppm. This findings were noted to be minimal, inconsistent and were not associated with effects on litter or pup weight. The Reproduction NOAEL for this study was therefore confirmed as 75 ppm (5mg/kgbw/day).

Histopathological review of the lesions founded in kidney from adults rats of the F1B generation and from weanling rat of the F2B generation were carried out by Offer in 1985. This revision suggest and occasional yellowish discoloration in proximal convoluted tubules associated with the dietary administration of Endosulfan at dose level ≥ 3 ppm in male adult rats and at dose level of 75 ppm in female adults rats of theF1B generation. In addition traces of minimal granular pigmentation in cells of proximal convoluted tubules were present in F1B generation adult rats at dose level of 75ppm.from the 75 ppm treatment group only. Besides, no evidence of histopathological changes were seen in the F2B generation weanling rats treated with 75 ppm of Endosulfan.

The teratogenic potential of Endosulfan was evaluated in rats and in rabbits. In rats the lowest NOAEL for maternotoxicity was established in 0.66 mg/kgbw/day based on clinical signs and reduced in body

weight showed at the mid dose tested.(Mc Kenzie, 1980). Developmental effects were reduced a skeletal variations and minimal anomalies at the higher dose level.

On the other hand, Endosulfan did not showed any developmental effect on rabbits and only and the high dose tested maternal toxicity was evident.

B.6.6.: Summary of acceptable reproduction studies

Study	**Dosage**	**NOAEL**		**LOAEL**		**Main Adverse effect**	**References**
		Ppm	**Mg/kg/day**	**ppm**	**Mg/kg/day**		
Multigeneration study							
Two generation reproduction toxicity in rats.	ppm: 0, 3, 15, 75 mg/kgbw/day 0.2,1, 4.99 for males and 0.24, 1.23, 6.18 for females	Parental =15 Reprod =75	Parental = 1m 1.23f Reprod= 1m 1.23f	Parental =:75 Reproduction >75	Parental=: 4.99m 6.18f Reproduction >4.99m >6.18f	Parental LOAEL: based histopathologic and organ weights changes showed in livers and kidney from F0 and F1Bgeneration Reproduction toxicity no effects	Edwards et al., (1984) AgrEvo:IIA, 5.6.1/3 ---------- Offer., (1985) AgrEvo, IIA: 5.6.1/4 (histopathological review of the kidney in adults rats of the F1Bgeneration and in weanling rat of the F2B generation
Developmental studies							
Teratology study with FMC 5462 rats	0. 0.66, 2 and 6 mg/kg bw/day		Maternal =0.66 Develop=2		Maternal:=2 Develop=6	Maternal toxicity based on clinical signs (face-rubbing and alopecia) and reduced in body weigh gain. Develop toxicity: based on reduce mean foetal weights and lengths and significant skeletal variations .No teratogenic effects	McKenzie (1980) AgrEvo: IIA, 5.6.2.1/3)
Embryotoxicity in the Wistar rats	0. 0.66, 2 and 6 mg/kg bw/day		Maternal:= 2 Develop:= 2		Maternal=6 Develop=6	Maternal toxicity;. based on deaths (4 dams), clinical signs (tonoclonic convulsions , increase salivation, blood-crusted nose) and decreased body weight Develop: based on minor anomalies as	Albrech & Baeder, 1993 AgrEvo: IIA, 5.6.2.1/4

B.6.6.: Summary of acceptable reproduction studies

Study	Dosage	NOAEL		LOAEL		Main Adverse effect	References
		Ppm	Mg/kg/day	ppm	Mg/kg/day		
						fragmentation of thoracic vertebral centra. No teratogenic effects	
Teratology study with FMC 5462 rabbits	0, 0.3, 0.7, 1.8 mg/kgbw/day		Maternal =0.7 Develop:=1.8		Maternal=1.8 Develop≥1.8	Maternal: based on deaths (4 animals) and clinical signs (noisy, rapid breathing, hyperactivity and convulsions) Developmental toxicity no effects	McKenzie et al., 1981 AgrEvo: IIA, 5.6.2.2/1

Reevaluation of studies included at the original monograph

B.6.6.1 Multigeneration reproductive studies

Kennedy. *et al.,* (1965) (AgrEvo, IIA,5.6.1/1)

Dates of experimental work: starts 2 April, 1964. Data of report: 30 December 1965.

The objective of the study was to evaluate the effects of Thiodan on the reproductive function of multiple generations in the rat.

The report does not claim adherence to a specific guidelines.

The study was performed prior the requirements for GLP

The study has to be considered as not acceptable. Since there is neither specification of the purity or the stability of the thiodan administered to the animals. Furthermore at least three treatment groups and a control group should be used instead the only two groups tested and the highest dosage employed did not elicit any evidence of toxicity.

Material and methods

The test substance was Thiodan. The test diet were prepared by first combining a weight amount of test material with a standard pulverised stock ratio (Wayne mouse Breeder diet), so that the final concentrations of test material in the diets were 2 ppm and 50 ppm respectively.

The animals employed were Sprague-Dawley rats (body weight: 64 g for males/ 62 g females) A total of 96 rats (32 males/32 females) were selected from a larger population of weanling to form two control groups (C-I and C-II) and two test groups of parental generation animals, Test I and Test II, that receiving endosulfan in the diet at concentration of 2 and 50 ppm respectively. Animals of the F0 generation in all group were maintained on their respective diets without interruption until their sacrifice, which followed the weaning of the F1b litters.

Matting trials were initiated when the F0 generation rats were 100 days old (78 days on test). Each of the 16 females in every group was mated randomly with a male from within the same group. The F1a litters obtained were weaned at 21 days post-partum. The parental females were then given a ten-day rest period and again mated, procedure being repeated in order to obtain the F1b litters. 8 males and 16 females from the F1b litters o each group was selected at weaning for use as F1 generation parental animals. The same method to obtain F2 and F3 generation was followed.

Clinical conditions, body weights, survival and abnormal behaviour on F0 generation, (fertility, gestation and lactation) were determined. In all pups abnormalities at birth, survival and stillborn members. were examined. After weanling of the F1b litters, all male and female parental animals from each group were sacrificed and subjected to complete gross and microscopic pathologic examination. The following tissues and organs were evaluated: hearth, trachea, lungs, liver, pancreas, oesophagus, stomach, intestinal tract, spleen, lymph nodes, kidneys, urinary bladder, testes, ovaries, prostate, seminal vesicles, uterus, vagina, pituitary , adrenal, thyroid and salivary glands, skeletal muscle ,eyes, brain, bones and peripheral and optic nerves.
An analysis of variance was first conducted , and significant effects disclosed by the treatment were further studies by “t”- test.

Results

The results of the first, second and third generation of a three-generation reproduction study using Thiodan revealed no abnormalities among the F0, F1 and F2 parental animals or their F1a/F1b; F2a,/F2b; F3a/32b progeny, respectively. Findings among test animals were comparable with those of control for all parameters investigated.

Conclusions

In all three generation, no adverse effects which could be correlated with the oral ingestion of thiodan at 2 or 50 ppm either parental animals or their progenies.

The study is considered as not acceptable thus the purity and the stability of the thiodan administered does not notified. At least three dose levels and a concurrent control shall be used and, furthermore, the highest dosage employed did not elicit any evidence of toxicity. Also, the dosages employed are referred to mg/kg/diet. It has not been possible to relate diet concentration of endosulfan to mass of endosulfan /kg/bw animals/day.

Edwards et al., 1982 (AgrEvo: IIA, 5.6.1/2)

Data of report: 2 December 1982.
The report does not claim adherence to specific guidelines.
The study was performed prior to GLP regulations.

The study is considered as additional information, thus it is a preliminary study to determine the maximum tolerated dosage of endosulfan suitable for use in a subsequent multigeneration study.

Materials and methods

The test material Endosulfan (97% purity) was dissolved in a small volume of acetone and mixed with corn oil. The mixture was administered to four groups of 10 males and 10 females each of the F0 and F1 generation of Charles River rats at dose levels of 0, 50, 75, and 100 ppm (equivalent in the first week to 4.10, 6.25 and 8.26 mg/kg/day in males and 4.19, 5.92 and 8.36 mg/kg/day in females; and 3.79, 5.58, 7.49 mg/kg/day in males and 4.49, 7.11, 9.59 mg/kg/day in females respectively, in the last week) from 2 weeks prior mating until sacrifice shortly after weaning.

The animals were observed daily for mortality and clinical symptoms, control of body weight and feed consumption as well as mating performance, pregnancy rate and gestation period. At termination, macroscopic examination, liver weight determination, litter data recordings (number, sex, and offspring weight, external abnormalities, survival rate, weight development, external and internal abnormalities at sacrifice 28 days post partum), were carried out.

Statistical methods to analyse the litter data were non-parametric test (Hollander & Wolfe, 1973). Analysis of covariance (Suedecor & Cochran, 1967) and a William's test (1971/2) were used to analyse the liver weights.

Results

Among adults there were no clinical signs attributable to treatment.. Group mean food consumption of males was unaffected by treatment In contrast, during the first week of treatment, all tested females showed a slightly lower food consumption in comparison to control animals, differences although not strictly dosage-related were more accentuated at 70 and 100 ppm than 50 ppm (table B.6.6.1-1).

Table B.6.6.1-1: Group mean food consumption in females (g/rat/day)

Week	0ppm	50ppm	75ppm	100ppm
1	20	18	16	17
2	20	20	21	20

Besides, at these two concentrations lower mean weekly body weight were apparent during the pre-mating treatment and subsequently through to week 6. During gestation at 75 and 100 ppm slightly lower weights gains were again observed. At 50 ppm there was a slight initial retardation but by day 17 of pregnancy weight gain was comparable to the control value. Mean value at weaning were comparable with the control value at 100 ppm, and respectively higher and lower at 50 and 75 ppm (Table B.6.6.1-2).

Table B.6.6.1-2: Group mean bodyweights of dams with viable young during gestation an lactation

	Gestation						Post-partum					
	Nº females	**0**	**7**	**14**	**17**	**20**	**Nº females**	**G0**	**0/1**	**7**	**14**	**21**
0ppm	9[a]	234	268	302	328	364	9[b]	236	284	279	302	290
50ppm	10	233	263	298	327	364	10	233	273	290	302	286
75ppm	9	224	250	286	310	345	8[c]	225	268	274	283	269
100ppm	10	224	252	287	312	344	10	224	269	275	291	281

G0= bodyweights of day 0 of gestation used for calculating post partum changes.

[a] Excluded one dam with viable young, day 20 bodyweight not recorded

[b] Excluded one dam showing total litter loss

[c] Excluded one dam sacrificed day 11 post-partum

Mating performance, pregnancy rate and the duration of gestation were all unaffected by treatment.

At terminal autopsy mean liver weights were significantly higher among females in all test group (table B.6.6.1-3) in comparison than the control value

Table B.6.6.1-3: Mean liver weights (g)

Group	Males	Females
0ppm	26.097	12.385
50ppm	26.864	14.842**
75ppm	23.978	14.039*
100ppm	26.914	15.011**

** p<0.01; * p<0.05

Among litter data parameters are summarised in table B.6.6.1-4;. At t birth, mean litter weight at 100 ppm was slightly lower than control value, but not statistically significant. Thereafter, during lactance there was a generally dosage-related tendency for slightly increased pup losses, resulting in lower litter sizes and for lower mean pup weight gains. The combination of these differences resulted in a significantly lower litter size weights at 75 ppm and a grater extent at 100 ppm from day 4 post-partum.

Table B.6 6.1-4: Group mean litter data

	0ppm	50ppm	75ppm	100ppm
At birth				
Litter size	13.7	14.7	13.6	12.4
Cumulative loss %	1.7	2.3	0.8	0.6
Litter weight (g)	76.2	80.5	76.8	71.0
Mean pup weight (g)	5.6	5.7	5.7	5.8
At day4				
Litter size	13.6	13.8	12.9	11.6
Cumulative loss %	2.4	5.5	5.4	8.3
Litter weight (g)	114.4	110.7	**97.4***	**87.2****
Mean pup weight (g)	8.5	8.0	7.6	7.6
At day 8				
Litter size	13.2	13.4	12.4	11.3
Cumulative loss %	4.8	8.0	8.7	10.4
Litter weight (g)	172.8	165.5	**141.1***	**128.2***
Mean pup weight (g)	13.2	12.3	11.5	11.4
At day 12				
Litter size	13.2	13.1	12.1	11.0
Cumulative loss %	4.8	9.8	10.3	12.6
Litter weight (g)	260.5	240.7	**208.7***	**184.9****
Mean pup weight (g)	19.9	18.2	17.3	**16.6***
At day 21				
Litter size	13.2	13.1	12.0	10.6
Cumulative loss %	4.8	9.8	11.1	15.7
Litter weight (g)	474.1	436.0	**362.5***	**320.1***
Mean pup weight (g)	36.3	32.9	30.4	30.3
At day 24				
Litter size	13.2	12.1	12.0	**9.2***
Cumulative loss %	4.8	16.2	11.1	25.8
Litter weight (g)	623.6	520.6	**488.6***	**382.3****
Mean pup weight (g)	47.7	41.4	41.0	39.5
At day 28				

	0ppm	50ppm	75ppm	100ppm
Litter size	13.2	11.7	12	**9***
Cumulative loss %	4.8	18.4	11.1	26.5
Litter weight (g)	832.9	710.9	686.3	**529.4****
Mean pup weight (g)	63.6	58.2	57.5	55.8

$^{+}p< 0.05$; $^{++}p < 0.01$

There were no abnormalities among young or changes observed at autopsy examination thatwere considered attributable to treatment.

Conclusions

On the basis of the above findings it was concluded that 75 ppm (equivalent to 8.26 mg/kg/day and 8.36 mg/kg/day male/female respectively), would be suitable fur use as the highest dietary concentration in the subsequent multigeneration study.

Edward, *et al* , (1984). (AgrEvo, IIA, 5.6.1/3; AgrEvo: ANRA)

Dates of experimental work: This report was performed between 13 January 1982 and 20 April 1883. Data of report: 19 July 1984.

The original study design employed was intended to fulfil the requirements that existed at the time of different regulatory agencies including the " American Environmental Protection Agency " and the Bureau of Chemical Safety ".

GLP: Yes

The study is acceptable.

Material and Methods

The test substance was endosulfan technical grade (97% purity)was dissolved in a small volume of acetone and mixed with corn oil .The mixture was administered in the diet to COBS CD (SD) BR Charles River strain rats at concentrations of 0, 3, 15 and 75 ppm for two mating generations (F0 and F1B), with two mating phases in each. In the premating period for the F0 generation, these dietary concentration 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. 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. Group sizes were 32/sex/ghroup for F0 and 28 sex/dose for F1B.

Rats of F0 generation were treated with the test substance for at least 84 days prior to mating until they were approximately 18 weeks of age. The animals were mated on the basis of 1:1 for a period of 20days. On or shortly after day 21 post partum, any F1A young were sacrificed to histopathological examination. Shortly following (approximately 10 days), the selected weaning F1A pups and the F0 generation were re-mated for a period of 20 days. At day 21 post-partum 28 male and 28 female pups per group were selected to form the basis of F1B generation and the excess pups were sacrificed. Shortly after theF1B had weaned, F0 generation was sacrificed and histopathological examen was performed from all specific organs.

In the selected F1B rats, a similar dietary and matting regimen was used as for the F0 generation. On or shortly after day 21 post partum, any F2A young were sacrificed to histopathological examination. Shortly following (approximately 10 days), the selected weaning F2A pups and the F1B generation were re-mated for a period of 20 days. After day 21 post-partum all F2B pups and F1B generation adults were sacrificed to histopathological examination.

Tissues of all adults and the selected pups from the control group at 75 ppm were subjected to histopathological examination(see adendum), and testes and the accessory organs of all animals failing to induce pregnancy at the second mate, and ovaries of females without young at the second mate, were also examined histopathologically.

All animals were regularly examined for signs associated with treatment, and determinations of body weigh and , food and water consumption were made alt 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, ileum, kidneys, lever, lungs, lymph nodes, mammary glands, seminal vesicles, skin, testes, thymus, thyroids, urinary bladder, uterus, vagina, mid colon, ovaries, pancreas, pituitary and prostate.

Statistical analysis was carried out: non-parametric method (Hollander & Wolfe, 1973) to analysed the variance to organ weight; heterogeneity of variance was indicated by Bartlett test (1937), and the intergroup comparisons were performed using the Williams test (1971/2).

Results

No clinical sign or mortality related to Endosulfan administration was observed during the study. Single mortalities in the F0 females at 0, 3 and 15 ppm and F1B control females.

There were no adverse effect on food consumption , only males of F1 generation males treated at 75 ppm showed a slightly reduction in comparison with the control.

Body weights and body weights gains in control and treated animals from F0 F1 and F2 generation were generally similar during the study.

Macroscopic autopsy among F0 generation males at 75 ppm there was a slightly increased incidence both of animals showing enlarged livers and of animals showing enlarged kidney.

At 75 ppm statistically significant increases in relative liver weights in males of F0 generation and F1B generation females were observed .In addition, kidney weights at the same dose levels among both F0 and F1B generation males were slightly higher than control animals (Table B.6 6.1-5/B.6.5.1-6) However, in the absence of any treatment- related microscopic changes among tissues from F1B adults, the differences in liver and kidney weights were considered to represent a marginal effect of treatment.

Occasional other significant differences were slightly increased heart weight among F0 males at dose level ≥ 15ppm and slightly higher brain weights among F0 females at 75 ppm. There was no consistent pattern among values for these organs to indicate any treatment relationship.

Table B.6 6.1-5: Group mean organ weights of adults from F0 generation

	Males				**Females**			
	0ppm	**3ppm**	**15ppm**	**75ppm**	**0ppm**	**3ppm**	**15ppm**	**75ppm**
Liver weight (g)								
absolute	25.661	26.744	26.496	28.346	14.031	14.082	13.701	14.957
Relative bw	26.181	25.970	27.070	**28.026***	13.815	13.927	13.808	**15.198****
Heart weight(g)								
absolute	1.7477	1.8671	1.8357	1.9023	1.2024	1.1885	1.1717	1.1931
relative	1.7763	1.8395	**1.8562***	**1.8908****	0.7845	0.7791	0.7769	0.7889
Kidney weight (g)								
absolute	4.6491	4.8815	4.8031	5.2596	2.7627	2.7157	2.6696	2.7739
relative	4.7064	4.7962	4.8664	**5.2243****	2.7316	2.6950	2.6852	2.8079
Brain weight (g)								
absolute	2.0614	2.0897	2.0781	2.0847	1.8507	1.8585	1.8551	1.8830
relative	2.0643	2.0854	2.0813	2.0839	1.8448	1.8540	1.8582	**1.8897***

$^{+}p< 0.05$; $^{++}p < 0.01$

Table B.6 6.1-6: Group mean organ weights of adults from F1B generation

	Males				**Females**			
	0ppm	**3ppm**	**15ppm**	**75ppm**	**0ppm**	**3ppm**	**15ppm**	**75ppm**
Liver weight (g)								
absolute	25.859	27.176	24.910	26.229	13.118	13.675	14.095	14.818
relative	25.857	26.122	25.298	26.898	13.181	13.497	**14.218****	**14.821****
Kidney weight (g)								
absolute	4.6219	47775	4.6692	4.6616	2.6348	2.6915	2.5204	2.7865
relative	4.6248	4.6394	4.7186	**5.1704****	2.6431	2.6683	2.5365	2.7869

$^{+}p< 0.05$; $^{++}p < 0.01$

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 no related to the dose of Endosulfan, and pup mortality , litter size and sex ratios were similar in control and treated groups..

However, at 75 ppm through lactation to weaning there was a general tendency during both mates of F0 generation for slightly lower mean litter weight with occasional statistically significant differences Although there was no consistent underlying pattern of differences among values for pup weight, or litter size, the tendency possibly represented a marginal impairment o overall litter growth (see table B.6.6.1-7).

Table B.6 6.1-7: Group mean litter weights of adults from F0 generation (g)

	0ppm	3ppm	15ppm	75ppm
1st mating				
At birth	67.4	72.3	72.6	60.4
At day 4	104.6	107.5	106.2	100.3
At day 8	170.3	174.7	174.5	157.6
At day 12	235.8	245.9	243.3	218.4
At day 21	434.4	449.4	436.5	**392.6***
2st mating				
At birth	76.1	74.7	73.4	73.4
At day 4	121.0	115.8	113.0	112.1
At day 8	191.2	185.6	177.6	172.5
At day 12	272.7	2.648	**2.528***	**2.408****
At day 21	491.3	483.5	468.6	443.8

$^{+}$ p< 0.05; $^{++}$p < 0.01

Conclusion

Under the conditions of the present study , there was no evidence of interference with fertility and pregnancy rate at the high dose tested. **The NOAEL for parental toxicity was 15 ppm** (1-1.23mg/kgbw/day in males and females respectively),based on the following effect showing at 75 ppm. (4.99-6.18mg/kgbw/day in males and females respectively):

1. slight decreased in food consumption in male rats
2. ;slight increase of enlarged kidney and enlarged livers in males of F0 ,
3. slightly increase in liver weights in F0 generation males and both F0 and F1B generation females;
4. slight increase of kidney weights from males of F0 and F1 generation

No effects on reproduction parameters were noted at any dose tested, only a minimal reduction in litter weight was observed at 75 ppm. This findings were noted to be minimal, inconsistent and were not associated with effects on litter or pup weight. The **Reproduction NOAEL for this study was therefore confirmed as 75 ppm (5mg/kgbw/day).**

Offer, J.M., 1985 (AgrEvo, IIA, 5.6.1/4; AgrEvo: ANRA)

Addendum to report IIA, 5.5.1/3.

Data of addendum: 22 March 1985

This work was a Histopathological review of the kidneys from adult rats of F1B generation previously examined in the original report (IIA, 5.5.1/3). In addition, this review was extended to adults rats form 3 and 15 ppm treatment groups not previously examined in the original report and kidneys from weanling rats in the F2B generation from the control and 75 ppm treatment group .

The histopathology review of the kidney has been undertaken following the observation of yellowish discoloration and pigment deposits in renal proximal convoluted tubules.

Results

The incidence and distribution of all renal changes from F1B generation adult rats are given in table B. 6.6.1-8. Changes considered to be treatment-related included:

1.- occasional cells of proximal convoluted tubules showing yellowish discoloration of their cytoplasm in male rats from all treatment groups and female from the 75 ppm treatment group.
2.-Another type of darker more particulate granular and/or clumped pigment in cells of the convolutions and straight portions of the proximal convoluted tubules from the 75 ppm treatment group only.

Table B. 6.6.1-8: Renal changes in F1B generation adult rats

Dietary inclusion (ppm)	**0**		**3**		**15**		**75**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**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
Mineral foci		1		1			1	1
Occasional basophilic tubules:				1			1	
Haemorrhage in pelvis:		1					1	
Increased pelvic dilatation:		2						2
Total number of rats examined	28	28	28	28	28	28	28	28

M = males
F= females

All other changes were considered to be unrelated to treatment.
No evidence of yellowish discoloured cells or granular/cumpled pigments were detected in F2B wealings rats from the 75 ppm treatment group.

Conclusion

0ccasional yellowish discoloration in proximal convoluted tubules were associated with the dietary administration of Endosulfan at dose level ≥ 3 ppm in male adult rats and at a level of 75 ppm in female adults rats of theF1B generation. In addition traces of minimal granular pigmentation in cells of proximal convoluted tubules were present in F1B generation adult rats at dose level of 75ppm.from the 75 ppm treatment group only.

No evidence of histopathological changes were seen in the F2B generation weanling rats treated with 75 ppm of Endosulfan.

B.6.6.2 Developmental studies

B.6.6.2.1 Rats

Haley *et al* (1972) (AgrEvo, IIA, 5.6.2.1/1)
Data of report: 18th July 1972.
The report does not claim adherence to specific guidelines.
The study was performed prior to **GLP** regulations

The study is considered as not acceptable because not offer the required information for evaluating teratogenic studies (OCDE 414) At least three dosage levels and a control group should be used should be tested instead two and he higher dosage should be induce ideally induce some overt maternal toxicity such a slight weight loss. On the other hand, the low dose should not induce observable effects attributable to the test substance and in this case.

Material and methods
Thiodan technical (purity 98%) was dissolved to corn oil and administered orally by gavage to Charles River albino at dosages of 0 (20 females), 0.5 (20 females) and 1.5 (23 females) mg/kg/day. All test animals were given the material test daily from the sixth day of the gestation period through the 15 day inclusive (a total of 10 doses. The day of gestation 0 was defined as the day of insemination (confirmed pregnant by sperm-positive results of vaginal examinations) The sacrifice of animals was day 20 by asphyxiation.

Each animal was observed daily for mortality and reactions, and body weight was determined every third day (days 6, 9, 12, 15 and at sacrifice).

Foetal swellings and implantation sites were counted. Special attention was paid to resorption sites or any other uterine abnormalities, as well the number of corpora lutea. Number of viable foetuses, foetal abnormalities, foetal skeletal development and foetal internal development were also investigated. Statistical methods are not mentioned.

Results
The study revealed no significant differences between test and control dams exposed to Thiodan during organogenesis.

No treatment-related differences for the above-mentioned parameters were observed on foetuses in all parameters evaluated. There was only one foetus with hematoma in the 1.5 mg/kg/day treatment group. All other foetuses obtained appeared outwardly normal. No treatment related skeletal abnormalities were observed between test and the control groups.

Conclusions

No significant maternal and developmental toxicity was observed attributable to the administration of endosulfan technical up to a dose level of 1.5 mg/kg bw/day, the highest dosage tested, in rats.

However, this study can not be acceptable for many reason; Teratogenic studies required al least three dose levels and a concurred control; The highest dose level should be chosen with the aim to induce toxicity and finally, there isn't any information about housing and preparations of animals and statistical methods.

Gupta *et al*., (1978) (AgrEvo: IA, 5.6.2.1/2; AgrEvo: ANRA; Calliope: IIA, 5.6.2/01)

This paper has been published in Acta Pharmacol Toxicol (1978). Vol. 42: 150-152.

No guideline method was available at the time of the study.

The study was performed prior to **GLP** regulations.

The study is to be considered as not acceptable because the purity, the stability of endosulfan technical, strain and age of animals were not specified. At least three dosage group should be used and the highest dosage level should ideally induce some overt maternal toxicity and the low dose level should not induce observable effects attributable to treatment.

Material and methods

Female albino rats were mated with males until copulation was confirmed by the presence of sperm and this was donated as day zero of pregnancy.

Endosulfan of technical grade suspended in corn oil was given orally from day. 6, through day 14 of gestation in doses of 0 .0, 5 and 10 mg/kg bw/day to female albino rats . The number of females copulates was 20, 26 and 32 females/dose level respectively and the number of pregnant at these dose levels was 18, 20 and 21, respectively. The animals were killed by ether inhalation on day 21 of gestation.

The pregnant were weighed on day zero and also from day 6 through day 14 of gestation as well as before and after caesarean section.

The parameters evaluated included: gross pathology on females (viscera, uteri, resorption of foetuses), and on foetuses (skeletal abnormalities, gross visceral inspection and internal malformations).

Statistical analysis of the data :Student's test The values were considered significant at $P < 0.05$.

Results

No marked changes in behaviour and appearance were observed. The body weight of the dams treated was comparable to that of the controls. Increasing mortality at 5.0 mg/kg /day (1 dead of 25 dams) or 10 mg/kg/day (5 of 25) was the only sign of toxicity in both groups. None of the animals showed aborted foetuses.

Although a few of the animals showed sperm on zero day of pregnancy no foetuses were observed upon sacrifice. The uteri of such animals were invariably enlarged and one of the uteri was filled with fluid. This enlargement could be due to the effect of endosulfan on the female sex hormones.

There was a significant increase in foetal mortality and resorption sizes in endosulfan treated rats. No significant change in foetal weight or gross abnormalities was observed.

Table B.6.6.2.1-1: Incidence of embryotoxic effects in rats receiving endosulfan

Parameter	**0 mg/kg**	**5 mg/kg**	**10mg/kg**
Number of females with sperm day 0	20	26	32
Number of survival	20	25	27
Number of pregnant	18	20	21
Total number of death foetuses	0	2	1
Total number of resorptions	5	26	29
% of litters with resorptions	5.5	20*	22.8*

* $p \leq 0.01$

Slight increases in the incidences of cerebral hypoplasia and enlargement of the renal pelvis were observed on visceral examination, but these effects were not considered to be related to treatment as they were also seen in control animals and the increases were small and were not dose-dependent. No other increase in the incidence of visceral abnormalities was reported.

Skeletal abnormalities revealed a statistically significant increase of absent 5 th sternebrae and in foetuses with incomplete ossification . The missing 5^{th} sternebrae was significantly high in both treated groups. These defects were observed after a high dose level used primarily to highlight the teratogenic effects to dose levels toxic to the dams. Other skeletal anomalies as incomplete ossification has been summarised in table B.6.6.2.1-2 but although they were statistically significant different form the control rats there were considered as incidental effects that there was not a dose relation ship. An extra rib is a frequent observation at dose level ≥ 5mg/kg/bw/day and could be regarded as a indicator of teratogenic potency. However, that this effect occurred also in control animals the explication could be due the fact the dams has been stressed sufficiently to express the developmental stability inherent in the specie.

Table 6.6.2.1-2: Skeletal abnormalities

Parameter	**0mg/kg/bw/day**	**5mg/kg/bw/day**	**10mg/kg/bw/day**
Nº foetuses examined			
% litters with abnormalities	15.7	26.6*	22.2
Sternebrae 5^{th} absent	3	**5***	**6***
Extra rib " 14 th rib"	2	3	3
Foetuses with incomplete calcification	6	9*	0
Wavy rib	0	1	0
Percent of foetuses with abnormalities	19.9	31.0*	24.9*

$p \leq 0.01$

Conclusions

Under the conditions of this study, the administration of Endosulfan to female rats caused a dose related increase in mortality. In addition, skeletal abnormalities have been developed at the low dose employed. Owing this results, it is not possible to set up a NOAEL for maternal or developmental toxicity and the study has been considered as not acceptable.

Mackenzie. *et al*., (1980) (AgrEvo: IIA, 5.6.2.1/3; AgrEvo: ANRA)

Dates of experimental work: The study was performed between 28 January1980 and 24– March 1980.Data of report: October 2, 1980.

No reference to a specific guideline.

GLP: Yes.

The study is acceptable

Material and methods

Endosulfan (FMC 5462), purity 97.3%, was dissolved in corn oil and administered via gavage to groups of 25 CD Sprague Dawley albino rats at dose levels of 0, 0.66, 2 and 6 mg/kg bw/day on days 6-19 of gestation. When mortality was observed in the high dose group, was added ten mated animals to the group On the other hand, when maceration occurred in six litters form the control group five mated animals were added to the control group to ensure that a minimum of 20 litters would be available for skeletal evaluation in that group

Pregnancy was checked daily for the presence of a vaginal plug or sperm in the vaginal smear. The day a vaginal plug or sperm was found was considered day 0 of gestation.

The body weight of each animal was determined on gestation days 0, 6, 9, 12, 15, 18 and at the time of sacrifice on day 20.

Each animal was observed twice daily throughout the test period for any abnormalities in behaviour, appearance, or any indication of toxicity, including changes in food intake, morbidity and mortality.

The necropsy included the investigation of reproductive tract and examination for gross external and internal abnormalities, also through examination of all viable foetuses. Dams that died during the test were examined similarly.

Depending on data, different statistical tests to evaluate dam body weight were used: Steel et al., (1960); Hollander & Wolfe, (1973) Dunn (1964).

Results

The survival of test animals is summarised in table B.6.6.21-3. The observed mortality in the high dose group could not be directly attributed to the test compound since the deaths of 5 of the 7 animals appeared to be due to technical factors. However although no pharmacotoxic signs were reported for these animals, their deaths may have been compound-related as indicated by a loss in body weight prior to death.

Table B.6.6.2.1-3.: Survival of mated rats treated with FMC 5462

	0 mg/kg	**0-66 mg/kg**	**2 mg/kg**	**6 mg/kg**
Nº of treated rat	30	25	25	35
Rats alive on day 20	29	25	25	28
%rats alive on day 20	97	100	100	80
6-8 gestation day	0	0	0	3
9-11 gestation day	1	0	0	1
12-14 gestation day	0	0	0	3
15-17 gestation day	0	0	0	0
18-20 gestation day	0	0	0	0

FMC 5462 caused pharmacotoxic effects in pregnant rats treated orally with 6 mg/kg/bw. These effects included rough coat, lethargy, flaccidity, hyperactivity and a response characterised by the rat rubbing its face. Maternal toxicity was also evident in the mid dose groups (2 mg/kg/day), although to a lesser extent. Uncorrected and corrected (for gravid uterine weight) day 20 day weights and body weights changes were significantly decreased ($p \leq 0.01$) in rats from the high dose group and corrected body weight and weight change also were significantly decreased ($p \leq 0.05$) in the 2 mg/kg/day. group.

Pregnancy maintenance, implantation efficiency, litter size and sex ratio were not affected. The percent live foetuses were significantly reduced and the number and percent of resorbed foetuses was significantly increased in animals treated with 2 mg/kg/day. However, these differences did not appear to be dose-related. Mean foetal weight and length were significantly reduced in litters of dams treated with 6 mg/kg/ day.

Table B.6.6.2.1-4: Summary of mean data from uterine examinations performed on day 20 of gestation

Parameters	**0 mg/kgbw/day**	**0.66 mg/kgbw/day**	**2 mg/kgbw/day**	**6 mg/kgbw/day**
Dams on study	30	25	25	35
Dams with implantation's	28	23	25	27
Mean nº of implantations/litters	15	14	16	15
Mean nº of live foetuses/litter	15	14	14	15
Mean % live foetuses	97.2	94.8	**91.5***	97.8
Mean foetal weight (g)	3.8	4	3.9	**3.5****
Mean foetal length	3.8	3.9	3.9	**3.7***
Mean nº of resorbed foetuses/litters	0.4	0.5	1.4*	0.3
Mean % resorbed foetuses	2.8	5.2	**8.5***	2.2

* $p \leq 0.05$; ** $p \leq 0.01$

No soft tissue abnormalities were present in foetuses from animals treated with 0.66 and 2 mg/kg/day, and no gross abnormalities were present in foetuses from animals treated with 2 mg/day.

Skeletal examinations were summarised in table B.6.6.2.1-5. Common minor skeletal variations were presented in all groups. Statistically significant variations included the presence of misaligned (fourth) sternebrae in the 0.66 and 2 mg/kg groups and significant increase in the incidence of small 4th, unossified 5th and poorly ossified and unossified 6th sternebrae in the high dose group.

Table B.6.6.2.1-5. Summary of Skeletal variations in rat

mg/kgbw/day	**0**	**0.66**	**2**	**6**
Sternebrae misaligned				
1	0.3	0.0	0.0	0.0
2	0.0	0.0	0.3	0.2
3	0.0	1.5	0.6	0.0
4	0.0	**3.3***	**3.1***	2.3
5	0.7	0.9	1.9	0.6
Small sternebrae				
1	0.6	0.0	4.1	1.6
2	61.9	66.0	64.3	76.7
3	0.3	0.0	0.6	0.5
4	18.7	11.0	5.0	**20.2****
Unossified sternebrae				
1	0.3	0.0	0.3	0.2
2	0.7	0.3	0.0	0.2
3	0.0	0.0	0.0	0.2
4	0.0	0.0	0.0	0.5
5	15.0	14.9	7.3	**34.0****
6	5.0	4.1	1.4	**12.4***
Sternebrae poorly ossified				
2	0.0	0.7	0.0	0.5
3	0.3	0.0	0.0	0.0
4	0.3	0.0	0.0	0.5
5	1.9	1.7	0.2	2.6
6	17.0	19.2	0.3	**29.5***

* $p \leq 0.05$; ** $p \leq 0.01$

Developmental malformations which were present at and incidence which was not statistically different from the controls, included microstomia in one foetuses at the low dose level and the following abnormalities in the high dose group: clubbed left (one foetus from each of two litters), skin of upper forelimb webbed to chest (one foetus from one litter), edema (one foetus form one liltter and five foetuses form another litter), lordosis (the same five foetuses in one litter which had edema) and cardiovascular abnormalities foetuses (two foetuses from two litters).

Conclusions

Maternal NOAEL was 0.66 mg/kgbw/day based on a dose related decreased in body weight and evident maternal toxicity showed at dose level ≥ 2 mg/kgbw/day

Developmental NOAEL was 2mg/kgbw/day based on significant reduced in mean foetal weight and length in litters of dams treated with 6 mg/kg/ day and skeletal variations showed at this dose level.
An additional review of this study by the U.S EPA concluded that replacement of animals during or after the study made it difficult to interpret the data and derive a NOAEL for this study. The U.S. EPA has recommended a repeat of this study.

Albrecht, & Baeder, (1993) (AgrEvo: IIA,5.6.2.1/4; AgrEvo: ANRA)
Dates of experimental work: the study was performed between 11 February 1993 and 19-April, 1993. Data of report: 18 November 1993.
The test guideline used was the **OECD 414** (1981), in compliance with EPA Guidelines, adopted in 1984.
GLP: yes.

The study is acceptable.

Material and methods
Hoe 002671- substance technical (purity 97.3%) was dissolved in sesame oil and administered orally by gavage to groups of 20 female Wistar, at dose levels of 0, 0.66, 2.00 and 6.00 mg/kg once daily from days 7 to 16 gravidity. The mating ratio was one male to one female. The day of sperm detection counted as day 1 of gravidity all survival females killed on day 21 post copulation by stunning and exsanguination.

All animals were subjected to determinations of clinical examinations, behaviour and general health condition, food consumption and body weight gains. After opening of the uterus, the live and dead foetuses, the conceptuses undergoing resorption and the placenta, and the corpora lutea on the ovaries were counted and examined macroscopically. The foetuses were then examined morphologically for developmental disturbances.

For the purpose of establishing comparisons with the control group, body weight, body weight gains and organ weight were evaluated by standard MANOVA, and the relative food consumption by the non-parametric linear mode of Puri & Sen (1985). The Mantel-Haenszel test was used fur analysing implants in corpora lutea, ratios of live and dead foetuses and conceptuses undergoing resorptions. The mean foetal weights, crown-rump lengths and placental weights for each litter were evaluated with a multivariance analysis. With each of these methods the probability of error for each group of parameters was 5%. The findings at autopsy and at the body cross-section and skeletal examinations were evaluated for foetus and litters separately by the Fisher test ($p < 0.05$: statistical significant) for comparing the data with historical controls the methods used in the same order were: WALD (1943), Wilks (1942) and Rosenkranks (1988).

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, and one of these dams also had a blood crusted nose on the day on which it died. The fourth dam died without any particular clinical signs of intolerance 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.

Administration of Endosulfan had no effect on the intrauterine development of the conceptuses. At delivery, most of the live foetuses were normally developed, and their body weights and body lengths were comparable with those of the control foetuses The presence of empty implantation sites only in one dam from 2 mg/kgbw/day group and another from the high dose group points to early intrauterine death of the conceptuses shortly after implantation, but the findings should be evaluated as isolated occurrences unrelated to the test substance. This is a justifiable assumption, since there was no increase in intrauterine mortality among the other dams in either of these dose groups and no doe-effect relationship can be derived. Even in earlier control groups up to 10% of the dams have been found to have empty implantation sites only.

The morphological examination oft he foetuses of the dams treated with Endosulfan did not reveal an increase in the incidence of malformations. The oedematous, retarded foetus in the 6 mg/kgbw/day group showing brachygnathia superior with a relatively small alveolar cavity in the upper jaw combined with cleft palate, bending of both hind-feet in the tarsal joints, wavy clavicles on both sides, and the bent and shortened scapula, must be rather as an isolated spontaneous occurrence.

The fragmentation of thoracic vertebral centra in even foetuses from three litters at 6 mg/kg as considered a minor anomaly. This effect was considered to be treatment related. Other minor anomalies (see table B.6.6.2.1-5) also occur spontaneously with similar frequency in control foetuses and there were not considered treatment-related. In addition, skeletal variations as anlage of a rib on the 7^{th} cervical or 1^{st} lumbar vertebra and the anlage of a 14 th thoracic vertebra with an analogous rib on one or both sides remained within the limits of the spontaneous rate, thus no casual connection with endosulfan administration.

No delaying ossification was observed of the live foetuses in the test group.

Table B.6.6.2.1-5: Incidence by Foetuses/litters of skeletal defects

Dose level (mg/kgbw/day)	**0**	**0.66**	**2**	**6**
Extra rib (variations)				
At 7th cervical vertebra Short-unilateral Foetuses	1(0.7%)	0 (0%)	3 (2.4%)	0 (0%)
litters	1 (5%)	0 (0%)	3 (15.8%)	0 (0%)
At 1st lumbar vertebra Short and/or normally long uni-or bilateral Foetuses	37(27.2%)	40 (28.2%)	50* (40.0%)	38(34.2%)
litters	12 (60%)	15 (75%)	17* (89.5%)	13(86.7%)
Thoracic vertebra centra (minimal anomalies)				
Fragmented Foetuses	1 (0.7%)	1 (0.7%)	0 (0%)	7* (6.35)
litters	1 (5%)	1 (5%)	0 (0%)	3 (20%)

* $p < 0.05$

Conclusions

The NOAEL for maternotoxicity in this study was 2 mg/kg/day, based on deaths, clinical signs (tonoclonic, convulsions and hypersalivation) and decreased bodyweight seen at 6 mg/kg/day.

The NOAEL 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 for malformations were observed in this study.

B.6.6.2.2 Rabbits

Mackenzie (1981). (AgrEvo: IIA, 5.6.2.2/1; AgrEvo: ANRA)

Dates of experimental work: this report was performed between February 8, 1981 and March 13, 1981.

Data of report: July 27, 1981.

No reference to a specific guideline

GLP: yes.

The study is acceptable

Material and Methods

The test compounds was FMC 5462 (97.3% purity). It was dissolved in corn oil (0.5 ml). FMC or vehicle was administered daily by oral gavage to mated New Zealand White rabbits at doses of 0, 0.3, 0.7 and 1.8 mg/kg/day on days 6-28 of gestation. Each dose level group consisted of 20 animals. 4/20 animals in the high dose group died during treatment, as consequence of these deaths a further 6 rabbits were treated with 1.8 mg/kg/day endosulfan. Day 6 was considered the first day of treatment and the time of sacrifice was day 29.

All animals were observed daily for signs of toxicity and all foetuses examined for external, skeletal and soft tissue anomalies and developmental variations.

Different statistical methods were carried out to evaluate dam body weight: Steel & Torrie (1960) and Hollander & Wolf (1973). Number of corpora lutea, number of implants, implantation efficiency, number and percent of live, resorbed, and dead foetuses, and sex ratio were analysed using the Krustal-Wallis test (Hollander & Wolf, 1973) and Dunn´s Test (Dunn, 1964). The number of litters with foetal gross, visceral, and skeletal abnormalities were analysed by the method discrete by Bishop et al., (1964). Percents of litters with foetal gross, visceral, and skeletal abnormalities were analysed using the Krustal-Wallis test.

Results

The survival of animals in this study is summarised in table B.6.6.2.2.-1 Four rabbits at the high dose tested died on gestation days 7, 10, 21 and 29 respectively. A probable cause of deaths was not established, but black, tar-like material was present in the intestines and the liver and kidneys were reported to have a pale appearance. Histopathological examination of these tissues revealed vacuolization of the hepatocytes. This findings was considered incidental and is associated with a variety of systemic disturbances. The colonic content of this animals suggested that this may have been caused by bleeding into the bowel, which also would explain the pale appearance of the liver and kidney.

B.6.6.2.2-1: Survival of mated rabbits treated with FMC 5462

FMC (mg/kg)	Nº rabbit treated	Rabbits dead on gestation days						Rabbits alive on day 29	
		6-9	10-13	14-17	18-21	22-25	26-28	number	Percent
0	20	0	0	0	0	0	0	20	100
0.3	20	0	0	0	0	0	0	20	100
0.7	20	0	0	0	0	0	0	20	100
1.8	26	1	1	0	1	0	1	22	84.6

The high dose was associate with signs of maternotoxicity including noisy and rapid breathing, hyperactivity and convulsions. and 3 of these animals died during treatment.

There were no statistically significant differences in mean body weight with endosulfan treatment.

Pregnancy maintenance, implantation, litter size, sex ratio, mean foetal weight and length, and the number and percent of live and resorbed foetuses were not significantly different from the control at any dose tested. There were no dead foetuses in any of the treatment groups or the control group.

No major gross, soft tissue or skeletal malformations occurred in any of the treatment groups. A single incidence of associated craniofacial malformation was reported in the control group. No gross external observations were reported in the high dose or low dose group. At he only observation in the mid dose group was a kindled tail which occurred in two foetuses in one litter.

One foetus in the control group and six foetuses from two litters in the high dose group had the he left carotid artery arising from the innominate. Common skeletal variations and minor anomalies were present in all treatment groups and in the control group in a nontreatment-pattern. control foetuses also showed this abnormality. Common skeletal variations and minor anomalies occurred with a similar incidence in control and treated foetuses.

Conclusions

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

Under the conditions of this study, endosulfan did not showed any embryotoxic or teratogenic effects , thus the NOAEL for developmental effects could be set up at dose level ≥1.8mg/kgbw/day

An additional review of this study by the U.S EPA concluded that replacement of animals during or after the study made it difficult to interpret the data and derive a NOAEL for this study. The U.S. EPA has recommended a repeat of this study.

B.6.7 Delayed neurotoxicity/Neurotoxicty (IIA, 5.7)

Summary

A summary of the only two acceptable neurotoxic studies are presented below.

In an acute delayed neurotoxicity study in hens, Endosulfan did not produced any clinical signs of neurotoxicity at the LD_{50} calculated (96 mg/kg)

On the other hand, a neurotoxicological screening was performed in Wistar rat. The NOAEL was established in 12.5-1.5 mg/kg/day in male and females respectively based on clinical signs as general discomfort, squatting posture and irregular respiration showed at 25 mg/kg bw in males and 3 mg/kg bw in females Deaths occurs at the highest dosage tested in male (6/10) and female (1/10) rats and non-compound related effects on motor activity were observed at non lethal doses.

Table B.6.7:.Summary of acceptable neurotoxicity studies with Endosulfan

Study	Dose levels	Comments	Reference and years
Acute Delayed Neurotoxicity in hens.	0,40,60,90,110, 135mg/kg	LD_{50} value of the 96 mg/Kg	Roberts & Phillipps (1983) AgrEvo: IIA, 5.7/1
Neurotoxicological screening in Wistar rat.	Males=0, 6.25, 12.5, 25, 50 and 100 mg/kg/day Females=0, 0.75, 1.5, 3, 6 and 12 mg/kg bw/day	NOAEL=12.5-1.5 mg/kg/day in male and females respectively	Bury, 1997 *Not taken account in the original monograph*

Evaluation of studies not included at the original monograph

Bury D.(1997)

Dates of experimental work. July 29, 1996- November 6, 1996. Date of report: July 7, 1997.

This study has been submitted by the main notifier on January 2001 in order to evaluate the neurotoxicological potential of endosulfan in rats after single oral exposure. The neurotoxicological screening consists of a comprehensive clinical and functional examination (Functional Observational Battery, FOB), an automated measurement of motor activity and neuropathology.

The present study was conducted in compliance with OECD Guideline for the Testing of Chemicals (Revised Proposal for a new Guideline).
GLP:Yes

The study is acceptable

Material & Methods
Groups of 10 male and 10 female Wistar rats received endosulfan (98.6 % purity) by oral gavage at single doses of 0, 0.75, 1.5, 3, 6 and 12 mg/kg bw/day and 0, 6.25, 12.5, 25, 50 and 100 mg/kg/day for females and males, respectively.

Behaviour, clinical signs and mortality of the animals were recorded twice every day (in the morning and in the afternoon), on weekends and public holidays only once. Animals found dead were necropsied as soon as possible and examined for macroscopically visible changes. Body weights and food consumption were recorded weekly.

The neurotoxicological screening, Functional Observational Battery (FOB), and automated measurement of motor activity, was carried out 7 days prior to treatment (measurement 1: study day -7), within 8 hours after administration (measurement 2: study day 1) as well as 7 days (measurement 3: study day 8) and 14 days (measurement 4: study day 15) after administration of the test substance.

Three weeks after administration of the test substance 10 male and 10 female controls as well as 5 male and 5 female animals (4 animals of the 100 mg/kg body weight group) of each group were killed by whole body perfusion and dissected for neuropathological examination. The remaining animals were killed by carbon dioxide asphyxiation, dissected and examined for macroscopically visible changes.

Results
Clinical signs and behaviour
The male animals of the 50 and 100 mg/kg body weight groups and the female animals of the 6 and 12 mg/kg body weight groups showed clinical symptoms such as decreased spontaneous activity, squatting posture, sunken flanks, bristled coat, stilted and uncoordinated gait, irregular respiration and panting, stupor, prone position, straddled hind limbs, increased fright reaction, narrowed palpebral fissures, coarse tremor, tonococlonic convulsions, and increased salivation.

Decreased spontaneous activity, squatting posture, sunken flanks, bristled coat, stilted gait, irregular respiration, panting, straddled hind limbs, and narrowed palpebral fissures were observed in females of the 3 mg/kg body weight group. Endosulfan-specific neurological signs, such as tremor and convulsions, were not observed at this dose level.
Male animals of the 25 mg/kg body weight group exhibited irregular respiration, decreased spontaneous activity, squatting posture and stilted gait. Endosulfan-specific neurological signs, such as tremor and convulsions, were not observed at this dose level.
The symptoms had reversed one day after administration of the test substance.

All animals of the control groups, females of the 0.75 and 1.5 mg/kg body weight groups as well as male animals of the 6.25 and 12.5 mg/kg body weight groups showed no clinical symptoms during the whole study.

Body weights were statistically significant increased in male animals of the 50 and 100 mg/kg body weight group at study days 1 and 8. Also females of the 6 and 12 mg/kg body weight groups showed increases in body weights at study day 1. Absolute and relative food consumption remained normal throughout the study.

Functional Observational battery (FOB)

Clinical signs were observed at measurement 2 (day 1: within 8 hours after administration). Male animals of the 50 and 100 mg/kg body weight group as well as female animals of the 6 and 12 mg/kg body weight group showed clinical symptoms such as stilted and uncoordinated gait, irregular respiration and panting, decreased spontaneous activity, squatting posture, sunken flanks, prone position, straddled hind limbs, stupor, bristled coat, narrowed palpebral fissures, coarse tremor, tonicoclonic convulsions, increased salivation and fright reaction.

Stilted gait, irregular respiration, decreased spontaneous activity, and squatting posture were observed in male animals of the 25 mg/kg body weight group.

Females of the 3 mg/kg body weight group exhibited stilted gait, irregular respiration, panting, decreased spontaneous activity, squatting posture, sunken flanks, straddled hind limbs, bristled coat, and narrowed palpebral fissure.

The symptoms had reversed in the surviving animals one day after administration of the test substance.

All animals of the control groups, females of the 0.75 and 1.5 mg/kg body weight groups as well as male animals of the 6.25 and 12.5 mg/kg body weight groups showed no clinical symptoms during the whole study.

Rearings

The number of rearings counted in a period of 2 minutes / animal showed no statistically significant differences between the control animals and the treated animals.

Forelimb and hindlimb grip strength

Absolute forelimb and hindlimb grip strength scores [g] remained normal in all groups throughout the study.

Landing foot-spread

Absolute landing foot-spread scores [mm] were comparable in all groups.

Motor activity

Motor activity was statistically significant increased in male animals of the 6.25 and 12.5 mg/kg body weight groups and decreased in female animals of the 1.5 mg/kg body weight group at measurement 2 (study day l). At measurement 3 (study day 8) motor activity values were increased in females of the 0.75 and 1.5 mg/kg body weight groups.

Male animals of the 12.5 mg/kg body weight group showed decreases in motor activity values at measurement 4 (study day 15). In all cases there were no dose dependency. Therefore, a compound-related effect is not evident.

Necropsy

Necropsy of the animal found dead revealed light discoloured liver, spleen and kidneys, lobular demarcation of the liver, diffuse reddened stomach and small intestine mucosa, a reddish-black mass or yellowish mucous in the small intestine and red or orange discoloured lungs. The animals killed at the end of the study by carbon dioxide asphyxiation showed no macroscopically visible changes,

Neuropathology

Histological examination was performed on 5 high dose males and 5 high dose females and controls.
Histologically, the central and peripheral nervous system including brain at 5 coronary levels, spinal cord at two levels, proximal and distal nerve segments, trigeminal and spinal root ganglia, optic nerve and retina, olfactory epithelium, pituitary gland, as well as two specimens of skeletal muscle did not exhibit any compound-induced changes.
Particularly in the hipocampus and cerebral cortex, no seizure-induced lesions were observed.
Very sporadically (1 control, 2 treated animals), a short segment of a single fiber in the tibial nerve longitudinal plastic section was found to be in decay, exhibiting several myelinic whorls and axonal globules. This lesion is regarded as a spontaneous background alteration. The teased fiber preparations (single and/or multiple isolated fibers) of tibial nerves did not indicate any damage.

To an individually different degree, some artificial (postvital) spreading and disintegration of myelin lamellae in longitudinal nerve sections was observed in several control and treated animals. This rather common artefact, which has to be differentiated from any intravital myelin change, results from inevitable delays in specimen isolation after the perfusion procedure and/or from the dehydration and embedding procedure.

Summarising, no neuropathological effects induced by the single dose treatment with the test-compound were observed after the 3 weeks observation period.

Conclusion

A single oral dose of 25, 50 or 100 mg/kg body weight of endosulfan was administered to male rats and 3, 6 or 12 mg/kg body weight to female rats produced mortality in the high doses of male and female animals and a dose-related increase in clinical signs. These clinical signs were reversible, visible only on the day of dosing, and supposed to be caused by the known affinity of endosulfan to brain-receptors of the neurotransmitter γ-amino-butyric acid (GABA). At 50 and 100 mg/kg body weight (males), and 6 and 12 mg/kg body weight (females) various serious neuropharmacological effects including coarse tremor and tonicoclonic convulsions occurred. At 25 mg/kg bw (males) and 3 mg/kg bw (females) the clinical effects were typical signs of general discomfort such as stilted gait, squatting posture, and irregular respiration,

No compound-related effects on motor activity were observed at any time-point at non-lethal doses. No effects were found on the rearing frequency, fore- or hindlimb grip strength or on landing foot-spread. No histopathological effects were found in any part of the central or peripheral nervous system.

A single dose of 12.5 mg/kg body weight for the male Wistar rat and 1.5 mg/kg body weight for the female Wistar rat of endosulfan did not cause any effects. Therefore the (NOAEL) was 12.5 mg/kg body weight for the male animals and 1.5 mg/kg for the female animals.

B.6.8 Further toxicological studies (IIA, 5.8)

B.6.8.1 Toxicity of endosulfan-metabolites

Endosulfan plant metabolites have been also identified in mammalian organisms. They were the sulphate, diol, lactone, hydroxyether and ether. Although they have been tested along with the active substance, AgrEvo has sponsored further toxicity studies with them.

Endosulfan-sulphate (Hoe 51327)

Endosulfan-sulphate has been tested for acute oral and dermal toxicity in rats as well as for subchronic toxicity in rats and dogs.

Results from endosulfan-sulphate toxicity studies are summarised in table B.6.8.1-1.

Rat acute toxicity studies were considered acceptable. These studies provided an oral LD_{50} of 25-50 mg/kg b.w. and a dermal LD_{50} of 280 mg/kg b.w, both values in female rats. According to Commission Directive 92/21/EEC, endosulfan-sulphate should be classified as T, R25, R24 “toxic if swallowed and in contact with skin ”.
Endosulfan was classified as T+, R28“ very toxic if swallowed” on the basis of an oral LD_{50} of 10 mg/kg b.w; and Xn, R21 “harmfull in contact with skin” on the basis of a dermal LD_{50} of 500 mg/kg b.w, both values in female rats.

Subchronic toxicity studies were not considered acceptable because there were some deficiencies in their performance and besides, the purity of the test substance was not reported in both of them. Nevertheless, results from these studies were taking into account as a useful information in order to get a fuller endosulfan-sulphate toxicological profile. Thus, the most relevant NOAEL was considered to be 0.75 mg/kg bw/day, based on results from the 90-day study in dogs. According to this value (NOAEL < 50 mg/kg bw/day) endosulfan-sulphate could be considered as a toxicologically significant metabolite.
For endosulfan, the most relevant subchronic NOAEL was considered to be 0.6 mg/kg bw/day, based on results from 1-year study in dogs.

In conclusion, from the available data it can be said that endosulfan-sulphate showed a toxicological profile very similar to that of endosulfan being considered a toxicologically significant metabolite.

Therefore, if this metabolite had been included in the residue definition, after ECCO 104, further toxicity studies are required.

Table B.6.8.1-1: Summary of endosulfan-sulphate toxicity studies

Study	Test system	Dosage	Results	Comments	Reference
Acute oral toxicity	Wistar rats (m/f)	25, 31.5, 50, 63, 100, 200, 400, 800 mg/kg bw.	LD_{50} = 568 mg/kg bw (m) LD_{50} = 25-50 mg/kg bw (f)		Ehling & Leist 1991b (IIA, 5.8.1.1/10)
Acute dermal toxicity	Wistar rats (m/f)	250, 315, 400, 1600, 2500, 4000 mg/kg bw.	LD_{50} = 2740 mg/kg bw (m) LD_{50} = 280 mg/kg bw (f)		Ehling & Leist 1991d (IIA, 5.8.1.1/11)
90-day, dietary toxicity	Sprague Dawley rats (m/f)	3, 10, 30, 50, 500 ppm 0.3, 1, 3, 5, 50 mg/kg bw/day	NOAEL: 10 ppm ≈ 1 mg/kg bw/day (f) 30 ppm ≈ 3 mg/kg bw/day (m) LOAEL: 30 ppm ≈ 3 mg/kg bw/day (f) 50 ppm ≈ 5 mg/kg bw/day (m)	Increased liver weight Purity not reported.	Wolf & Calandra 1965 (IIA, 5.8.1.5/2)
90-day, dietary toxicity	Beagle dogs (m/f)	0.075, 0.75, 2.5/1.5 mg/kg bw/day. 2.5 mg/kg bw/day (up to day 18) and 1.5 mg/kg bw/day from day 20)	NOAEL: 0.75 mg/kg bw/day (m/f) LOAEL: 2.5/1.5 mg/kg bw/day (m/f)	Salivation, muscular tremors and tonic-clonic convulsions Purity not reported.	Cervenka *et al* 1964 (IIA, 5.8.1.5/1)

Endosulfan-diol

Endosulfan-diol has been tested for acute oral and dermal toxicity in rats, ocular and dermal irritation in rabbits, skin sensitization in guinea pigs, subchronic toxicity in rats and dogs as well as *in vitro* and *in vivo* genotoxicity. All studies were considered acceptable.

Results from endosulfan-diol toxicity studies are summarised in table B.6.8.1-2.

The rat acute toxicity studies provided an oral LD_{50} >5000 mg/kg b.w. and a dermal LD_{50} >2000 mg/kg b.w. Based on these results, endosulfan-diol is considered "not classified", according to Commission Directive 92/21/EEC.

With respect to dermal and eye irritation, endosulfan-diol is considered as not irritant

Endosulfan-diol produced sensitisation in guinea pig by means the maximisation test but this effect was not observed when the Buehler test was applied. Therefore, according to Commission Directive 92/21/EEC, endosulfan-diol is classified as Xn, R43 "harmful, sensitising agent". However, it should be taken into account that endosulfan, the parent compound, was not a sensitising agent.

The most relevant subchronic NOAEL, for endosulfan-diol, was considered to be 8 mg/kg b.w./day, based on results from the 90-day study in rats. According to this value (NOAEL < 50 mg/kg bw/day) endosulfan-diol could be considered as a toxicologically significant metabolite. Nevertheless, this value

was higher than the most relevant subchronic NOAEL of the parent compound, 0.6 mg/kg bw/day, based on results from 1-year study in dogs.

The available information showed that endosulfan-diol could be considered as non-genotoxic *in vitro and in vivo* for somatic cells.

In conclusion, based on results from subchronic toxicity studies, endosulfan-diol should be considered a toxicologically significant metabolite. Nevertheless, results from the remaining studies together with the fact that its subchronic toxicity was lower than that of the parent compound, endosulfan, support its lack of toxicological significance. Therefore, although this metabolite has been included in the residue definition, after ECCO 104, no further toxicity studies are required.

Table B.6.8.1-2: Summary of endosulfan-diol toxicity studies

Study	Test system	Dosage	Results	Comments	Reference
Acute oral toxicity	Wistar rats (m/f)	5000 mg/kg bw.	LD_{50}>5000 mg/kg bw (m/f)		Ehling & Leist 1991 a (IIA, 5.8.1.2/1)
Acute dermal toxicity	Wistar rats (m/f)	2000 mg/kg bw.	LD_{50}>2000 mg/kg bw (m/f)		Ehling & Leist 1991c (IIA, 5.8.1.2/2)
Dermal irritation	New Zealand White rabbits (f)		Not irritant.		Hammerl 1996c (IIA, 5.8.1.4/3)
Ocular irritation	New Zealand White rabbits (f)		Not subjet to labelling requeriments.		Hammerl 1996c (IIA, 5.8.1.4/4)
Skin sensitization (maximisation test)	Pirbright-White guinea pig (f)		Irritant. Sensitisation by skin contact		Hammerl 1996 a (IIA, 5.8.1.4/1)
Skin sensitization (Buehler test)	Pirbright-White guinea pig (f)		No sensitising		Hammerl 1996b (IIA, 5.8.1.4/2)
90-day, dietary toxicity	Wistar rats (m/f)	100, 500, 1000, 10000 ppm	NOAEL: 100 ppm 7.8 mg/kg bw /day (m) 8.0 mg/kg bw /day (f) LOAEL: 500 ppm 40.2 mg/kg bw /day (m) 40.7 mg/kg bw /day (f)	Haematotoxicity and liver toxicity	Ebert and Hack, 1996 (IIA, 5.8.1.5/4).
90-day, dietary toxicity	Beagle dogs (m/f)	100, 1000, 10000 ppm 9.1, 89.4, 910.6 mg/kg bw /day (m) 8.4, 82.9, 870.9 mg/kg bw /day (f)	NOAEL: 100 ppm 9.1 mg/kg bw /day (m) 8.4 mg/kg bw /day (f) LOAEL:1000 ppm 89.4 mg/kg bw /day (m) 82.9 mg/kg bw /day (f)	Bile duct proliferated with fibrosis	Stammberger 1994 (IIA, 5.8.1.5/3).
Bacterial gene mutation (plate incorporation assay)	*Salmonella typhimurium* TA1535, TA100, TA1538, TA98, TA1537, *Escherichia coli* WP2 *uvrA*	1st experiment: 4, 20, 100, 500, 2500, 10000 µg/plate (±S9) 2nd experiment: 0.16, 0.8, 4, 20, 100, 500 µg/plate (±S9)	Negative	Precipitation and toxicity at 500 µg/plate (±S9).	Stammberger, 1992 (IIA, 5.8.1.3/1)

Study	Test system	Dosage	Results	Comments	Reference
In vitro UDS assay	Human cell line A 549	1st experiment: 0.3, 1, 3, 10, 30, 100, 300 µg/mL (±S9) 2nd experiment: 0.03, 0.1, 0.3, 1, 3, 10, 30µg/mL (±S9) The exposure time was 3 h.	Negative	An increasing cytotoxicity was observed from 100 µg/mL (-S9) and from 10 (+S9)	Stammberger, 1993 a (IIA, 5.8.1.3/2)
In vivo MN assay	Bone marrow cells from male and female NMRI mice strain NMRKf (SPF71).	A single oral gavage dose (500, 2500, 5000 mg/kg)	Negative	No toxicity in mice or bone marrow. The maximum tolerated dose considered was 5000 mg/kg	Stamberger, 1993b (IIA, 5.8.1.3/3)

Endosulfan-lactone

Endosulfan-lactone has been tested for acute oral toxicity in rats in three studies. These studies were not considered acceptable because there were some deficiencies in their performance and besides, the purity of the test substance was not reported in any of them. Nevertheless, results from these studies were taking into account as a useful information in order to get the endosulfan-lactone toxicological profile.

Results from endosulfan-lactone toxicity studies are summarised in table B.6.8.1-3.

The lower oral LD_{50} was considered to be 105 mg/kg bw in male rats. According to Commission Directive 92/21/EEC, endosulfan-lactone should be classified as T, R25 “toxic if swallowed”.
Endosulfan was classified as T+, R28“ very toxic if swallowed” on the basis of an oral LD_{50} of 10 mg/kg b.w.

In conclusion, endosulfan-lactone should be considered a toxicologically significant metabolite, based on results from acute toxicity studies, although its acute toxicity was lower than that of the parent compound, endosulfan. Therefore, if this metabolite had been included in the residue definition, after ECCO 104, further toxicity studies are required.

Table B.6.8.1-3: Summary of endosulfan-lactone toxicity studies

Study	Test system	Dosage	Results	Comments	Reference
Acute oral toxicity	Wistar rats (m)	80, 125, 200, 320, 500 mg/kg bw.	LD_{50} = 165 mg/kg bw (m)	Deficiencies were observed at performance of the study. Purity not reported	Hollander and Kramer 1975a (IIA, 5.8.1.1/2)
Acute oral toxicity	Wistar rats (f)	100, 160, 250, 400, 630mg/kg bw.	LD_{50} = 290 mg/kg bw (f)	Deficiencies were observed at performance of the study. Purity not reported	Hollander and Kramer 1975b (IIA, 5.8.1.1/7)

Study	Test system	Dosage	Results	Comments	Reference
Acute oral toxicity	Wistar rats (m/f)	80, 100, 125, 160 mg/kg bw.	LD_{50} = 105 mg/kg bw (m) 115 mg/kg bw (f)	Deficiencies were observed at performance of the study. Purity not reported	Kramer and Weigand 1971 (IIA, 5.8.1.1/1)

Endosulfan-hydroxyether

Endosulfan-hydroxyether has been tested for acute oral toxicity in rats. The study was not considered acceptable because there were some deficiencies in its performance and besides, the purity of the test substance was not reported. Nevertheless, results from this study were taking into account as a useful information in order to get the endosulfan-hydroxyether toxicological profile.

Results from endosulfan-hydroxyether toxicity studies are summarised in table B.6.8.1-4.

The oral LD_{50} was considered to be 1750 mg/kg bw in female rats. According to Commission Directive 92/21/EEC, endosulfan-hidroxyether should be classified as Xn, R22 “harmfull if swallowed”.
Endosulfan was classified as T+, R28“ very toxic if swallowed” on the basis of an oral LD_{50} of 10 mg/kg b.w.

In conclusion, endosulfan-hydroxyether should be considered as a no toxicologically significant metabolite, based on results from the acute toxicity study. Nevertheless, if this metabolite had been included in the residue definition, after ECCO 104, further toxicity studies might be required.

Table B.6.8.1-4: Summary of endosulfan-hydroxyether toxicity studies

Study	Test system	Dosage	Results	Comments	Reference
Acute oral toxicity	Wistar rats (f)	630, 1000, 1600, 2500, 4000 mg/kg bw.	LD_{50} = 1750 mg/kg bw (f)	Deficiencies were observed at performance of the study. Purity not reported	Hollander and Kramer 1975d (IIA, 5.8.1.1/5)

Endosulfan-ether

Endosulfan-ether has been tested for acute oral toxicity in rats. The study was not considered acceptable because there were some deficiencies in its performance and besides, the purity of the test substance was not reported. Nevertheless, results from this study were taking into account as a useful information in order to get the endosulfan-ether toxicological profile.

Results from endosulfan-ether toxicity studies are summarised in table B.6.8.1-5.

The oral LD_{50} was considered to be >15000 mg/kg bw in female rats. According to Commission Directive 92/21/EEC, endosulfan-ether should be no classified.

In conclusion, endosulfan-ether should be considered as a no toxicologically significant metabolite, based on results from the acute toxicity study. Therefore, if this metabolite had been included in the residue definition, after ECCO 104, further toxicity might be required.

Table B.6.8.1-5: Summary of endosulfan-ether toxicity studies

Study	Test system	Dosage	Results	Comments	Reference
Acute oral toxicity	Albino rats (f)	100, 320, 1000, 3200, 15000, 15000 mg/kg bw.	LD_{50} >15000 mg/kg bw (f)	Deficiencies were observed at performance of the study. Purity not reported	Hollander and Kramer 1975e (IIA, 5.8.1.1/6)

B. 6.10.- Summary of mammalian toxicology and proposed ADI, AOEL, ArfD and drinking water limit (Annex IIA 5.10)

B.6.10.1 Summary of Mammalian Toxicology

The pharmacokinetics and metabolism of endosulfan have been investigated in rats, mice, goats, sheep and cattle.

In rats, the intestinal absorption after a single oral dose of 2 mg/kg b.w. of endosulfan was estimated to be 60% for males and 70% for females. The maximum concentrations of endosulfan in blood were attained at 7 hours in male rats (0.25 µg/ml) and at 18 hr in female rats (0.2 µg/ml). Elimination from blood in male rats was biphasic with biological half lives of 8 h and, from the second day, 110 h and, monophasic in female rats with a biological half life of 75 h. After seven days endosulfan levels in blood had dropped down to 15% (males) and 23% (females) of the peak values.

After a single i.v. dose of 0.5 mg/kg b.w of endosulfan administered to the rat, the concentration in blood after 5 minutes was 0.18 mg/ml. Elimination of endosulfan and its metabolites was triphasic in male rats with biological half lives initially of 0.8 hours, then between 6 hours and 3 days post application of 12.5 hours and from day 3 onwards of 157 hours. In the female rats, elimination was biphasic with biological half lives of 1.2 and after 6 hours of 47 hours. After six days endosulfan levels in blood had dropped to the limit of detection of 0.014 µg/ml.

The tissue distribution and accumulation of endosulfan have been studied in different species. The liver and the kidney were the target organs in all species studied.

^{14}C-residues after a single oral dose of 2 mg/kg to the rat, seven days after application, accounted for 3.7% of the dose in the males and 4.7% in the females. Approximately half of this amount (1.5% of the dose) was found in kidneys (1.8 µg/g) and liver (0.2 µg/g in males and 0.5 µg/g in females). Beside, the levels of endosulfan found after 7 days of dosing were 0.05 µg/ml in blood and 0.16 µg/g in peritoneal fat. In all other organs the levels were below the detection limit of 0.1 µg/g.

In a oral multiple dose toxicokinetic study in rat at a dose level of 5 mg/kg/14 days, only 1.5% of the total residue was present in kidneys and liver after 14 days. The concentrations of endosulfan were 3 µg/g in kidneys, 1 µg/g in liver, 0.5 µg/g in visceral fat, 0.2 µg/g in subcutaneous fat, 0.05 µg/g in muscle and 0.07 µg/g in brain.

In mouse, a toxicokinetic study has been conducted at a single oral dose of 4 mg/kg b.w. and at a multiple oral dose of 2.4 mg/kg b.w. In both studies, a high accumulation has been observed 24 days after treatment in the liver, the fat and the spleen.

An oral multiple dose (1 mg/kg/day endosulfan for 28 days) study has been conducted in goat. One day after treatment, endosulfan was present in amounts of 0.29 mg/kg in kidneys, 0.20 mg/kg in gastrointestinal tract, 0.12 mg/kg in liver, 0.06 mg/kg in brain, 0.04 mg/kg in muscle and spleen, 0.01

mg/kg in lung and heart and 0.02 mg/kg in milk. Within 15 days, concentrations had dropped to below 0.01 mg/kg except in the kidneys with concentrations of 0.02 mg/kg. Twenty one days after dosing endosulfan could not be detected any more.

In cow, one study has been conducted at an oral multiple dose of 0.3, 3 or 30 ppm, during 14 days. The highest residues were measured in the liver. Analysis in blood showed a gradual rise reaching a plateau after 21 days. In the recovery period of 14 days the residue levels came down significantly, though in most cases not yet below detection limit.

The excretion of endosulfan in rats, following an oral single dose of 2 mg/kg b.w., was rapid. Approximately 80-90 % of the dose was eliminated at 24 h. The major portion was found in the faeces.

Administration of a single oral dose of the α- or β-isomer of ^{14}C-endosulfan to rats revealed some quantitative differences in their route of elimination. In this experiment female rats were gavaged with 2 mg/kg of the ^{14}C-labelled α- or β-isomer, while male rats were given a single oral dose of 1.2 mg/kg of the ^{14}C-labelled α- or β-isomer after a bile cannula was implanted. In females, faecal and urinary excretion of the first five days accounted for 88% of the dose. 120 hours after dosing, 75% of the α-isomer was excreted with faeces and 13% with urine, while 68% of the β-isomer was excreted in faeces and 19% in urine.

In the rat oral multiple dose toxicokinetic study at single dose level of 5 mg/kg b.w. during 14 days, no significant difference were observed in elimination between α- and β-endosulfan. In both cases total elimination via urine and faeces amounted to 65% 24h after dosing, while another 8% was eliminated in the next two weeks.

In mouse, after an oral single dose of 4 mg/kg b.w., 44% of the dose had been excreted in urine and faeces, 24 h after dosing. This amount had increased to 91% after 5 days and to 94% after 24 days.
In mouse oral multiple dose (2.4 mg/kg b.w./24 days), excretion occurred predominantly in the faeces.
Urinary excretion averaged only 10% of total excretion. This relative proportion increased slowly to a maximum of 25% at the end of treatment.

Endosulfan is converted in the animal organism to the following metabolites: endosulfan-sulphate, endosulfan-diol, endosulfan-ether, endosulfan-hydroxyether and endosulfan-lactone. A number of unidentified polar metabolites are probably the conjugates of the metabolites.

The cytochrome-P450 group of enzymes was not significantly activated by endosulfan. This was the outcome of an experiment where 5 mice were dosed with 5 mg/kg for three days and their livers examined on day 4. A similar outcome was observed in rats.

In a 30-day feeding study in rat at 360 and 720 mg/kg b.w., samples of liver, kidneys and blood of the rats were analysed for α-endosulfan, β-endosulfan, endosulfan-sulphate, endosulfan-hydroxyether, endosulfan-lactone and endosulfan-diol. α-endosulfan is stored only temporarily in the kidneys and β-endosulfan is hardly if at all stored (α/β in kidneys was 230/1, whereas α/β in administered test substance

was 2/1). Endosulfan-hydroxyether and endosulfan-diol were only present as traces or not at all. Presence of the metabolites endosulfan-sulphate and endosulfan-lactone in liver and kidneys indicates active metabolism. Storage in the kidneys proved to be temporary. Reversibility is also shown by levels found after 30-day recovery period. Only traces of α-endosulfan and two metabolites could still be found in the kidneys at that time point.

In a metabolism experiment with endosulfan and its metabolites applied by oral and intraperitoneal route to Sprague-Dawley rats, semi-quantitative metabolism and excretion in faeces, urine and bile was determined. The majority of orally applied endosulfan (α-E and β-E) was excreted unchanged with faeces in the first 48 hr. In addition, the lactone (EL), the hydroxy-ether (HE) and some sulphate (ES) were found. The ratio found was α-E or β-E/ES/HE/EL as 10/0.3/0.3/1.

The applied metabolites were excreted in faeces unchanged, as the lactone, as an unidentified metabolite (M_2) and as the hydroxyether. In urine less unchanged endosulfan isomers, but more lactone (EL), unidentified metabolite (M_1) as well as some sulphate (ES) were present.

The ratio α-E /ES/HE/EL found was 3/1/1/2, while the ratio β-E/ES/HE/EL was 2/1/6/20. On the third day only both endosulfan-isomers and the lactone were present in urine.

In bile only the lactone and the unidentified substance (M_1) could be found from α-E in a ratio of EL/ M_1 is 5/1 and from β-E in a ratio of EL/ M_1 is 1/30. Metabolism of β-endosulfan was different, as shown by analysis in urine and bile, and also faster than metabolism of α-endosulfan. In the first 24 hours the 1/1 ratio in a mixed sample of α/β-endosulfan did not change in faeces, whereas in urine this ratio had become 5:1. Therefore β-endosulfan is more quickly metabolised.

Endosulfan has been thoroughly tested for acute toxicity, primary irritation and sensitisation potential.
According to results:

- The endosulfan acute oral LD_{50} was 10-22.7 mg/kg bw in female rats.
- The endosulfan acute dermal LD_{50} was 500 mg/kg b.w. in female rats.
- The endosulfan acute inhalation LC_{50} was 0.0126 mg/l air for 4 hours in female rats.
- Endosulfan was designated as not irritating to skin. Not classified according to EU criteria.
- Endosulfan was designated as not irritating to eyes. Not classified according to EU criteria.
- Endosulfan was not considered a contact allergen. Not classified according to EU criteria.

Therefore, endosulfan should be considered as “very toxic if swallowed” (T+, R28), “harmful in contact with skin” (Xn, R21) and “very toxic by inhalation” (T+, R26).

Endosulfan has been thoroughly tested for short-term toxicity.
According to results:

- The oral NOAEL in rat was 3.85 mg/kg bw/day for males (90-day study).
- The oral NOAEL in mouse was 2.3 mg/kg bw/day (90-day study).

- The oral NOAEL in dog was 0.65mg/kg bw/day for males and 0.57 mg/kg bw/day for females (one-year study).
- The dermal NOAEL in rat was 3 mg/kg bw/day for males (28-day study).
- The inhalation NOEL in rat was greater than 0.002 mg/L/day (29-day study).

The lowest relevant oral NOAEL was 0.6 mg/kg bw/day based on violent muscular contractions of the abdominal muscles observed at 2.3 mg/kg bw/day.

The genotoxic potential of endosulfan has been investigated in numerous *in vitro* and *in vivo* tests. However, the genotoxicity evaluation was confined to studies carried out with technical endosulfan of stated purity.

The major features of endosulfan, based in data from these studies are the following:

7. Endosulfan does not induce gene mutation in bacterial or mammalian cells; and it appears to be non-mutagenic for yeast, however, results from the acceptable study cannot be considered conclusive because of its conduct.
8. Endosulfan was not clastogenic in cultured human lymphocytes following a short treatment but a continuous treatment without metabolic activation was not carried out.
9. Endosulfan did not induce DNA damage in bacteria (rec-assay) or in cultured mammalian cell (UDS); however, negative results from the acceptable yeast mitotic gene conversion assay cannot be considered conclusive because of its conduct.
10. Endosulfan is non-clastogenic in mammalian somatic cells *in vivo.*
11. It cannot be concluded that endosulfan is not mutagenic for germ cells.

    The treatment of male mice with technical endosulfan (purity 97.03%) at the highest dose of 16.6 mg/kg for 5 consecutive days induced a single isolated increase in dominant lethal mutations in females from one mating interval (36-42 days) post treatment (Pandey *et. al*, 1990). The mating interval (sixth week) indicates that damage that can result in dominant mutation is induced specifically in spermatogonia. The lack of detail in this published study could make the significance of the isolated finding questionable. However, data from other studies support that endosulfan target is spermatogonial cells. These data, from two published studies on chromosomal aberrations in germ cells, were not included in Table B.6.4-1 because the purity of the test substance was not stated. Negative in rats (Dikshith and Datta, 1978) and positive in mice (Rani and Reddy, 1986) results were reported. It should be taken account all rats dosed at 36.6 and 55 mg/kg died before 24 h and two rats dosed with 22 mg/kg died after 72 h, and no toxicity data were reported for mice treated at similar doses of 22, 32 and 42 mg/kg. Besides, although the treatment period was the same in both studies (5 consecutive days), rats were sacrificed immediately and mice 60 days after treatment, and mouse spermatocytes at meiotic metaphases were presumed to have been spermatogonia at the time of treatment.
12. Endosulfan induced sperm abnormalities in rodents. The postulated mechanism for sperm abnormalities is that endosulfan impairs testicular functions by altering the enzyme activities responsible for spermatogenesis, thereby influencing intratesticular spermatid count and causing low sperm production and sperm deformities, being the young growing animals more susceptible than adult animals to exposure.

In conclusion, it can be said that endosulfan is not mutagenic *in vitro* and *in vivo* for somatic cells. Nevertheless, some positive results obtained in studies *in vivo* with germ cells suggest that endosulfan induces mutations specifically in spermatogonia. Therefore, in order to confirm these results or support the lack of mutagenicity, further testing are required, i. e. a chromosomal aberration assay in rodent germ cells that ensures spermatogonial cells to be exposed to endosulfan.

The long-term toxicity and carcinogenicity of endosulfan have been studied in rats and mice.
In the rat combined chronic-carcinogenicity study, the NOAEL was established at 0.6 mg/kg bw/day (m) and 0.7 mg/kg bw/day (f) based on effects observed at 2.9 mg/kg bw/day (m) and 3.8 mg/kg bw/day (f). These effects were increased incidence of enlarged kidneys in females; blood vessel aneurysms mainly in males, and enlarged lumbar lymph nodes in males. Endosulfan did not show any carcinogenic potential.

In the mouse carcinogenicity study the NOAEL was established at 0.84 mg/kg bw/day (m) and 0.97 mg/kg bw/day (f) based on effects observed at 2.51 mg/kg bw/day (m) and 2.86 mg/kg bw/day (f). These effects were an increased mortality and significant decreases in the relative lung and ovary weights after 12 months of treatment, in females; and a decreased body weight over a period of 24 months in males.

Therefore, the lowest relevant chronic oral NOAEL was considered to be 0.6 mg/kg bw/day (104-week combined chronic-carcinogenicity study in rats). No evidence of carcinogenicity was seen in the rat or mouse. In addition,the ECCO Meeting 102 concluded that data on tumour-promoting potential were not of concern due to the lack of carcinogenicity seen in standard chronic studies

In the two generation oral study in rats, no effects on reproduction were observed. Therefore, the reproductive NOAEL was established at 75 ppm, 4.99 mg/kg bw/day (m) and 6.18 mg/kg bw/day (f), which was the highest dose tested and parentally toxic.

The lowest relevant developmental NOAEL was established at 2 mg/kg bw/day, based on skeletal variations or minimal anomalies observed in rats at 6 mg/kg bw/day, which was the highest dose tested associated with severe maternal toxicity. There was no evidence of teratogenicity.
In rabbits, developmental or teratogenic effects were not observed at 1.8 mg/kg bw/day, which was the highest dose tested associated with severe maternal toxicity.

The neurotoxicity NOAEL in rat was established at 12.5 mg/kg/day (m) and 1.5 mg/kg/day (f), based on clinical signs as general discomfort, squatting posture and irregular respiration, showed at 25 mg/kg bw (m) and 3 mg/kg bw (f).Endosulfan did not produced any clinical signs of delayed neurotoxicity in hens up to the calculated LD_{50} of 96 mg/kg bw.

Some toxicity studies were carried out with endosulfan plant metabolites, which have been also identified in mammalian organisms. They were the sulphate, diol, lactone, hydroxyether and ether.
According to results:

- Endosulfan-sulphate showed a toxicological profile very similar to that of endosulfan being considered a toxicologically significant metabolite. Therefore, if this metabolite had been included in the residue definition, after ECCO 104, further toxicity, mainly genotoxicity, studies are required.

- Endosulfan-diol should be considered a toxicologically significant metabolite based on results from subchronic toxicity studies. Nevertheless, results from the remaining studies together with the fact that its subchronic toxicity was lower than that of the parent compound, endosulfan, support its lack of toxicological significance. Therefore, although this metabolite has been included in the residue definition, after ECCO 104, no further toxicity studies are required.
- Endosulfan-lactone should be considered a toxicologically significant metabolite, based on results from acute toxicity studies, although its acute toxicity was lower than that of the parent compound, endosulfan. Therefore, if this metabolite had been included in the residue definition, after ECCO 104, further subcronic toxicity and genotoxicity studies are required.
- Endosulfan-hydroxyether should be considered as a no toxicologically significant metabolite, based on results from the acute toxicity study. Nevertheless, if this metabolite had been included in the residue definition, after ECCO 104, further subcronic toxicity and genotoxicity studies might be required.
- Endosulfan-ether should be considered as a no toxicologically significant metabolite, based on results from the acute toxicity study. Therefore, if this metabolite had been included in the residue definition, after ECCO 104, further subcronic toxicity and genotoxicity studies might be required.

The ECCO Meeting 102 concluded that endosulfan does not induce inmunotoxic effects. Positive results obtained in some studies were considered as derived from a general toxicity.

The ECCO Meeting 102 concluded that, on the weight of evidence, endosulfan is not an endocrine disruptor.

. B 6.10.1.2 Overall Evaluation of Mammalian Toxicology

B.6.10.2: Summary of mammalian toxicology studies with Endosulfan

Study	Dose levels	NOAEL		LOAEL		Target organs/main effects	Reference
		ppm	mg/kg/day	ppm	mg/kgw/day		
Subchronic studies							
90-day, diet, rat.	10, 30, 60 and 360 mg/kg feed (equal to 0.64, 1.9, 3.8 and 23 mg/kg/day for males and 0.75, 2.3, 4.6 and 27 mg/kg/day for females)		3.85 (m)		23.41 (m)	Haematological changes	Barnard *et al*., 1985** (AgrEvo IIA, 5.3.2.1/2)
90-day, diet, mouse CD-1	2, 6, 18, and 54 mg/kg feed (equal to 0.24, 0.74, 2.13 or 7.3 mg/kg/day for males and 0.27, 0.80, 2.39 or 7.5 mg/kg/day for females		2.3 (m/f)		7.4 (m/f)	Lethality and neurological signs	Barnard *et al*., 1984 (AgrEvo IIA, 5.3.2.4/1)
1-year, diet, Beagle dog	3, 10, 30 ppm.(equivalent to 0.23, 0.77 and 2.3 mg/kgbw/day)	10	0.65 m 0.57 f	30	2.3	LOAEL based on the clinical signs (violent muscular contractions of the abdominal muscles),and reductions in body weights.	Brunk (1989; 1990)* (AgrEvo: 5.3.2.3/3).
Long-term studies							
Combined chronic-carcinogenic study in Charles River rats Oral.104 weeks.	ppm: 0,3,7.5, 15 and 75 mg/kg/day 0, 0.1, 0.3, 0.6 and 2.9 for males and 0, 0.1, 0.4, 0.7 and 3.8 in females	Chronic NOAEL 15(m/f)	Chronic NOAEL 0.6 m 0.7f	Chronic LOAEL 75(m/f)	Chronic LOAEL 2.9m 3.8f	Chronic LOAEL , based on the low body weight gains in both sexes, increase in the incidence of enlarged kidneys in females; increase in the incidence of blood vessel aneurysms mainly in males and increased incidence of enlarged lumbar lymph nodes in males) at 75 ppm No carcinogenic potential	Ruckman SA et al., (1989) AgrEvo: IIA, 5.5.1/4)** (AgrEvo: ANRA) Hack et al., (1995) ** AgrEvo:IIA, 5.5.1/6) (Published)
Carcinogenicity study in NMRI mice. Oral, 24 months.	ppm: 0, 2, 6, 18 mg/kg/day 0.28, 0.84 and 2.51 for males and 0.32, 0.97 and .2.86 for females)	Chronic NOAEL 6	Chronic NOAEL 0.84 (m) 0.97 (f)	Chronic LOAEL 18	Chronic LOAEL 2.51 m 2.86 f	Chronic LOAEL based on an increase mortality in females decreased body weight in males over a period of 24 months ,and signficant decrease in the relative lung and ovary weights in female mice after 12 months of treatment No carcinogenic potential	Donaubauer, HH (1989a, 1989b, 1990) (AgrEvo: IIA, 5.5.2/1/2/3) (AgrEvo: ANRA)** Hack et al., (1995)** (Published) (AgrEvo: IIA, 5.5.1/6)
Reproduction studies							

B.6.10.2: Summary of mammalian toxicology studies with Endosulfan

Study	Dose levels	NOAEL		LOAEL		Target organs/main effects	Reference
		ppm	mg/kg/day	ppm	mg/kgw/day		
Two generation reproduction toxicity in rats.	ppm: 0, 3, 15, 75 mg/kgbw/dy 0.2,1, 4.99 for males and 0.24, 1.23, 6.18 for females	Parental =15 Reprod =75 Develp=15	Parental =1m and 1.23f Reprod= 4.99 m and 6.18f Develp=1 m and 1.23f	Parental = 75 Reproduction≥75 Develp=75	Parental =4.99m and 6.18f Reprod. ≥4.99 m and 6.18f Develp= 4.99m and 6.18f	Parental LOAEL: based histopathologic and organ weights changes showed in livers and kidney from F0 and F1b generation Reproduction toxicity not observed Developmental toxicity :based on decrease in litter weight	Edwards et al., (1984) AgrEvo:IIA, 5.6.1/3** ---------- Offer., (1985) AgrEvo, IIA: 5.6.1/4** (histopathological review of the kidney in adults rats of the F1b generation and in weanling rat of the F2b generation.
Teratology study with FMC 5462 rats	0. 0.66, 2 and 6 mg/kg bw/day		Maternal =0.66 Develop =2		Maternal: =2 Develop =6	Maternal:toxicity based on clinical signs (face-rubbing and alopecia) and reduced in body weigh gain. Develop toxicity: based on reduce mean fetal weights and lenghts and significant skeletal variations. No teratogenic effects.	McKenzie (1980) AgrEvo: IIA, 5.6.2.1/3)**
Embryotoxicity in the Wistar rats	0. 0.66, 2 and 6 mg/kg bw/day		Maternal: = 2 Develop: = 2		Maternal =6 Develop: =6	Maternal toxicity;. based on deaths (4 dams), clinical signs (tonoclonic convulsions , increase salivation, blood-crusted nose) and decreased body weight. Develop: based on minor anomalies as fragmentation of thoracic vertebral centra. No teratogenic effects.	Albrech & Baeder, 1993 AgrEvo: IIA, 5.6.2.1/4**
Teratology study with FMC 5462 rabbits	0, 0.3, 0.7, 1.8 mg/kgbw/day		Maternal: =0.7 Develop: =1.8		Maternal: =1.8 Develop: ≥1.8	Maternal: based on deaths (4 animals) and clinical signs (noisy, rapid breathing, hyperactivity and convulsions) Developmental toxicity: no effects	McKenzie et al., 1981 AgrEvo: IIA, 5.6.2.2/1**
Neurotoxicity study							

B.6.10.2: Summary of mammalian toxicology studies with Endosulfan

Study	Dose levels	NOAEL		LOAEL		Target organs/main effects	Reference
		ppm	mg/kg/day	ppm	mg/kgw/day		
Neurotoxicological screening in Wistar rat.	Males=0, 6.25, 12.5, 25, 50 and 100 mg/kg/day Females=0, 0.75, 1.5, 3, 6 and 12 mg/kg bw/day		NOAEL =12.5m 1.5f		LOAEL = 25m and 3f	LOAEL based on clinical signs as general discomfort, squatting posture and irregular respiration.	Bury, 1997*

*Studies not included at the original monograph.
**Re-evaluated studies.

B.6.10.3 Acceptable Daily Intake (ADI)

An ADI of 0.006 mg/kg bw/day was derived using the NOAEL of 0.6 mg/kg bw/day from the 104-wee koral rat study and the assessment factor of 100.

B.6.10.4 Acceptable Operator Exposure Level (AOEL)

An AOEL of 0.004 mg/kg bw/day was derived using the NOAEL of 0.6 mg/kg bw/day from the 1-year oral dog study, applying the correction factor for oral absorption of 60% and the assessment factor of 100.

B.6.10.5 Acute Reference Dose (ArfD)

An ARfD of 0.015 mg/kg bw/day was derived using the NOAEL of 1.5 mg/kg bw/day from the rat neurotoxicity study and the assessment factor of 100.

B.6.11.1. Other toxicity studies of preparations

Summary

Two new studies of repeated dose dermal 21-28 day toxicity study in rats have been submitted by Hoechst Aktiengesellschaft with two different preparations: Endosulfan water dispersible powder and Endosulfan emulsifiable concentrate (33.3%).

The NOAEL established for Endosulfan water dispersible powder (50%) in female Wistar rats was 40 mg/kg b.w. based on the mortality observed at 80 mg/kg as well as on the clinical signs of intoxication (dacryohaemorrhoea and blood-crusted snout) and decrease in the activity of serum cholinesterase observed at this dose level. **A NOAEL could not be established for endosulfan water dispersible powder (50%) in male Wistar rats.**

The NOAEL established for Endosulfan emulsifiable concentrate (33.3%) in female rats was 9 mg/kg b.w based on one dead observed at 12 mg/kg b.w. At this dose level some females showed transient toxic signs of the nervous system and slightly decrease of plasma cholinesterase activity. **The NOAEL established for Endosulfan emulsifiable concentrate (33.3%) in male rats was 54 mg/kg**

b.w based on the toxic signs observed at 81 mg/kg b.w. (nervous toxic signs and increase in the aspartate aminotransferase activity)

B.6.11.1a.1 Repeated dose dermal toxicity : 21/28-day study in rat with ENDOSULFAN WATER DISPERSIBLE POWDER (50%) (Hoe 002671 OI WP50 A501) (study not evaluated at the original monograph)

Ebert, E. ,1988 (Hoechst Aktiengesellschaft; Report No. 88.1209)

Dates of experimental work: males: 27th October 1987-17th December 1987; females: 28th October 1987-18th December 1987.

Date of report: 3th October 1988

Objectives: The present dermal toxicity study in rats was conducted in order to characterise the toxicological profile of endosulfan water dispersible powder (50%) (Hoe 002671 OI WP50 A501) after repeated dermal exposure.

Guidelines: OECD guidelines No. 410 (1981) and EPA pesticide assessment guidelines series 82-2 (1984). There were no deviations from the protocol.

GLP: Yes

The study is acceptable.

Materials and Methods

Endosulfan water dispersible powder (50%) was applied 21 times over a test period of 30 days (on weekdays from Mondays to Fridays) in form of 2-32% aqueous dispersions at a constant application volume of 2 ml/kg b.w. to the shaved, intact dorsal skin of Wistar rats.

The daily exposure period was 6 hours under an occlusive bandage. After removal of the bandage the treated skin areas were washed with warm water. The controls were treated analogously with the vehicle (deionised water).

The following test groups were used during this study:

Table B.6.11.1a.1-1.- Dose levels of physiological saline (control group) and endosulfan water dispersible powder (50%) (treated groups) and animal numbers in each group

Group	Dose		Number of animals			
			Main group		Satellite group	
	Males	Females	Males	Females	Males	Females
Group 1: Control group	2 ml/kg b.w.	2 ml/kg b.w.	6	6	5	5
Group 2: Treated group	40 mg/kg b.w.	40 mg/kg b.w.	6	6	-	-
Group 3: Treated group	160 mg/kg b.w	80 mg/kg b.w.	6	6	5	5
Group 4: Treated group	640 mg/kg b.w	160 mg/kg b.w	6	6	5	5

The main group animals were killed one day after the last dermal treatment, the satellite group animals were killed 25 days (males) or 24 days (females) after the last treatment with the substance. The satellite group is observed for reversibility, persistence, or delayed occurrence of toxic effects post-treatment.

Behaviour and general health condition were observed daily. The animals were examined weekly for neurological disturbances, opacity of the refracting media of the eyes, damage to the oral mucosa and impairment of dental growth. At the same time, the dermal and eye irritancy of the test substance was assessed according to the scores of OECD guidelines No. 404 and No. 405, respectively. Body weights and food consumption were recorded twice weekly and water consumption once weekly.

Haematological examinations, clinical chemistry and urinalysis were carried out at the termination of the treatment in main group animals and at the end of the recovery period in satellite group animals.

At autopsy, the animals were examined macroscopically for changes in skin, orifices, eyes, teeth, oral mucosa and internal organs, the major organs were weighed and the relative organ weights calculated. A wide selection of tissues, and also the treated skin of each animal, were subjected to a histological examination.

Data were evaluated statistically at the level of significance $p<0.05$ with the aid of a program package for evaluating toxicological studies. The statistical methods used in each case are given on the computer printouts (parametric method of Sidak, ditributed-free method by Nemenyi/Sidak, ditributed-free method by Nemenyi/Dunnett, parametric method by Dunnett, two-sided T-test, two-sided Wilcoxon-test).

Findings

Behaviour, general health condition, food and water consumption of all surviving animals showed no substance-related impairment. Neurological disturbances, changes in the eyes or oral mucosa, and disturbances of dental growth could not be observed.

There were no clinical signs of intoxication or mortality among the males in any dose group.

In the females, only the 40 mg/kg b.w. group was free of mortality or visible clinical signs of intoxication. On day 22, one animal in the 80 mg/kg b.w. group showed dacryo-haemorrhoea and blood-crusted snout; on day 21 of the study, one animal in this dose-group died. Between days 3 and 24 of the study, 3 animals died in the highest female dose group (160 mg/kg b.w) without showing previous clinical signs of intoxication.

Special behavioural, clinical signs and ocular examinations gave no indications of substance-related changes in the surviving animals from all treatment groups.

During the first two weeks of treatment, testing for primary dermal irritation yielded very slight signs of irritation (very slight erythema) in individual animals from the 80 mg/kg b.w. group and in many of the animals from the 160/640 mg/kg b.w. groups. These signs of irritation had already receded by the end of the second week of the study and were only present in some individual cases during the third week of the study (Table B.6.11.1a.1-2).

Table B.6.11.1a.1-2.- Dermal reactions observed in male and female rats after treatment with endosulfan water dispersible powder (50%)

Day	Dermal irritation score (No. of animals with dermal reactions/Total no. of animals)							
	Males				Females			
	Control	40 mg/kg	160 mg/kg	640 mg/kg	Control	40 mg/kg	80 mg/kg	160 mg/kg
2-3	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)
4	0 (0/11)	0 (0/6)	0 (0/11)	1 (3/11)	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)
5	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)
6	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)	0 (0/11)	0 (0/6)	1 (1/11) 2 (1/11)	1 (4/10) 2 (1/10)
7	0 (0/11)	0 (0/6)	1 (1/11)	1 (8/11)	0 (0/11)	0 (0/6)	0 (0/11)	1 (4/10)

8	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)	0 (0/11)	0 (0/6)	1 (1/11)	1 (4/10)
9	0 (0/11)	0 (0/6)	1 (1/11)	1 (5/11)	0 (0/11)	0 (0/6)	1 (2/11)	1 (5/10)
10	0 (0/11)	0 (0/6)	1 (3/11)	1 (8/11)	0 (0/11)	0 (0/6)	1 (4/11)	1 (5/10)
11	0 (0/11)	0 (0/6)	1 (3/11)	1 (7/11)	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/10)
13	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)	0 (0/11)	0 (0/6)	1 (5/11)	1 (3/9)
14	0 (0/11)	0 (0/6)	0 (0/11)	1 (1/11)	0 (0/11)	0 (0/6)	0 (0/11)	1 (3/9)
15	0 (0/11)	0 (0/6)	0 (0/11)	1 (1/11)	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/9)
16	0 (0/11)	0 (0/6)	1 (1/11)	0 (0/11)	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/9)
17	0 (0/11)	0 (0/6)	0 (0/11)	1 (2/11)	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/9)
18	0 (0/11)	0 (0/6)	1 (1/11)	0 (0/11)	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/9)
19-52	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)	0 (0/11)	0 (0/6)	0 (0/11)	0 (0/11)

0: No erythema, no oedema
1: Very slight erythema
2: Well-defined erythema

From the 2nd week of treatment until the end of the study, body weight gains were slightly retarded in the males from the highest dose group (640 mg/kg b.w). Body weight gains remained unaffected by the test substance in all other treatment groups (Table B.6.11.1a.1-3 and Table B.6.11.1a.1-4)

Table B.6.11.1a.1-3.- Mean body weights of male rats treated with three dose level of endosulfan water dispersible powder (50%)

Day	**Males**			
	Control (n=11)	**40 mg/kg b.w. (n=6)**	**160 mg/kg b.w. (n=11)**	**640 mg/kg b.w. (n=11)**
-5	248 ± 10	251 ± 8	248 ± 8	249 ± 7
1	286 ± 11	288 ± 12	282 ± 12	281 ± 11
3	285 ± 14	287 ± 12	279 ± 12	273 ± 14
7	301 ± 13	302 ± 12	295 ± 14	288 ± 13
10	306 ± 15	307 ± 13	296 ± 17	287 ± 13* (1)
15	319 ± 18	318 ± 14	309 ± 17	301 ± 11* (1)
18	322 ± 21	320 ± 14	311 ± 19	301 ± 11* (1)
22	333 ± 20	334 ± 14	321 ± 18	309 ± 11* (1)
25	324 ± 23	315 ± 9	311 ± 18	298 ± 11* (1)
30	345 ± 23	338 ± 10	328 ± 19	317 ± 12* (1)
32	345 ± 18		326 ± 13	313 ± 7* (2)
37	357 ± 21		336 ± 13	323 ± 9* (2)
39	367 ± 22		345 ± 14	333 ± 10* (2)
43	381 ± 22		359 ± 16	344 ± 11* (2)
46	370 ± 19		350 ± 14	335 ± 12* (2)
51	395 ± 20		373 ± 16	358 ± 12* (2)
53	404 ± 17		377 ± 16 * (2)	365 ± 17* (2)

*Statistically significant at $p<0.05$ with respect to control animals.
(1) parametric method by Sidak
(2) parametric method by Dunnett

Table B.6.11.1a.1-4.- Mean body weights of female rats treated with three dose level of endosulfan water dispersible powder (50%)

Day	**Females**			
	Control (n=11)	**40 mg/kg b.w. (n=6)**	**80 mg/kg b.w. (n=11)**	**160 mg/kg b.w. (n=11)**
-6	227 ± 9	226 ± 5	227 ± 12	228 ± 4
1	237 ± 10	232 ± 12	239 ± 12	236 ± 8
3	234 ± 8	226 ± 9	226 ± 8	216 ± 7* (1)
8	237 ± 11	235 ± 8	231 ± 14	231 ± 10
10	235 ± 11	232 ± 8	230 ± 15	231 ± 9
14	237 ± 12	236 ± 13	238 ± 16	231 ± 10
17	238 ± 14	242 ± 8	240 ± 18	240 ± 7

Day	Females			
	Control (n=11)	40 mg/kg b.w. (n=6)	80 mg/kg b.w. (n=11)	160 mg/kg b.w. (n=11)
22	239 ± 13	246 ± 12	243 ± 13	242 ± 7
24	219 ± 12	222 ± 11	220 ± 14	216 ± 9
30	241 ± 13	250 ± 11	247 ± 16	246 ± 15
31	243 ± 17		255 ± 12	259 ± 18 (a)
36	244 ± 16		262 ± 17	255 ± 14 (a)
38	250 ± 16		265 ± 16	260 ± 20 (a)
42	251 ± 17		267 ± 19	260 ± 20 (a)
45	245 ± 18		256 ± 15	252 ± 16 (a)
50	255 ± 17		272 ± 16	264 ± 24 (a)
52	261 ± 20		277 ± 14	264 ± 19 (a)

*Statistically significant at $p<0.05$ with respect to control animals.
(1) parametric method by Sidak
(a) no statistic because number of animals <4

In both sexes, from all treatment groups, food and water consumption was not affected by the test substance.

Haematology and urinalysis showed no substance-related changes.

Statistical evaluation of clinical chemistry at the end of the treatment and recovery periods revealed a series of significant changes as shown in Table B.6.11.1a.1-5 and Table B. 6.11.1b.1-6. At the end of the treatment and at the end of the recovery period, the activity of serum cholinesterase (CHE) showed a dose-related decrease in the females from the 80 and 160 mg/kg b.w. groups. This effect was possibly caused by reduced biosynthesis in the liver parenchyma and could thus be interpreted as an impairment of hepatic function. There were no indications of cholinesterase inhibition, since a comparable effect of the substance on erythrocytes and brain cholinesterase was not observed. The chlolesterol and total lipid levels of the females in the 160 mg/kg b.w. group were slightly increased at the end of the treatment. All other statistically significant changes in clinical chemistry parameters were only faintly marked and without any dose relationship, and are thus to be evaluated as non-substance-related and of no toxicological significance.

Table B.6.11.1a.1-5.- Clinical chemistry evaluation in male rats at the end of the treatment and recovery period

	Males			
Parameter	**Control**	**40 mg/kg**	**160 mg/kg**	**640 mg/kg**
End of treatment				
Urea (mmol/L)	9.2±0.6	7.8±0.6* (1)	9.1±0.8	9.4±0.9
Creatinine (µmol/L)	56±6	46±3* (2)	51±8	50±6
Protein (g/L)	53±2	53±2	52±3	51±1* (2)
Serum CHE (U/L)	467±39	407±31* (1)	426±45	405±30* (1)
Brain CHE (U/kg)	4267±147	4538±455	4579±264	4891±263* (1)
α_1-globulin	0.168±0.014	0.157±0.025	0.131±0.023* (1)	0.143±0.015
α_2-globulin	0.063±0.011	0.058±0.005	0.060±0.012	0.045±0.012* (1)
End of recovery				
Potassium	6.1±0.5		6.0±0.8	5.3±0.3* (2)

*Statistically significant at $p<0.05$ with respect to control animals.
(1) parametric method by Dunnett
(2) distributed-free method by Nemenyi/Dunnett

Table B.6.11.1a.1-6.- Clinical chemistry evaluation in female rats

at the end of the treatment and recovery period

Parameter	Females			
	Control	**40 mg/kg**	**80 mg/kg**	**160 mg/kg**
End of treatment				
Cholesterol (mmol/L)	0.82±0.11	0.85±0.11	0.90±0.20	1.25±0.24* (1)
Total lipids (g/L)	3.02±0.42	3.20±0.25	3.20±0.61	4.12±0.49* (1)
ASAT (GOT) (U/L)	111±24	86±8	92±15	84±15* (1)
Serum CHE (U/L)	1318±246	1005±248	952±121	709±79* (1)
End of recovery				
Sodium	147±2		150±2* (3)	149±2 (a)
Calcium	2.57±0.04		2.49±0.06* (2)	2.55±0.07 (a)
Chloride	104±1		107±1* (3)	106±1 (a)
Serum glucose	12.9±0.5		11.4±1.1* (2)	11.9±1.4 (a)
Serum CHE	1031±125		782±124* (2)	798±175 (a)
Albumin	0.669±0.021		0.624±0.031* (2)	0.651±0.029 (a)
α_2-globulin	0.048±0.003		0.059±0.007* (3)	0.043±0.004 (a)
Albumine./globuline	2.025±0.193		1.679±0.224* (2)	1.868±0.227 (a)

*Statistically significant at $p<0.05$ with respect to control animals.
parametric method by Sidak
two-sided T-test (independent sample)
(3) two-sided Wilcoxon-test (independent sample)
(a) no statistic because number of animals <4

The absolute and relative organ weights showed no substance-related changes. Based on macroscopic and microscopic examinations, no substance-related pathomorphological organ changes could be observed. Histological examination revealed no substance-related pathomorphological changes on the treated skin areas.

Apart from signs of cardiovascular insufficiency, the females found dead in the 80 and 160 mg/kg b.w. groups showed no pathomorphological abnormalities which might have accounted for the cause of death.

Conclusions

Based on the results of this subchronic dermal toxicity study of endosulfan water dispersible powder (50%) in the Wistar rat, the no observed adverse effect level (NOAEL) has been established in 40 mg/kg b.w. for the females. This value is based in the following results:

- Repeated dermal treatment with 80 and 160 mg/kg b.w. caused mortality among the females. In addition, one female in the 80 mg/kg b.w. group showed clinical signs of intoxication such as dacryohaemorrhoea and blood-crusted snout during the treatment phase. The activity of serum cholinesterase showed a dose-related decrease in the females from the 80 and 160 mg/kg b.w.

A NOAEL could not be established for male rats as the only effect observed at the highest dose (640 mg/kg b.w.) was a reduction of body weight gains from the 2nd week of treatment.

B.6.11.1b.1 Repeated dose dermal toxicity: 21/28-day study in rat with ENDOSULFAN EMULSIFIABLE CONCENTRATE (33,3%) (Hoe 002671 OI EC34 A101) (Study not evaluated at the original monograph)

Thevenaz, Ph., Luetkemeir, H. and Chevalier, H.J., 1988 (Hoechst Aktiengesellschaft, Repot No. 88.1735)

Dates of experimental work: 7th September 1987 / 4th November 1987

Date of report: 4th October 1988

Objectives:The purpose of this subchronic 4-week repeated dose dermal toxicity study was to assess the toxicological profile of endosulfan emulsifiable concentrate when applied to rats dermally for 6 hours/day, 5 days/week for a total of 21-22 applications.

Guidelines: EPA pesticide assessment guidelines series 82-2 (1984), OECD guidelines No. 410 (1981) and EEC Directive 84/449 Part B.9. There were no deviations from the protocol.

GLP: Yes

The study is acceptable.

Materials and Methods

Endosulfan emulsifiable concentrate (Endosulfan EC) was applied to albino wistar male and female rats for 6 hours/day (5 days per week) for a total of 21 or 22 applications, respectively, over a 29-day or 30-day period, respectively. The study design included a main group and a satellite (recovery) group, for a total of 15 rats at each dose level. From the 15 rats per sex for each dosage group, 10 were assigned to terminal sacrifice, and 5 to sacrifice after a 4-week recovery period. The doses applied are reported in Table B.6.11.1b.1-1.

Table B.6.11.1b.1-1 Protocol of dosage for the subchronic dermal toxicity study with Endosulfan EC to the rat.

Group	Substance	Males	Females
Group 1: Control	Vehicle (aqueous 4% carboxymethylcellulose solut.)	2 ml/kg b.w.	2 ml/kg b.w.
Group 2:	Formulation base (HOE 002671 OI ECOO A302, administered in the vehicle)	81 mg/kg b.w.	36 mg/kg b.w.
Group 3: Treated	Endosulfan EC	27 mg/kg b.w.	9 mg/kg b.w.
Group 4: Treated	Endosulfan EC	54 mg/kg b.w.	12 mg/kg b.w.
Group 5: Treated	Endosulfan EC	81 mg/kg b.w.	18 mg/kg b.w.
Group 6: Treated	Endosulfan EC	-	36 mg/kg b.w.

Endosulfan EC or the formulation base was applied at 2ml/kg b.w. on the shaved skin and covered with a semi-occlusive dressing and left for 6 hours. After removal of the bandage, excess test article was removed carefully with lukewarm water and dried with a disposable paper towel. The animals of the control group were treated with the vehicle alone under the same conditions.

Mortality and clinical systemic signs were observed at least once daily. Local clinical signs were observed daily prior to the following application according to guidelines scores. Food consumption and body weights were observed weekly. Haemathological examinations, clinical chemistry and urinalysis were carried out in all animals at the end of treatment or recovery period. All animals were necropsied and descriptions of all macroscopic abnormalities were recorded. The major organs were weight at necropsy. A wide selection of tissues, and also the normal and treated skin of each animal, were subjected to a histological examination.

The following statistical methods were used to analyze the body weights, food consumption, organ weights and clinical laboratory data:

Univariate one-way analysis of variance was used to assess the significance of intergroup differences.

If the variables could be assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on pooled variance estimate was applied for the comparison between the treated groups and the control groups.

The Steel-test (many-one rank test) was applied when the data could not be assumed to follow a normal distribution.

For the overall spontaneous mortality data, the Fisher's exact test for 2x2 tables was applied.

Findings

One female from group 4 (12 mg/kg b.w.) and one female from group 5 (18 mg/kg) were found dead during treatment week 4. Four females from group 6 (36 mg/kg) were found dead during treatment weeks 1, 2 and 4. This incidence of mortality in the high female dose groups and some clinical observations clearly suggest treatment-related deaths, though no pathomorphologic lesions in these animals could be distinctly attributed to the administration of the test article.

Among the males no treatment-related mortality occurred in any dose group.

No systemic signs were detected during the daily observation session preceeding the application of the test article.

The following essential transient signs, slight to severe in intensity, were observed after the end of the application period: tremor, straub-tail, trismus, saltatory spasms, extension spasms, tetanoid spasms. Onset occurred within one hour after daily application, and duration did not exceed approximately 30 min. Occurrence was limited to male group 5 and female groups 5 and 6, with isolated cases recorded in female group 4. One female from group 6 died following one spasm attack. No comparable signs were observed during the recovery period.

Effects on skin are summarised in Table B.6.11.1b.1-2 and Table 6.11.1b.1-3. In dosage group 2 (treated with formulation base), erythema of minimal to moderate incidence and/or intensity was observed during both treatment and recovery periods in male and female rats. Mean erythema values increased towards the end of the treatment period in males and females, with concomitant slight oedema from week 3 of experiment in male rats. Oedema was no longer detectable following recovery week 1, and erythema decreased from moderate to marginal mean values over the duration of the treatment-free (recovery) period.

During the second half of the treatment period to male and female rats, marginal to slight erythema was observed in dosage groups 3 to 6, with concomitant marginal oedema occurring in groups 4 and 5. During the recovery period, marginal erythema persisted during week 1, whereas oedema was non longer detectable.

Histological examination revealed minimal to moderate hyperkeratosis and inflammation of the treated skin in rats of all groups. The severity and incidence of these findings was more prominent in rats of group 2 treated with formulation base.

No changes were seen in the control group at the application site during the whole experiment.

Table B.6.11.1b.1-2.- Mean values for dermal reactions observed in male animals

Group	E=erythema O=oedema	Treatment period (day)				Recovery period (day)			
		1-7	8-14	15-21	22-30	31-37	38-44	45-51	52-58
1 vehicle (control)	E	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0

2 81 mg/kg formulation base	E	0.2	1.1	1.3	1.5	0.7	1.4	0	0
	O	0	0	0.3	0.8	0.2	0	0	0
3 27 mg/kg Endosulfan EC	E	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0
4 54 mg/kg Endosulfan EC	E	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0
5 81 mg/kg Endosulfan EC	E	0	0	0.4	0.2	0	0	0	0
	O	0	0	0	0	0	0	0	0

0: No erythema, no oedema
1: Very slight erythema

Table B.6.11.1b.1-3.- Mean values for dermal reactions observed in female animals

Group	E=erythema O=oedema	Treatment period (day)				Recovery period(day)			
		1-7	8-14	15-21	22-31	32-38	39-45	46-52	53-59
1 vehicle (control)	E	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0
2 36 mg/kg formulation base	E	0	0.5	0.4	0.8	0.8	0.5	0.4	0.1
	O	0	0	0	0	0	0	0	0
3 9 mg/kg Endosulfan EC	E	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0
4 12 mg/kg Endosulfan EC	E	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0
5 18 mg/kg Endosulfan EC	E	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0
6 36 mg/kg Endosulfan EC	E	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0

0: No erythema, no oedema 1: Very slight erythema

In males and females, mean food consumption values were comparable in control and all treated groups during both treatment and recovery periods.

In male and females, mean body weight values were similar in control and all treated groups during both treatment and recovery periods.

The assessment of hematological data indicated no changes of toxicological significance at the end of the treatment, nor at the end of the treatment-free (recovery) period.

For biochemical data the following effects were noted at the end of the treatment:

- slightly increased aspartate aminotransferase activity (by 18%) for males of group 5 (81 mg/kg b.w.)
- slightly increased alkaline phosphatase activity (by 46%) for females of group 6 (36 mg/kg b.w.)
- slightly decreased albumin level (by 6to 8%) for females of groups 5 and 6 (18 and 36 mg/kg b.w., respectively)
- slightly decreased albumin to globulin ratio (by 11%) for females of group 6 (36 mg/kg b.w.)
- slightly decreased plasma chlolinesterase activity (by 22 to 32%) for females of groups 4, 5 and 6 (12, 18 and 36 mg/kg b.w., respectively).

These findings were considered to be treatment related and probably indicative of faint impairment of hepatic function.

At the treatment-free (recovery) period the findings were unremarkable with the exception of a slightly decreased albumin and total protein concentration for females of group 6 (36 mg/kg b.w.).

For urinalysis data no treatment-related changes were noted at the end of the treatment and the recovery period.

At the end of both treatment and recovery periods, the analysis of absolute mean organ weights and of mean organ to body and to brain weight ratios revealed no consistent treatment-related effects either in males nor in females.

No pathomorphologic signs of systemic toxicity were noted in the rats treated with the formulation base of endosulfan. No pathomorphologic signs of local toxicity were noted in the rats treated with endosulfan.
In comparison with hyperkeratosis and inflammation noted at the application site in the groups 1, 3, 4, 5 and 6, which were considered to reflect mechanical irritation occurring during hair clipping and shaving, the severity and/or incidence of these findings were increased in group 2. This was considered to be treatment-related and to reflect a slight irritating effect of the formulation base.
All other pathomorphological findings noted in this study were considered to be incidental, since they are commonly observed in rats of this strain and age.

Conclusions

The no-observable adverse effect level (NOAEL) during this subchronic 4-week repeated-dose dermal toxicity study was considered to be 54 mg/kg b.w. in male rats and 9 mg/kg b.w. in female rats. These NOAEL are established based on the following results:
- One female dead at 12 mg/kg b.w. At this dose level some females showed transient toxic signs of the nervous system and plasma cholinesterase activity decreased slightly.
- Males at the dose level 81 mg/kg b.w. elicited nervous toxic signs and the aspartate aminotransferase activity was increased by 18% .

At the application site, the test substance elicited marginally to slightly irritating effects due to the formulation base. There were nearly completely reversible within the one-month recovery period.
Clinical observations and biochemical investigations provided evidence that the nervous system and in addition, to a lesser extent, the liver, are target systems in rats treated with Endosulfan EC.

B.6.12 Dermal absorption (Annex IIIA, point 7.3)

Summary

In vivo d*ermal* penetration study in rats, animals were given a single dose of a similar-to-field-use EC35 formulation of ^{14}C-endosulfan at actual levels of 0.09, 0.98 or 10.98 mg ^{14}C-endosulfan /kg body weight for 10 hours. After 24, 48, 72 and 168 hours 4 animals/group were sacrificed and disposition of ^{14}C was measured. Skin absorption was proportional to the dose but was in all doses less than 50%, at the high dose of 10.98 mg/kg only 20%. Only little residue was still present in the skin after one week, indicating that the penetration process was completed in one week. Little also remained in other parts of the body. The rate of elimination of ^{14}C-material was low at 24 hr, then accelerated with a peak at 48 hours and subsequently slowed down again. Two thirds of the eliminated radioactivity was excreted in the faeces and one third in the urine. (Craine, 1986, **7.3/03**; Craine, 1988, **7.3/04**).

In monkey, the excretion and metabolism kinetics after dermal exposure are similar to that in the rat. A plateau in blood is reached after 36 hours, earlier than in the rat. Dermal penetration is also slow (Lachmann 1987, **7.3/01**).

The rate of penetration of an experimental ^{14}C-endosulfan-EC35 formulation through isolated human and rat skin was assessed *in vitro* following a single application of 1.0, 0.1 or 0.01 mg endosulfan/cm² to the epidermal surface. Penetration of endosulfan through the skin started after a lag time of generally less than one hour at a steadily decreasing rate. The highest mean penetration rate was between 1 - 8 hr, was dose dependent and found to be 4.3 times higher through rat skin than through human skin. Analysis of the receptor fluid in the high dose groups further revealed interesting differences in degradation products, showing that the human skin preparation had more residual detoxifying capacity than the rat skin preparation (Noctor and John 1995, **7.3/02**). These results are in accordance with the *in vivo* dermal penetration study on rats. The skin appears to have a significant depot function, slowing down the rate of penetration significantly. Longer storage in the skin may enhance possible dermal detoxification.

Reevaluation of studies included at the original monograph

Monkey dermal single dose toxicokinetic study			
Autor(s):	Lachmann G & Siegemund B.	**Study design:**	The animals were housed in metabolic cages. Acclimatisation period: 2 weeks. Fed and tap water plus freshly squeezed orange juice *ad libitum*. Blood samples were collected in intervals from 1 to 96 h after treatment; urine and faeces in 24-h intervals until 96 h after treatment. Skin of the application site, untreated skin, brain, liver, kidney, muscle and fat tissues were collected at sacrifice. The radioactivity of blood, plasma, faeces, urine and tissues was measured by LSC; pattern of metabolites was performed by chromatography on silica gel and exposed to x-ray films (autoradiography); and quantification of metabolites was performed by HPLC analysis.
Study Title:	**Hoe 002671-(5a, 9a-^{14}C). Dermal absorption of ^{14}C-endosulfan in Rhesus monkeys.** 7.3/01		
Testing facility:	[redacted]		
Report Number:	A36685		
Study duration:	From February 1987 to May 8th 1987.	**Dose:**	2.2 and 3.3 mg/kg
Date of report:	1987	**Vehicle/Solvent:**	Blank solution
Test Substance:	THIODAN EC (^{14}C-labelled endosulfan)	**Route:**	dermal
Batch Nº.:	Hoe 002671 00 ZE98 0005	**Statistics/ Measurements:**	
Radiochemical purity:	98%		
Test Animals:	Male Rhesus monkeys	**GLP:**	Yes
Origin:	[redacted]	**Guideline:**	
Bodyweight:	4.5-5 kg	**Deviation:**	
Groups:	2 animals	**Acceptability:**	The study is not acceptable

Findings

Total recovery of radioactivity was about 50 %. Levels in blood and plasma reached a plateau of 25 and 35 mg/kg respectively 36 hours after treatment. A total of about 8 % of applied radioactivity were excreted via urine and faeces over the entire period. Excretion rate had not declined by the time of sacrifice. While concentrations in muscle and especially in brain was below blood levels, it was higher in kidney, fat, and liver (factors 3, 9, and 19 respectively). Only low amounts of unmetabolised substance were excreted, especially with urine, where the main metabolite was the endosulfan-diol. The second biggest portion in urine was an unidentified metabolite which is supposed to be endosulfan hydroxycarboxylic acid. This unidentified compound was the most important metabolite in faeces. Radioactivity metabolites of ^{14}C-endosulfan in faeces and urine are summarised in Table B.6.12-1.

Table 6.12-1: Radioactivity metabolites of ^{14}C-endosulfan in faeces and urine.

	URINE in % total radioactivity of the respective sampling interval								
		0 - 24 h			**24 - 48 h**			**48 - 72 h**	
	retention time (min)	**native**	**G**	**G+S**	**native**	**G**	**G+S**	**native**	**G**	**G+S**
x	2.7	28.4	59.5	55.2	50.2	75.7	68.5	41.3	77.6	68.3
endosulfan-diol	9.8	67.0	37.2	44.8	41.3	24.3	31.5	49.9	22.4	31.7
β-endosulfan	16.4	1.9	1.2	-	4.1	-	-	4.2	-	-
α-ensulfan	18.5	2.6	2.1	-	4.4	-	-	4.6	-	-
	URINE in % administered dose								
	retention time (min)	**native**	**G**	**G+S**	**native**	**G**	**G+S**	**native**	**G**	**G+S**
x	2.7	0.63	1.33	1.23	0.28	0.41	0.37	0.16	0.31	0.27
endosulfan-diol	9.8	1.50	0.83	1.00	0.23	0.13	0.17	0.20	0.09	0.13
β-endosulfan	16.4	0.04	0.02	-	0.02	-	-	0.02	-	-
α-ensulfan	18.5	0.06	0.05	-	0.02	-	-	0.02	-	-
	FAECES in % total radioactivity of the respective sampling interavl								
		24 - 48 h			**48 - 72 h**			**72 - 96 h**	
	retention time (min)	**native**	**G**	**G+S**	**native**	**G**	**G+S**	**native**	**G**	**G+S**
x	2.2	70.7	100	100	86.3	100	100	74.1	100	100
endosulfan-diol	9.9	6.7	-	-	-	-	-	-	-	-
β-endosulfan	15.9	8.7	-	-	5.7	-	-	6.7	-	-
α-ensulfan	18.1	13.9	-	-	8.0	-	-	10.8	-	-
y	19.9	-	-	-	-	-	-	8.4	-	-
	FAECES in % administered dose								
	retention time (min)	**native**	**G**	**G+S**	**native**	**G**	**G+S**	**native**	**G**	**G+S**
x		0.40	0.56	0.56	0.21	0.25	0.25	0.41	0.56	0.56
endosulfan-diol	15.92.2	0.04	-	-	-	-	-	-	-	-
β-endosulfan	9.9	0.05	-	-	0.01	-	-	0.04	-	-
α-ensulfan	18.1	0.08	-	-	0.02	-	-	0.06	-	-
y	19.9	-	-	-	-	-	-	0.05	-	-

G: hydrolysis with glucuronidase. G+S: hydrolysis with glucuronidase + arylsulphatase.

Conclusions

After single dermal application of endosulfan to Rhesus monkeys a plateau in blood is reached after about 36 hours. Higher concentrations are found 96 hours after treatment in kidney, fat and liver. Mainly metabolites are eliminated via urine and faeces.

Comparative study on the penetration through human and rat skin			
Autor(s):	**Noctor JC & John SA.**	**Study design:**	Pieces of excised skin (rat and human) were partially thawed and cut to a uniform thickness (ca 0.4 mm) using a dermatome. The resulting section consisted of intact epidermis and a portion of dermis. Skin sections were used immediately or stored flat at ca -20°C until used. On the day prior to dose application, the skin sections were thawed and mounted in a „Franz type“ static in vitro dermal penetration cell. Dose application and dose levels: Immediately prior to dose application the receptor chamber was refilled with a known volume of acidified ethanol/water (1 : 1 v/v), pH 5.5. The test substance was applied to the epidermal surface of each skin preparation by syringe.
Study Title:	(^{14}C)-endosulfan: Rates of penetration through human and rat skin determined using an *in vitro* system. **7.3/02**		
Testing facility:	[redacted]		
Report Number:	A54103		
Study duration:	From June 2nd 1993 to May 8th 1995	**Dose:**	0.01, 0.1 or 1.0, or 10.0 mg/cm^2
Date of report:	1995	**Vehicle/Solvent:**	water
Test Substance:	ENDOSULFAN 35 EC (^{14}C labelled endosulfan alpha- and beta-isomer and non-radiolabelled endosulfan, alpha- and beta-isomer)	**Route:**	dermal
Batch N°.:	22022 II 23023 II Hoe 052618 00 ZB99 0007 Hoe 052619 00 ZB99 0006	**Statistics/ Measurements:**	
Radiochemical purity:	99-99.8%		
Test Animals:	Skin preparations of Sprague Dawley rats and human	**GLP:**	Yes
Origin:		**Guideline:**	US EPA. Federal Register 48, no 230, 40 CFR, part 160, Nov 1983 and Pesticides Ass. Guidel., F, Human and Domestic Animals
Bodyweight:	211-262 g	**Deviation:**	
Groups:	3 groups, 24 animals/group	**Acceptability:**	The study is acceptable

Results

Following a single application of (^{14}C)-endosulfan to rat skin preparations, penetration rates of 0.220, 0.764 and 5.160 µg endosulfan/cm^2/h were observed at the low, intermediate and high dose levels, respectively. The extrapolated lag times were 0.644, 1.497 and 1.089 h at the low, intermediate and high dose levels, respectively. Following a single application of (^{14}C)-endosulfan to rat skin preparations, 95.75, 75.91 and 40.23% of the applied dose (low, intermediate and high dose levels, respectively) was recovered in the receptor fluid after 72 h. The overall recovery of radioactivity in these groups was 110.8, 94.10 and 94.68% of the applied dose, respectively. When skin preparations were washed at 10 h post-application, a mean of 51.05% of the applied dose was recovered in washings, and the residual activity

was subsequently recovered from receptor fluid (9.130%), terminal washings (7.467%) and skin (20.61%). The penetrant was identified by HPLC as mainly beta-endosulfan (81.4%) with some alpha-endosulfan (3.05%), indicating a lack of extensive detoxification/degradation within the epidermal membrane. Following a single application of (^{14}C)-endosulfan to human skin preparations, 60.55, 29.39 and 19.97 % of the applied dose (low, intermediate and high dose levels, respectively) was recovered in the receptor fluid after 72 h. The overall recovery of radioactivity in these groups was 93.53, 87.21 and 75.97 % of the applied dose, respectively. When skin preparations were washed at 10 h post-application, a mean of 58.71 % of the applied dose was recovered in washings, and the residual radioactivity was subsequently recovered from receptor fluid (4.013%), terminal washings (0.833%) and skin (4.277%). The penetrate was identified by HPLC as mainly beta-endosulfan (27.33 %) and endosulfan diol (34.0 %) with some endosulfan sulphate (8.27%) and an unidentified component (17.23 %), indicating that detoxification/ degradation was occurring within the human epidermal preparation. Mean cumulative penetration following a single application of ^{14}C-endosulfan to preparations of isolated skin are summarised in Table B.6.12-2. Mean recovery of radioactivity 72 h after a single application are summarised in Table B.6.12-3.

Table B.6.12-2: Mean cumulative penetration following a single application of ^{14}C-endosulfan to preparations of isolated skin.

cumulative amount of ^{14}C-endosulfan equivalents (µg/cm^2)								
Time (h)	**A1 rat 1.0 mg/cm^2**	**A2 rat 1.0 mg/cm^2 10h wash**	**B rat 0.1 mg/cm^2**	**C rat 0.01 mg/cm^2**	**D1 human 1.0 mg/cm^2**	**D2 Human 1.0 mg/cm^2 10 h wash**	**E human 0.1 mg/cm^2**	**F human 0.01 mg/cm^2**
1	5.587	4.043	1.686	0.839	0.748	ND	0.073	0.050
2	27.14	19.68	5.166	2.234	5.798	1.363	0.418	0.212
4	68.44	59.38	13.97	4.887	25.82	5.839	4.931	0.815
8	110.9	101.6	29.30	7.257	56.43	13.05	5.081	1.560
10	126.0	116.3	34.86	7.880	67.08	15.41	6.265	1.826
16	170.2	137.7	47.43	8.907	87.62	21.75	8.715	2.615
24	217.7	139.3	57.40	9.379	110.1	26.43	11.95	3.383
48	317.8	129.7	71.24	9.696	146.2	32.74	20.78	5.112
72	395.3	89.71	76.03	9.665	191.7	39.43	29.19	6.075

ND: Not detected

Table B.6.12-3: Mean recovery of radioactivity 72 h after a single application of ^{14}C-endosulfan to preparations of isolated skin.

	% of administered dose							
specimen	**A1 rat 1.0 mg/cm^2**	**A2 rat 1.0 mg/cm^2 10h wash**	**B rat 0.1 mg/cm^2**	**C rat 0.01 mg/cm^2**	**D1 human 1.0 mg/cm^2**	**D2 human 1.0 mg/cm^2 10 h wash**	**E human 0.1 mg/cm^2**	**F human 0.01 mg/cm^2**
receptor fluid	40.23	9.130	75.91	95.75	19.97	4.013	29.39	60.55
washings (10h)	NA	51.05	NA	NA	NA	58.71	NA	NA
washings (72h)	23.77	7.467	3.905	1.727	49.32	0.833	44.28	25.59
skin	30.69	20.61	14.29	13.30	6.680	4.277	13.54	7.392
Total	94.68	88.26	94.10	110.8	75.97	67.83	87.21	93.53

NA: Not applicable.

Conclusions

After a single application of (^{14}C)-endosulfan, the rate of penetration of radioactivity through rat skin was 4.0, 5.7 and 3.1 times greater than that observed in human skin at the low, intermediate and high dose levels, respectively. In both species, rates of penetration were dose dependent, increasing in a non-linear manner with increasing dose level. Very little detoxification/degradation occurred in rat skin, but was more extensive in human skin preparations.

Rat dermal single dose toxicokinetic study			
Autor(s):	**Craine EM**	**Study design:**	Assessment of health condition. Rats housed single in a metabolism cage. Individual housing in metabolism cages in air-conditioned rooms, feed and water ad libitum . 4 animals were sacrificed each after 0.5, 1. 2, 4, 10, 03 24 hours; blood, urine from the bladder, skin of the application site, remaining skin and carcass were separated, liver, kidney and fat tissues sampled; urine and faeces excreted between treatment and sacrifice collected. The samples were analysed by LSC.
Study Title:	**A dermal absorption study in rats with ^{14}C-endosulfan.** 7.3/03		
Testing facility:	[redacted]		
Report Number:	A35730		
Study duration:	From April 1986 to July 1986	**Dose:**	0.1, 1.0, or 10.0 mg substance /kg bw
Date of report:	1986	**Vehicle/Solvent:**	water
Test Substance:	THIODAN 3EC (^{14}C labelled endosulfan)	**Route:**	dermal
Batch Nº.:	Not provided in the report	**Statistics/ Measurements:**	
Radiochemical purity:	94.6%		
Test Animals:	Male rats	**GLP:**	Yes
Origin:	[redacted]	**Guideline:**	US EPA. Federal Register 48, no 230, 40 CFR, part 160, Nov 1983 and Pesticides Ass. Guidel., F, Human and Domestic Animals
Bodyweight:	203-288 g	**Deviation:**	
Groups:	3 groups, 24 animals/group	**Acceptability:**	The study is acceptable

Results

No symptoms of irritation were observed at the application site. At all three levels about 80 % of the applied dose penetrated into the skin and were not removable with soap and water. Penetration occurred within 30 minutes after application and did not increase further with time of exposure. 90 % or more of the material penetrated, remained bound in the skin after 10 hours. Only about 8 % were absorbed by the body during this period. This rate increased to about 25 % after 24 hours. Low concentrations of radioactivity appeared in the blood and organs one hour after application. At later dates liver, kidney, and fat contained the largest portions of absorbed radioactivity. Only small amounts were eliminated with excreta during the first 10 hours. After 24 hours concentration in the excreta had increased to 13.5 % (0.1 mg/kg dose), 12.4 % (1 mg/kg dose), and 4.9 % (10.0 mg/kg dose). Faeces contained 2 to 3 times as much radioactivity as urine.

Conclusions

After single dermal application, endosulfan penetrates quickly into the skin of rats. The residue in the skin is slowly resorbed. Resorbed material is partly distributed in various organs, partly excreted within 24 hours after application.

Rat dermal single dose toxicokinetic study			
Autor(s):	**Craine EM, Elliot M**	**Study design:**	Assessment of health condition. Rats housed single in a metabolism cage. Individual housing in metabolism cages in air-conditioned rooms, feed and water ad libitum . 4 animals were sacrificed each after 24, 48, 72 and 168 hours; blood, urine from the bladder, skin of the application site, remaining skin and carcass were separated, liver, kidney and fat tissues sampled; urine and faeces excreted between treatment and sacrifice collected. The samples were analysed by LSC.
Study Title:	A dermal absorption study in rats with ^{14}C-endosulfan with extended test duration**. 7.3/04**		
Testing facility:	[redacted]		
Report Number:	A39677		
Study duration:	From June 16th 1986 to January 1987	**Dose:**	0.1, 1.0, or 10.0 mg substance /kg bw
Date of report:	1988	**Vehicle/Solvent:**	water
Test Substance:	THIODAN 3EC (^{14}C labelled endosulfan)	**Route:**	dermal
Batch Nº.:	Hoe 002671 00 ZE98 0002 Hoe 002671 0I ZE98 0003	**Statistics/ Measurements:**	
Radiochemical purity:	94.6%		
Test Animals:	Male rats	**GLP:**	Yes
Origin:	[redacted]	**Guideline:**	US EPA. Federal Register 48, no 230, 40 CFR, part 160, Nov 1983 and Pesticides Ass. Guidel., F, Human and Domestic Animals
Bodyweight:	211-262 g	**Deviation:**	
Groups:	3 groups, 24 animals/group	**Acceptability:**	The study is acceptable

Results

No symptoms of general intoxication were observed. No symptoms of irritation were observed at the application site. Low concentrations of radioactivity were observed in blood and tissues 24 hours after application. Peak concentrations were reached after 48 hours, they then decreased. Concentrations were dose dependant. Accumulation in the analysed organs could not be observed. Distribution of Radioactivity 168 h after application are summarised in Table B.6.12-4. Two third of the elimination was via faeces, one third via urine.

Table B.6.12-4: Radioactivity 168 hours after application of ^{14}C-endosulfan.

	0.1 mg/kg	**1.0 mg/kg**	**10 mg/kg**
% of applied dose removed by washing of application site after 10 h	28	47	69
% of applied dose that penetrate through skin	45	46	20
% of dose applied remaining in the treated skin	1.7	1.5	1.0
% of dose applied remaining in the body of the animal	2.5	2.3	1.4
% of penetrated dose excreted	94	95	94

Conclusions

After single dermal application, endosulfan penetrates into the skin of rats. Above a dose of 1 mg/kg bw percentage absorption decreases. Material transported from the skin into the body reaches maximum concentrations in blood and organs after 48 hours and then is rapidly eliminated via faeces and urine.

B.6.14 Exposure data (IIIA, 7.2)

B.6.14.1 Excel applicant

Operator exposure

Operator exposure, in the context of this section, refers to potential exposure to the person or persons involved in mixing, loading and/or spray application of a plant protection product.

Endocel 35 EC is applied using field crop sprayers and hand held sprayers.

- **ESTIMATES OF OPERATOR EXPOSURE UK MODEL**

Hand held sprayers- no PPE

A. PRODUCT DATA

1.	Name	Endocel 35 EC
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	2 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	1 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	2 litres product/ha
3.	Work rate	1 ha/day

4.	Number of operations	4/day
5.	Hand contamination	0.04 ml/day
6.	Protective clothing	NONE
7.	Transmission to skin	100%
8.	Dermal exposure to formulation	0.04 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	350 l spray/ha		
3.	Volume of surface contamination	50 ml/h		
		Hands	Trunk	Legs
4.	Distribution	25%	25%	50%
5.	Clothing	None	Perm.	Perm.
6.	Penetration	100%	20%	18%
7.	Dermal exposure	10	2.5	4.5
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	102 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	0.04 ml/day	102 ml/day
2.	Concentration of as	350 mg/ml	2 mg/ml
3.	Dermal exposure to as	14 mg/day	204 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	2.8 mg/day	40.8 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.02 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	2 mg/ml
4.	Inhalational exposure to as	0.24 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.24 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	43.84 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**0.731 mg/kg bw/day**

Hand held sprayers- with PPE (Gloves for mixer/loader and applicator)

A. PRODUCT DATA

1.	Name	Endocel 35 EC
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	2 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	1 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	2 litres product/ha
3.	Work rate	1 ha/day
4.	Number of operations	4/day
5.	Hand contamination	0.04 ml/day
6.	Protective clothing	Gloves
7.	Transmission to skin	1%
8.	Dermal exposure to formulation	0.0004 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	350 l spray/ha		
3.	Volume of surface contamination	50 ml/h		
		Hands	Trunk	Legs
4.	Distribution	25%	25%	50%
5.	Clothing	Gloves	Perm.	Perm.
6.	Penetration	1%	20%	18%
7.	Dermal exposure	0.125	2.5	4.5
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	42.75 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	0.0004 ml/day	42.75 ml/day
2.	Concentration of as	350 mg/ml	2 mg/ml
3.	Dermal exposure to as	0.14 mg/day	85.5 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	0.028 mg/day	17.1 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.02 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	2 mg/ml
4.	Inhalational exposure to as	0.24 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.24 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	17.368 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**0.289 mg/kg bw/day**

Estimates of operator exposure-German model

Endocel 35 EC, calculation of exposure for mixer/loader and spray application by tractor. No PPE

Maximum Application Rate (kg ai/ha) : 0.7

Specific Exposure and Work Rate

Mixing and Loading (mg/person x kg ai)	Spray Application (mg/person x kg ai)	Work Rate (ha/day)
$I^*_M = 0.0006$ $D^*_{M(H)} = 2.4$	$I^*_A = 0.001$ $D^*_{A(C)} = 0.06$ $D^*_{A(H)} = 0.38$ $D^*_{A(B)} = 1.6$	20

Expected Inhalation Exposure:

$I_M = I^*_M \times R \times A = 0.0006 \times 0.7 \times 20 = 0.0084$ mg/person/day

$I_A = I^*_A \times R \times A = 0.001 \times 0.7 \times 20 = 0.014$ mg/person/day

Expected Dermal Exposure:

$D_{M(H)} = D^*_{M(H)} \times R \times A = 2.4 \times 0.7 \times 20 = 33.6$ mg/person/day

$D_{A(H)} = D^*_{A(H)} \times R \times A = 0.38 \times 0.7 \times 20 = 5.32$ mg/person/day

$D_{A(C)} = D^*_{A(C)} \times R \times A = 0.06 \times 0.7 \times 20 = 0.84$ mg/person/day

$D_{A(B)} = D^*_{A(B)} \times R \times A = 1.6 \times 0.7 \times 20 = 22.4$ mg/person/day

Inhalation exposure = 0.0084 + 0.014 mg ai/person = **0.0224 mg ai/person**

Total dermal exposure = 62.16 mg ai/person

Total dermal exposure based on dermal absorption in humans of 20% = 12.432 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 12.454 mg ai/person

Total systemic exposure for a 70 kg person **= 0.178 mg ai/kg/day**

With PPE (Gloves 1% mixing/loading 1%; gloves during aplication 1%; protection cloth during aplication 5% and hear protection during aplication 50%):

Dermal exposure:

$D_{M(H)}$ = 0.336 mg/person/day (1%)
$D_{A(H)}$ = 0.0532 mg/person/day (1%)
$D_{A(C)}$ = 0.42 mg/person/day (50%)
$D_{A(B)}$ = 1.12 mg/person/day (5%)

Total dermal exposure = 1.9292 mg ai/person

Total dermal exposure based on dermal absorption in humans of 20% = 0.386 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 0.408 mg ai/person

Total systemic exposure for a 70 kg person **= 0.006 mg ai/kg/day**

- **ESTIMATES OF OPERATOR EXPOSURE UK MODEL**

Tractor mounted boom (with cab) with hydraulic nozzles- no PPE

A. PRODUCT DATA

1.	Name	Endocel 35 EC
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	2 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	1 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	2 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	100/day
5.	Hand contamination	1 ml/day
6.	Protective clothing	NONE
7.	Transmission to skin	100%
8.	Dermal exposure to formulation	1 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	350 l spray/ha		
3.	Volume of surface contamination	10 ml/h		
		Hands	Trunk	Legs
4.	Distribution	65%	10%	25%
5.	Clothing	None	Perm.	Perm.
6.	Penetration	100%	50%	15%
7.	Dermal exposure	6.5	0.5	0.375
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	44.25 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	1 ml/day	44.25 ml/day
2.	Concentration of as	350 mg/ml	2 mg/ml
3.	Dermal exposure to as	350 mg/day	88.5 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	70 mg/day	17.7 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.01 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	2 mg/ml
4.	Inhalational exposure to as	0.12 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.12 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	87.82 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**1.464 mg/kg bw/day**

Tractor mounted boom (with cab) with hydraulic nozzles- with PPE (Gloves for mixer/loader and applicator)

A. PRODUCT DATA

1.	Name	Endocel 35 EC
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	2 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	1 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	2 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	100/day
5.	Hand contamination	1 ml/day
6.	Protective clothing	Gloves
7.	Transmission to skin	1%
8.	Dermal exposure to formulation	0.01 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	350 l spray/ha		
3.	Volume of surface contamination	10 ml/h		
		Hands	Trunk	Legs
4.	Distribution	65%	10%	25%
5.	Clothing	Gloves	Perm.	Perm.
6.	Penetration	1%	50%	15%
7.	Dermal exposure	0.065	0.5	0.375
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	5.64 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	0.01 ml/day	5.64 ml/day
2.	Concentration of as	350 mg/ml	2 mg/ml
3.	Dermal exposure to as	3.5 mg/day	11.28 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	0.7 mg/day	2.256 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.01 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	2 mg/ml
4.	Inhalational exposure to as	0.12 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.12 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	3.076 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**0.051 mg/kg bw/day**

B.6.14.1bCalliope applicant

Operator exposure

The following assumptions have been used in calculation operator exposure:

Maximum application rate	610 g of a.i/ha, corresponding with 1,74 l of product/ha	
Spray volume	Proyected spray	400-1000 l/ha
	Pneumatic systems	80-150 l/ha
Maximum in-use a.i. concentration	Proyected spray	1,53 mg/ml
	Pneumatic systems	7,63 mg/ml
Container size	5 litres (63 mm neck diameter)	
Application techniques	Tractor mounted boom (with cab) with hydraulic nozzles	
	Tractor mounted boom (with cab) with rotary discs	
	Tractor mounted (whitout cab) air assisted: application volume 100l/ha	

Estimates of operator exposure-German model

Callistar, calculation of exposure for mixer/loader and spray application by tractor - No PPE

Maximum Application Rate (kg ai/ha): 0.61

Specific Exposure and Work Rate

Mixing and Loading (mg/person x kg ai)	Spray Application (mg/person x kg ai)	Work Rate (ha/day)
$I^*_M = 0.0006$ $D^*_{M(H)} = 2.4$	$I^*_A = 0.001$ $D^*_{A(C)} = 0.06$ $D^*_{A(H)} = 0.38$ $D^*_{A(B)} = 1.6$	20

Expected Inhalation Exposure:

$$I_M = I^*_M \times R \times A = 0.0006 \times 0.61 \times 20 = 0.00732 \text{ mg/person/day}$$
$$I_A = I^*_A \times R \times A = 0.001 \times 0.61 \times 20 = 0.0122 \text{ mg/person/day}$$

Expected Dermal Exposure:

$$D_{M(H)} = D^*_{M(H)} \times R \times A = 2.4 \times 0.61 \times 20 = 29.28 \text{ mg/person/day}$$
$$D_{A(H)} = D^*_{A(H)} \times R \times A = 0.38 \times 0.61 \times 20 = 4.636 \text{ mg/person/day}$$
$$D_{A(C)} = D^*_{A(C)} \times R \times A = 0.06 \times 0.61 \times 20 = 0.732 \text{ mg/person/day}$$
$$D_{A(B)} = D^*_{A(B)} \times R \times A = 1.6 \times 0.61 \times 20 = 19.52 \text{ mg/person/day}$$

Inhalation exposure = 0.00732 + 0.0122 mg ai/person = **0.01952 mg ai/person**

Total dermal exposure = 54.168 mg ai/person

Total dermal exposure based on dermal absorption in humans of 20% = 10.834 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 10.853 mg ai/person

Total systemic exposure for a 70 kg person **= 0.155 mg ai/kg/day**

With PPE (Gloves during mixing/loading1%; gloves during aplication 1%; protection cloth during aplication 5% and hear protection during aplication 50%):

Dermal exposure:

$D_{M(H)}$ = 0.293 mg/person/day (1%)
$D_{A(H)}$ = 0.04636 mg/person/day (1%)
$D_{A(C)}$ = 0.366 mg/person/day (50%)
$D_{A(B)}$ = 0.976 mg/person/day (5%)

Total dermal exposure = 1.681 mg ai/person

Total dermal exposure based on dermal absorption in humans of 20% = 0.3362 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 0.356 mg ai/person

Total systemic exposure for a 70 kg person **= 0.005 mg ai/kg/day**

- **ESTIMATES OF OPERATOR EXPOSURE UK MODEL**

Tractor mounted boom (with cab) with hydraulic nozzles- no PPE

A. PRODUCT DATA

1.	Name	Callistar
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	1.53 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	5 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	1.74 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	18/day
5.	Hand contamination	0.18 ml/day
6.	Protective clothing	NONE
7.	Transmission to skin	100%
8.	Dermal exposure to formulation	0.18 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	400 l spray/ha		
3.	Volume of surface contamination	10 ml/h		
		Hands	Trunk	Legs
4.	Distribution	65%	10%	25%
5.	Clothing	None	Perm.	Perm.
6.	Penetration	100%	50%	15%
7.	Dermal exposure	6.5	0.5	0.375
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	44.25 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	0.18 ml/day	44.25 ml/day
2.	Concentration of as	350 mg/ml	1.53 mg/ml
3.	Dermal exposure to as	63 mg/day	67.70 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	12.6 mg/day	13.54 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.01 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	1.53 mg/ml
4.	Inhalational exposure to as	0.092 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.092 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	26.232 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**0.437 mg/kg bw/day**

Tractor mounted boom (with cab) with hydraulic nozzles- with PPE (Gloves for mixer/loader and applicator)

A. PRODUCT DATA

2.	Name	Callistar
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	1.53 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	5 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	1.74 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	18/day
5.	Hand contamination	0.18 ml/day
6.	Protective clothing	Gloves
7.	Transmission to skin	1%
8.	Dermal exposure to formulation	0.0018 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	400 l spray/ha		
3.	Volume of surface contamination	10 ml/h		
		Hands	Trunk	Legs
4.	Distribution	65%	10%	25%
5.	Clothing	Gloves	Perm.	Perm.
6.	Penetration	1%	50%	15%
7.	Dermal exposure	0.065	0.5	0.375
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	5.64 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	0.0018 ml/day	5.64 ml/day
2.	Concentration of as	350 mg/ml	1.53 mg/ml
3.	Dermal exposure to as	0.63 mg/day	8.63 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	0.126 mg/day	1.726 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.01 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	1.53 mg/ml
4.	Inhalational exposure to as	0.092 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.092 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	1.944 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**0.0324 mg/kg bw/day**

- **ESTIMATES OF OPERATOR EXPOSURE UK MODEL**

Tractor mounted boom (with cab) with rotary discs- No PPE

A. PRODUCT DATA

3.	Name	Callistar
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	7.63 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	5 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	1.74 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	18/day
5.	Hand contamination	0.18 ml/day
6.	Protective clothing	NONE
7.	Transmission to skin	100%
8.	Dermal exposure to formulation	0.18 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	80 l spray/ha		
3.	Volume of surface contamination	2 ml/h		
		Hands	Trunk	Legs
4.	Distribution	75%	15%	10%
5.	Clothing	None	Perm.	Perm.

6.	Penetration	100%	5%	5%
7.	Dermal exposure	1.5	0.015	0.01
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	9.15 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	0.18 ml/day	9.15 ml/day
2.	Concentration of as	350 mg/ml	7.63 mg/ml
3.	Dermal exposure to as	63 mg/day	69.81 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	12.6 mg/day	13.962 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.005 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	7.63 mg/ml
4.	Inhalational exposure to as	0.2289 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.2289 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	26.791 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**0.447 mg/kg bw/day**

Tractor mounted boom (with cab) with rotary discs- - with PPE (Gloves for mixer/loader and applicator)

A. PRODUCT DATA

4.	Name	Callistar
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	7.63 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	5 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	1.74 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	18/day

5.	Hand contamination	0.18 ml/day
6.	Protective clothing	Gloves
7.	Transmission to skin	1%
8.	Dermal exposure to formulation	0.0018 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	80 l spray/ha		
3.	Volume of surface contamination	2 ml/h		
		Hands	Trunk	Legs
4.	Distribution	75%	15%	10%
5.	Clothing	Gloves	Perm.	Perm.
6.	Penetration	1%	5%	5%
7.	Dermal exposure	0.015	0.015	0.01
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	0.24 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	0.0018 ml/day	0.24 ml/day
2.	Concentration of as	350 mg/ml	7.63 mg/ml
3.	Dermal exposure to as	0.63 mg/day	1.83 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	0.126 mg/day	1.566 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.005 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	7.63 mg/ml
4.	Inhalational exposure to as	0.2289 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.2289 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	1.9209 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**0.032 mg/kg bw/day**

B.6.14.1c AgrEvo applicant

Operator exposure

The exposure to endosulfan in Thiodan-EC35 is predicted according to the German BBA-Model and the UK Model

Scenario 1: Tractor mounted boom sprayers in field crops

The maximum application rate in maize is 1.05 Kg a.s/ha (equivalent to 3.0 l product/ha) applied in 400 to 1000 l of water (depending on the growth stage of the crop).

Scenario 2: Airblast spraying in high crops with tractor –mounted equipment

The worst case for this scenario is airblast spraying in citrus orchards with a maximum application rate of 1.05 kg a.s./ha (equivalent to 3.0 l of product/ha) and a water volume of 1000 to 3000 l/ha.

Estimates of operator exposure-German model

Scenario 1: Tractor-mounted boom sprayers in field crop

Maximum Application Rate (kg ai/ha): 1.05

Specific Exposure and Work Rate

Mixing and Loading (mg/person x kg ai)	Spray Application (mg/person x kg ai)	Work Rate (ha/day)
$I^*_M = 0.0006$ $D^*_{M(H)} = 2.4$	$I^*_A = 0.001$ $D^*_{A(C)} = 0.06$ $D^*_{A(H)} = 0.38$ $D^*_{A(B)} = 1.6$	20

Expected Inhalation Exposure:

$I_M = I^*_M \times R \times A = 0.0006 \times 1.05 \times 20 = 0.0126$ mg/person/day

$I_A = I^*_A \times R \times A = 0.001 \times 1.05 \times 20 = 0.021$ mg/person/day

Expected Dermal Exposure:

$D_{M(H)} = D^*_{M(H)} \times R \times A = 2.4 \times 1.05 \times 20 = 50.4$ mg/person/day

$D_{A(H)} = D^*_{A(H)} \times R \times A = 0.38 \times 1.05 \times 20 = 7.98$ mg/person/day

$D_{A(C)} = D^*_{A(C)} \times R \times A = 0.06 \times 1.05 \times 20 = 1.26$ mg/person/day

$D_{A(B)} = D^*_{A(B)} \times R \times A = 1.6 \times 1.05 \times 20 = 33.6$ mg/person/day

Inhalation exposure = 0.0126 + 0.021 mg ai/person = **0.0336 mg ai/person**

Total dermal exposure = 93.24 mg ai/person

Total dermal exposure based on dermal absorption in humans of 20% = 18.648 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 18.682 mg ai/person

Total systemic exposure for a 70 kg person **= 0.267 mg ai/kg/day**

With PPE (Gloves 1% during mixing/loading/aplication, protection cloth during aplication 5% and hear protection during aplication 50%):

Dermal exposure:

$D_{M(H)}$ = 0.504 mg/person/day (1%)
$D_{A(H)}$ = 0.0798 mg/person/day (1%)
$D_{A(C)}$ = 0.63 mg/person/day (50%)
$D_{A(B)}$ = 1.68 mg/person/day (5%)

Total dermal exposure = 2.894 mg ai/person

Total dermal exposure based on dermal absorption in humans of 20% = 0.579 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 0.612 mg ai/person

Total systemic exposure for a 70 kg person **= 0.009 mg ai/kg/day**

- **ESTIMATES OF OPERATOR EXPOSURE UK MODEL**

Tractor mounted boom (with cab) with hydraulic nozzles- no PPE

A. PRODUCT DATA

5.	Name	Thiodan EC 35
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	2.625 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	1 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	3 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	150/day
5.	Hand contamination	1.5 ml/day
6.	Protective clothing	NONE
7.	Transmission to skin	100%

8.	Dermal exposure to formulation	1.5 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	400 l spray/ha		
3.	Volume of surface contamination	10 ml/h		
		Hands	Trunk	Legs
4.	Distribution	65%	10%	25%
5.	Clothing	None	Perm.	Perm.
6.	Penetration	100%	50%	15%
7.	Dermal exposure	6.5	0.5	0.375
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	44.25 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	1.5 ml/day	44.25 ml/day
2.	Concentration of as	350 mg/ml	2.625 mg/ml
3.	Dermal exposure to as	525 mg/day	116.16 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	105 mg/day	23.232 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.01 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	2.625 mg/ml
4.	Inhalational exposure to as	0.158 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.158 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	128.39 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**2.14 mg/kg bw/day**

Tractor mounted boom (with cab) with hydraulic nozzles- with PPE (Gloves for mixer/loader and applicator)

A. PRODUCT DATA

6.	Name	Thiodan EC 35
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	2.625 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	1 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	3 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	150/day
5.	Hand contamination	1.5 ml/day
6.	Protective clothing	Gloves
7.	Transmission to skin	1%
8.	Dermal exposure to formulation	0.015 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	400 l spray/ha		
3.	Volume of surface contamination	10 ml/h		
		Hands	Trunk	Legs
4.	Distribution	65%	10%	25%
5.	Clothing	Gloves	Perm.	Perm.
6.	Penetration	1%	50%	15%
7.	Dermal exposure	0.065	0.5	0.375
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	5.64 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	0.015 ml/day	5.64 ml/day
2.	Concentration of as	350 mg/ml	2.625 mg/ml
3.	Dermal exposure to as	5.25 mg/day	14.81 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	1.05 mg/day	2.962 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.01 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	2.625 mg/ml
4.	Inhalational exposure to as	0.158 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.158 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	4.17 mg/day
2.	Operator body weight	60 kg
3.	Operator exposure	**0.07 mg/kg bw/day**

Estimates of operator exposure-German model

Scenario 2: Airblast spraying in high crops with tractor-mounted equipment

Maximum Application Rate (kg ai/ha): 1.05

Specific Exposure and Work Rate

Mixing and Loading (mg/person x kg ai)	Spray Application (mg/person x kg ai)	Work Rate (ha/day)
$I^*_M = 0.0006$ $D^*_{M(H)} = 2.4$	$I^*_A = 0.018$ $D^*_{A(C)} = 1.2$ $D^*_{A(H)} = 0.7$ $D^*_{A(B)} = 9.6$	8

Expected Inhalation Exposure:

$$I_M = I^*_M \times R \times A = 0.0006 \times 1.05 \times 8 = 0.00504 \text{ mg/person/day}$$
$$I_A = I^*_A \times R \times A = 0.018 \times 1.05 \times 8 = 0.1512 \text{ mg/person/day}$$

Expected Dermal Exposure:

$$D_{M(H)} = D^*_{M(H)} \times R \times A = 2.4 \times 1.05 \times 8 = 20.16 \text{ mg/person/day}$$
$$D_{A(H)} = D^*_{A(H)} \times R \times A = 0.7 \times 1.05 \times 8 = 5.88 \text{ mg/person/day}$$
$$D_{A(C)} = D^*_{A(C)} \times R \times A = 1.2 \times 1.05 \times 8 = 10.08 \text{ mg/person/day}$$
$$D_{A(B)} = D^*_{A(B)} \times R \times A = 9.6 \times 1.05 \times 8 = 80.64 \text{ mg/person/day}$$

Inhalation exposure = 0.00504 + 0.1512 mg ai/person = **0.15624 mg ai/person**

Total dermal exposure = 116.76 mg ai/person

Total dermal exposure based on dermal absorption in humans of 20% = 23.352 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 23.508 mg ai/person

Total systemic exposure for a 70 kg person **= 0.336 mg ai/kg/day**

With PPE (Gloves during mixing/loading/aplication 1%,; protection cloth during aplication 5%; during aplication 20% and hear protection during aplication 50%):

Inhalation exposure = **0.0313 mg ai/person** (20%)

Dermal exposure:

$D_{M(H)}$ = 0.2016 mg/person/day (1%)
$D_{A(H)}$ = 0.0588 mg/person/day (1%)
$D_{A(C)}$ = 5.04 mg/person/day (10%)
$D_{A(B)}$ = 4.032 mg/person/day (5%)

Total dermal exposure = 9.3324 mg ai/person

Total dermal exposure based on dermal absorption in humans of 20% = 1.866 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 1.898 mg ai/person

Total systemic exposure for a 70 kg person **= 0.027 mg ai/kg/day**

- **ESTIMATES OF OPERATOR EXPOSURE UK MODEL**

Tractor mounted (whitout cab) air assisted: application volume 1000l/ha- No PPE

A. PRODUCT DATA

7.	Name	Thidan EC 35
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	1.05 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	1 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	3 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	150/day
5.	Hand contamination	1.5 ml/day
6.	Protective clothing	NONE
7.	Transmission to skin	100%
8.	Dermal exposure to formulation	1.5 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	1000 l spray/ha		
3.	Volume of surface contamination	400 ml/h		
		Hands	Trunk	Legs
4.	Distribution	10%	65%	25%
5.	Clothing	None	Perm.	Perm.
6.	Penetration	100%	2%	5%
7.	Dermal exposure	10	5.2	5
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	121.2 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	1.5 ml/day	121.2 ml/day
2.	Concentration of as	350 mg/ml	1.05 mg/ml
3.	Dermal exposure to as	525 mg/day	127.26 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	105 mg/day	25.452 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.05 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	1.05 mg/ml
4.	Inhalational exposure to as	0.315 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.315 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	130.767 mg/day
2.	Operator body weight	60 kg
3.	**Operator exposure**	**2.179 mg/kg bw/day**

Tractor mounted (whitout cab) air assisted: application volume 1000l/ha- - with PPE (Gloves for mixer/loader and applicator)

A. PRODUCT DATA

8.	Name	Thiodan Ec 35
2a.	Active Ingredient	Endosulfan
2b.	Concentration	350 mg/ml
3.	Formulation type	EC
4a.	Main solvent	
4b.	Concentration of solvent	
5.	Maximum in-use as concentration	1.05 mg/ml

B. EXPOSURE DURING MIXING AND LOADING

1a.	Container size	1 litre
1b.	Hand contamination/operation	0.01 ml
2.	Application dose	3 litres product/ha
3.	Work rate	50 ha/day
4.	Number of operations	150/day
5.	Hand contamination	1.5 ml/day
6.	Protective clothing	Gloves
7.	Transmission to skin	1%
8.	Dermal exposure to formulation	0.015 ml/day

C. EXPOSURE DURING SPRAY APPLICATION

1.	Application technique			
2.	Application volume	1000 l spray/ha		
3.	Volume of surface contamination	400 ml/h		
		Hands	Trunk	Legs
4.	Distribution	10%	65%	25%
5.	Clothing	Gloves	Perm.	Perm.
6.	Penetration	1%	2%	5%
7.	Dermal exposure	0.4	5.2	5
8.	Duration of exposure		6 h	
9.	Total dermal exposure to spray	63.6 ml/day		

D. ABSORBED DOSE

		Mix/load	Application
1.	Dermal exposure	0.015 ml/day	63.6 ml/day
2.	Concentration of as	350 mg/ml	1.05 mg/ml
3.	Dermal exposure to as	5.25 mg/day	66.78 mg/day
4.	Percent absorbed	20%	20%
5.	Absorbed dose	1.05 mg/day	13.356 mg/day

E. INHALED EXPOSURE DURING SPRAY APPLICATION

1.	Inhalation exposure	0.05 ml/h
2.	Duration of exposure	6 h
3.	Concentration of as	1.05 mg/ml
4.	Inhalational exposure to as	0.315 mg/day
5.	Percent absorbed	100%
6.	Absorbed dose	0.315 mg/day

F. PREDICTED EXPOSURE

1.	Total absorbed dose	14.721 mg/day
2.	Operator body weight	60 kg
3.	**Operator exposure**	**0.245 mg/kg bw/day**

B.5.14.d Summary of predicted exposure

Predicted total systemic exposures from a representative sample of "worst-case" applications are summarised in Table 5.14d

Table 5.14d: Estimed operator exposure from a representative sample of use conditions

Crop	Product Name	Application equipment	Total systemic exposure (mg/kg bw/day)			
			UK POEM		GERMAN	
			No PPE	PPE	No PPE	PPE
Field	Endocel 35 EC	Hand held sprayers	0.731	0.289	---	---
		Tractor mounted boom	1.464	0.051	0.178	0.006
Field	Callistar	Tractor mounted boom	0.437	0.032	0.155	0.005
		Tractor mounted boom wiht rotary disc	0.447	0.032	0.155	0.005
Field	Thiodan EC 35	Tractor mounted boom	2.14	0.07	0.267	0.009
High	Thiodan EC 35	Airblast spraying whit tractor mounted equipment	2.179	0.245	0.336	0.027

The AOEL for endosulfan has been proposed by the raporteur at 0.004 mg/kg bw/day

		UK POEM		German model	
		Total systemic exposure (mg/kg bw/day)	% AOEL	Total systemic exposure (mg/kg bw/day)	% AOEL
Endocel 35 EC Field crop-Hand held sprayers	No PPE	0.731	18275	---	---
	PPE	0.289	7225	---	---
Endocel 35 EC Field crop-Tractor mounted boom	No PPE	1.464	36600	0.178	4450
	PPE	0.051	1275	0.006	150
Callistar Field crop-Tractor mounted boom	No PPE	0.437	10925	0.155	3875
	PPE	0.032	800	0.005	125
Callistar Field crop-Tractor mounted boom with rotary disc	No PPE	0.447	11175	0.155	3875
	PPE	0.032	800	0.005	125
Thiodan EC 35 Field crop-Tractor mounted boom	No PPE	2.14	53500	0.267	6675
	PPE	0.07	1750	0.009	225
Thiodan EC 35 High crop_Airblast spraying	No PPE	2.179	54475	0.336	8400
	PPE	0.245	6125	0.027	675

In conclusion, based on estimates by the German and the UK operator exposure models, all uses of Endosulfan result in exposured over than the AOEL proposed .

B.6.15 References relied on

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA/5.2.4	Bremmer 1997[a] Primary Dermal Irritation in the rabbit Doc. No. A58442	YES	NO	AgrEvo	YES
IIA/5.2.5	Bremmer 1997b Primary Eye Irritation in the rabbit Doc. No. A58443	YES	NO	AgrEvo	YES
IIA/5.2.6	Arcelin 1996 Contac Hypersensitivity in albino guinea pigs. Maximization Test. Doc. No. A58132	YES	NO	AgrEvo	YES
IIA/5.3.2.1/2	Barnard *et al* 1985 13 week Toxicity study in rats followed by 4-week withdrawal period. Doc. No. A30700	YES	NO	AgrEvo	YES
IIA/5.3.2.3/3	Brunk 1989 1-year feeding study to Beagle dogs Doc. No. A40441	YES	NO	AgrEvo	YES
IIA/5.3.3.1/1	Ebert 1985[a] Subchronic dermal Toxicity in Wistar rats Doc. No. A30750	YES	NO	AgrEvo	YES
IIA/5.4	Jung, Weigand and Kramer 1983 Mouse micronucleus test following oral administration. Report No. 83.0458	YES	NO	AgrEvo	NO
IIA/5.4	Völkner, W. 2000 Chromosome aberration assay in bone marrow cells of the rat with Endosulfan Report No. 644101	YES	NO	AgrEvo	NO
IIA/5.4	Sinha, N., Narayan, R., Shanker, R. and Saxena, D.K. 1995 Endosulfan-induced biochemical changes in the testis of rats. Published in Vet. Human Toxicol., 37: 547-549.		YES		NO

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA/5.4	Khan, P:K: and Sinha, S.P. 1996 Ameliorating effect of vitamin C on murine sperm toxicity induced by three pesticides (endosulfan, phosphamidon and mancozeb) Published in Mutagenesis, 11: 33-36		YES		NO
IIA/5.4	Sinha, N., Narayan, R. and Saxena, D.K. 1997 Effect of endosulfan on the testis of growing rats. Published in Bull. Environ. Contam. Toxicol., 58: 79-86		YES		NO
IIA/5.4	Rupa, D.S., Reddy P.P. and Reddi, O.S. 1989 Chromosomal aberrations in peripheral lymphocytes of cotton field workers exposed to pesticides Published in Environmental Research, 49: 1-6		YES		NO
IIA/5.4	Rupa, D.S., Reddy P.P. and Reddi, O.S 1991b Clastogenic effect of pesticides in peripheral lymphocytes of cotton-field workers Published in Mutation Research, 261: 177-180		YES		NO
IIA/5.4	Fransson, R. 1990 Toxicological evaluation of the insecticide endosulfan Report No. A67384	NO	NO	AgrEvo	NO
IIIA/7.1/A39426	Ebert, E. 1988 Endosulfan water dispersible powder (50%) subchronic dermal toxicity (21 treatments in 30 days) in the Wistar rat Report No. 87.0664	YES	NO	AgrEvo	YES
IIIA/7.1/A39279	Thevenaz, Ph., Luetkemeier, H.J., Chevalier, H.J., Vogel, W. & Terrier Ch. 1988 Endosulfan emulsifiable concentrate subchronic (4-week) repeated dose dermal toxicity study in rats Report No. 88.1735	YES	NO	AgrEvo	YES