ANNEX B

ENDOSULFAN

B - 6 : TOXICOLOGY AND METABOLISM

B.6 Toxicology and metabolism

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

The pharmacokinetic and metabolism of endosulfan were investigated in rats, mice, goats, sheep and cow. Information on pigs and rabbits were provided only as review of the original paper.

In the following, residues were measured as radioactivity in body liquids, tissues and excreta. The measurement values were recalculated into µg-equivalents of the parent substance per g or ml on basis of the specific radioactivity of the test substance. The resulting values are given in mg/g or mg/ml.

Rats:

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

^{14}C endosulfan (α-or β-isomers) were rapidly excreted by female rats following single oral administration of 2 mg/kg, or via dietary administration at doses of 5 ppm. After single oral administration, greater than 85% of the administered radiolabel was excreted within 120h (> 70% after 48h), mainly in the faeces and two and a lesser extent in the urine. After dietary administration for 14 days, followed by a 14 days recovery period, recovery of the radiolabel was > 72% of the administered dose . Biliary excretion of radiolabel male rats administered 1.2 mg/kg endosulfan as a single dose approached 50% of the α-isomer and 49% the β-isomer; the tissue residues were generally greatest in the kidneys and liver, with smaller confined to the kidneys and, to a lesser extent, the liver with a half life of about 7 days for the excreta and tissues were very polar, and no bioaccumulation in the fatty tissue was found (Dorough et al., 1978).

Male Sprague-Dawley rats (24/group) were dermally exposed to C labelled endosulfan at 0.10,0.76 and 10.13 mg/kg without washing. Four animals from each group were sacrificed at 0.5, 1, 2, 4, 10 or 24 h and radioactivity was determinate in the collected excreta and various organs and tissues. There was no

skin irritation at the application site. Absorption of the dose into the skin was rapid and substantial at all doses but movement through the skin was slow and the 0.10, 0.76 and 10.13 mg/kg groups recorded respectively 73%, 73% and 88.8% of the absorbed dose still bound to the skin at 24 h. At 10 h each dose group had excreted less than 1% of the applied dose. At 24 h the excretion was 11%, 10% and 4% of the applied dose for the 0.10, 0.76 and 10.13 mg/kg groups respectively. The percentage absorption of applied dose ranged from 21.5% at a dose of 0.10 mg/kg, to 8.4% at a dose of 10.13 mg/kg (Craine, 1986).

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

Mouse

Following oral single dose of 4 mg/kg excretion of the gavaged endosulfan started later than excretion of endosulfan administered in the less fatty diet. Apart from this delay the elimination pattern was the same. After one day 44% of the gavaged dose 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. Retention of the administered dose went down from 50% after one day (gavage), 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).

Following 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. 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)

When administered to male Balb/c mice at a dose level of 0.3 mg/mouse endosulfan and its two isomers was not completely absorbed from the gastrointestinal tract but was excreted, along with the metabolites endosulfan sulfate and diol in the faeces. Only the diol metabolite was excreted via the urine; the sulfate metabolite was the only form of endosulfan found in tissues, with relatively large amounts in liver, small in intestine, and visceral fat and trace amounts in muscle and kidney. When fed

to Balb/c mice at a dietary levels of 10 ppm forup to 49 day, the sulfate metabolite was detected in the liver and visceral fat of all animals. Both isomers and the sulfate and diol metabolites of endosulfan were detected in the faeces, while the only endosulfan product detected in the urine of these animals in this early study was the diol metabolite. Following a single dose of ^{14}C-radilabelled to Balb/c mice at dose levels of up to 0.3 mg/mouse, approximately 65% of the radiolabel was recovered; the faeces accounted for the highest concentrations, followed (in the rank order) by visceral fat >urine>small intestine>Kidney>brain>expired carbon dioxide >blood (Deema et al., 1996).

Sheep

Following a single dose of 0.3 mg/kg body weight of ^{14}C-endosulfan, urinary excretion amounted to 41% of applied ^{14}C-material, faecal excretion to 50 % and 1 % was found in the milk. The entire radioactivity in milk appeared to be endosulfan-sulphate, the only fat soluble metabolite of endosulfan. The highest radioactivity found in the milk was 0.15 µg/g of milk and was three times as high as the maximum concentration in blood. Maximal excretion in faeces occurred on day 2, practically all of it was unchanged endosulfan. Urine did not contain parent material. The two metabolites found there were endosulfan-diol and endosulfan-hydroxy-ether.

The organs and tissues of the sheep killed after 40 days revealed concentrations of 0.02 –0.03 µg endosulfan /g in fat, kidney and liver. All remaining tissues had considerably lower levels. Total radioactivity found in organs and tissue accounted for less than 1% of the administered label. (Gorbach et al., 1965).

Following oral multiple dose of 0.3 mg/kg for 26 days, 50% the administered dose was found in faeces., 10% - 20 % of the administered dose was excreted as unchanged endosulfan in the faeces, another 10% was excreted as endosulfan-diol in the urine. Another 30% were also excreted in urine as unidentified metabolite and endosulfan sulphate. The balance must have been excreted as other metabolites. No endosulfan or endosulfan-sulphate was found in milk (Gorbach, 1965)

Goat

Following oral administration with 1 mg/kg/day endosulfan for 28 days the tissues were analysed for presence of α- and β-endosulfan and endosulfan-sulphate. Total residues were detected in kidneys (0.29 mg/kg), gastrointestinal tract (0.20), liver (0.12) brain (0.06) muscle and spleen (0.04), lung and heart (0.01) and milk (0.02) on the first day after dosing. Within 15 days concentrations had dropped to below (0.01 mg/kg) except in the kidneys (0.02). Twenty one days after dosing endosulfan could not be detected any more (Indraningsih *et al*., 1993).

Cow

Following oral multiple dose of ^{14}C-endosulfan 0, 0.3, 3 or 30 ppm to lactating Holtein cows. for 30 days, residue levels at the end of the dosing period were proportional to dose in all tissues indicating absence of bioaccumulation. 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 (Keller, 1959).

When milk cows were fed a combination of endosulfan isomers (5 ppm) and endosulfan sulphate (5 ppm) in their diets, daily for 30 days, Endosulfan sulphate was the only residue detected in the milk in amounts ranging from 0.01 to 0.16 ppm. The sulphate was also detected in fat (0.89 ppm), liver (0.63 ppm) and kidney (0.07 ppm) tissue samples of cows killed immediately following treatment. (FMC Corporation 1965)

Hereford steers feeding a powder form of endosulfan (0.15, 1.10, 2.50 and 5.00 mg/kg b.w./day) was mixed carefully into the rations. 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. .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.. No effect was seen on silage consumption or milk production; residues in milk were not detectable. (Beck, 1966)

Pig

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

Rabbits

The toxicokinetic profile was studied in rabbits after a single dose of 2 mg/kg of endosulfan. After injection, its concentration in plasma declined rapidly. Higher percentages of the dose were excreted in the urine for α-endosulfan (37 %) than for β-endosulfan (11 %) during 0-5 days. With reference to total endosulfan, 29 % of the dose was eliminated unchanged in urine up to 5 days. Very small amounts of endosulfan (2.7 % of α-endosulfan and 0.4 % of the β-isomer) were excreted unchanged in the faeces. No attempt was made to identify the other metabolites **(**Gupta & Chandra, 1975)

Dermal absorption studies were carried out in vivo on rats and monkeys (Craine1986,1988; Lachmann, 1987) and one rat-human vitro study (Noctor and John, 1995). The dermal absorption suggest that initial absorption is dose related, movement through skin is low; endosulfan continues to be absorbed from skin reservoirs after skin washing and penetration as per cent of rate is lower in human skin than

rat skin. All of these studies made reference to active substance and theirs formulates, thus will be evaluated in B.6.12 paragraph.

B.6.1.1 Absorption, accumulation, distribuiton and excretion studies

B.6.1.1.1 Rat

B.6.1.1.1 1 Oral and intravenous study

Kellner, H. M. & Eckert (1983a) (AgrEvo: IIA, 5.1.1.1/2)
Date of experimental work: From November 1981 to September 1982.

The study was performed prior to GLP regulations.

The study is acceptable.

Material and methods
Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (Endosulfan), with 98% of purity.

Groups of male and female SPF-Wistar rats (Breeder Winkelmann, Borchen, groups of 6 males and 5-6 females each), weighing 180-200 g, were dosed with 2 mg/kg b.w. (orally by gavage in cooking oil) or 0.5 mg/kg b.w. (intravenously, in 1,2-propanediol). The animals were housed in metabolism cages with a device for separate collection of urine and faeces in air-conditioned rooms (22 ºC, 45-55 % humidity), feed (milled Altromin R from Altrogge, Lage/Lippe) and tap water *ad libitum.* 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.

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 6.1.1.1-1. 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 6.1.1.1.1-1: 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 ($t_{½}$) 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 %.

Kellner, H. M. & Eckert (1983b) (AgrEvo: IIA, 5.1.2.1/1)

Date of experimental work: From November 1981 to September 1982.

The study was performed prior to GLP regulations.

The study is acceptable.

Material and methods

See Kellner, H. M. & Eckert (1983a)

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (Endosulfan).

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 6.1.1.1.1-2. Kinetic data on excretion are summarised in Table 6.1.1.1.1-3. Absorption after oral administration of 2 mg/kg b.w. are summarised in Table 6.1.1.1.1-4.

Table 6.1.1.1.1-2: 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 6.1.1.1.1-3: 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 6.1.1.1.1-4: 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.

Excretion and Distribution study

Dorough HW, et al (1978) (AgrEvo: IIA, 5.1.1.1/1)

.Date of experimental work: Not provided in the report.

The study was performed prior to GLP regulations

The study is not acceptable partially. In continuos feeding study, for 14 days followed by untreated feeding for another 14 days, the applicant use one animal only in each time-point of sacrifice (1-, 3-, 7-, 10- and 14-day).

Material and methods

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (α- and β-Endosulfan isomers). The percentage of purity has been not including the report.

Male and female albino rats (Laboratory Supply Co., Indianapolis Ind.) weighing 400 g (males, bile collection study) or 200-250 g (females, single oral dose and 14 days feeding) were used in this experiment. In single oral study, the animals received 2 mg/kg b.w. by gavage (in corn oil). In continuous feeding study, the quantities of ^{14}C-endosulfan in 4 prepared feeds were 5 ppm for either α- or β-endosulfan (for 14 days followed by untreated feeding for another 14 days, 1 animal each sacrificed on day 1, 3, 7, 10 and 14 of both parts of the study, all 3 types of treatment with both isomers separately), 25 ppm of α-endosulfan and 25 ppm of a 7:3 mixture of the either α- or β-endosulfan isomers. For the bile-collection studies, the animals received 1.2 mg/kg b.w. as a single oral dose. 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.

Results

Elimination of ^{14}C-endosulfan as a single oral dose or as a dietary supplement are summarised in Table 6.1.1.1.2-1. Residues in Tissues or female rats fed 5 ppm of α- or β-radiolabel isomers in diet are summarised in Table 6.1.1.1.2-2. Extraction characteristics of residues in excreta and tissues are summarised in Table 6.1.1.1.2-3. Analysis of apolar endosulfan ^{14}C equivalents are summarised in Table 6.1.1.1.2-4. 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 6.1.1.1.2-1: 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 6.1.1.1.2-2: 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 6.1.1.1.2-3: 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 6.1.1.1.2-4: 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.

B.6.1.1.2 Mouse

B.6.1 1.2.1 Oral Single-Multiple Dose

Christ, O. E. & Kellner, H. M. (1968) (AgrEvo: IIA, 5.1.1/2)

Date of experimental work: Start not provided in the report; end: December 31st 1968.

The study was designated to investigate the excretion and distribution of ^{14}C endosulfan in mice. The study was performed prior to GLP regulations.

The study is acceptable

Material and methods

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (Endosulfan). The percentage of purity has been not included in the report..Albino mice, weighing approximately 20 g, were 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 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.

Results

In the experiment a), cumulative elimination of radioactivity are summarised in Table 6.1.1.2.1-1. 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 6.1.1.2.1-2. 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 6.1.1.2.1-1: 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 6.1.1.2.1-2: 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.

B.6.1.1.2.2 Metabolism and excretion

Deema *et al.*, 1996 (AgrEvo: ANRA)

Journal of Economic Entomology, 59, 546-550.

The study is considered as additional information because is only a review of the published paper.

Material and Methods

1) Single dose study

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

Endosulfan and its two isomers was not completely absorbed from the gastrointestinal tract but was excreted, along with the metabolites endosulfan sulfate and diol in the faeces. Only the diol metabolite was excreted via the urine; the sulfate metabolite was the only form of endosulfan found in tissues, with relatively large amounts in liver, small in intestine, and visceral fat and trace amounts in muscle and kidney

2.- Repeat dose study

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

The sulphate metabolite was detected in the liver and visceral fat of all animals. Both isomers and the sulphate and diol metabolites of endosulfan were detected in the faeces, while the only endosulfan product detected in the urine of these animals in this early study was the diol metabolite

c) ^{14}C-radiolabelled distribution study

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

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

After a single dose of up to 0.3 mg C-endosulfan to Balb/c mice, about 65% of the radiolabel was recovered; the faeces accounted for the highest concentrations, followed (in the rank order) by visceral fat >urine>small intestine>Kidney>brain>expired carbon dioxide >blood).

B.6.1.1.3 Sheep

B.6.1.1.3.1 Single oral dose study

Gorbach, S. G. (1965) (AgrEvo: IIA, 5.1.2.4/1)

Date of experimental work: From June 20th 1964 to July 16th 1964.

The study was performed prior to GLP regulations.

The study is acceptable.

Material and methods

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (α- and β-Endosulfan isomers). The purity of the test substance has not been included in the report.

Milk sheep, (*Ovis aries*, Merino sheep, 3 animals per group) of 2.5 years old and weighing 40-66.5 kg were dosed with 0 and 15 mg/day (fed by means of gelatine capsules) were dosing during a period of 20 days (1 animal each group) or 26 days (other animals). Milk, tissue fat, faeces, urine and animal tissues were collected, and the samples were analysed by chromatography.

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.

Gorbach, S. G. , et al (1968) (AgrEvo: IIA, 5.1.1.3/1)

Date of experimental work: Not provided in the report.

The study was performed prior to GLP regulations.

The study is not acceptable. The number of animals used does not permit obtaining significant results.

Material and methods

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (α- and β-Endosulfan isomers).

3 East Friesian milk sheep (2 + 1 control) of 1.5-3 years old and weighing 49-52 kg b.w. were dosed with 0.3 and 0.26 mg/kg b.w. in fed (substance dissolved in acetone). 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.

Results

In blood the highest activity was measured 24 hours after application (Table 6.1.1.3.1-1). 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 6.1.1.3.1-2). 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 6.1.1.3.1-3.). 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 6.1.13.1-1: 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 6.1.1.3.1-2: 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 6.1.1.3.1-3: 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.

B.6.1.1.4 Goat

B.6.1.14.1 Residue study in lactating goat

Indranignsih, et al (1993) (AgrEvo: IIA, 5.1.2.5/1)

Date of experimental work: Not provided in the report.

The study was performed prior to GLP regulations.

The study is acceptable

Material and methods

Test substance: 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (Endosulfan). The purity of endosulfan has not been included in the report. 12 lactating feral goats (25-40 kg), each with one kid (3 animals and their respective kids per group) were placed in individual metabolism cages and fed *ad libitum* a diet of summer (*Stylosanthes hamata cv* 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.

Results

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 6.1.1.4.1-1.

Table 6.1.1.4.1-1: 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.6.1.1.5 Caw

B.6.1.1.5.1 Residue study in lactating cows

Keller, J (1959) (AgrEvo: IIA, 5.1.2.6/2)

Date of experimental work: Not provided in the report.

The study was performed prior to GLP regulations.

The study is considered acceptable

Material and methods

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (^{14}C-Thiodan). The purity of the test substance has not been included in the report.

12 mature, lactating Holstein cows (4 groups, 3 cows each) were dosed with 0.3, 3 and 30 ppm during a feeding period of 30 days. One animal of each group was held on a ^{14}C-Thiodan free diet during a recovery period (duration of the recovery period: 14 days). 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 analysed by LSC.

Results

Food consumption and milk production for each animal were within 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.

Beck, *et al* (1966) (ANRA)

J. Econ, Entomol., vol 59, nº 6: 1444-1550.

The study was performed prior to GLP regulations.

The study is considered as additional information because in a review of the original paper.

Material and methods

Test substance: 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (Endosulfan). The purity of the test substance is not provided.

Hereford steers, weighing 600-800 lb were fed individually, twice daily, with amounts of fattening ration equal to 1.0 % of their b.w. This quantity was consumed completely at each feeding. A powder form of endosulfan (0.15, 1.10, 2.50 and 5.00 mg/kg b.w./day) was mixed carefully into the rations. 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.

Results

Feeding study in cattle are summarised in Table 6.1.1.5.1-1. 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 6.1.1.5.1-1: Feeding study in cattle.

dose/effect (mg/kg/day)	0.15	1.10	2.50	5.00
Toxicity[1]				
Muscle convulsions			+[5]	+[6]
Salivation			+	+
Incoordination			+	+
Residues in omental fat (ppm)				
- Day 0	0	0	0	0
- 30 days (metabolic study)		0.5[3]		
- 60 days	0	1.0[4]	-	-
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.

FMC Corporation (1965) (AgrEvo: ANRA)

Date of report: 24 September 1965.

This report is only a review, thus is only considered as additional information.

Milk cows were fed a combination of endosulfan isomers (5 ppm) and endosulfan sulphate (5 ppm) in their diets ,daily for 30 days. Two cows were killed at the end of the treatment period. A further 2 animals were maintained on control diet for an additional 30 days prior to being killed. Milk samples were collected at various time intervals throughout the study.

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

Gupta, P. K. & Gupta, R. C. (1979) (Excel, 5/02)

Toxicology , 13 115-130.

The information provided is only a review of the original paper. is a review, thus is only considered as additional information.

When cows were fed 2.5-5 ppm of endosulfan for 30 days, 0.1-0.2 ppm of endosulfan sulphate was excreted in milk (FMC, 1965). Beck *et al*. (1966) using smaller doses and shorter treatment periods, could not detected any residues of the insecticide in the milk of cows which had been fed for 21 consecutive days of silage containing 0.41, 0.7 and 2.35 ppm of endosulfan.

B.6.1.1.6 Pig

Maier-Bode, 1966 (Excel, 5/01)
Residue Review, vol 22, item III

The information provided is only a review of the original paper. is a review, thus is only considered as additional information

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

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

B.6.1.1.7 Rabbit

Gupta & Chandra 1975. (Excel, 5.1.2/03)
Bull. Environm. Cont & Toxicol. Vol 14, nº 5.

The information provided is only a review of the original paper. is a review, thus is only considered as additional information.

The toxicokinetic profile was studied in rabbits after a single dose of 2 mg/kg of endosulfan. After injection, its concentration in plasma declined rapidly. Higher percentages of the dose were excreted in the urine for α-endosulfan (37 %) than for β-endosulfan (11 %) during 0-5 days. With reference to total endosulfan, 29 % of the dose was eliminated unchanged in urine up to 5 days. Very small amounts of endosulfan (2.7 % of α-endosulfan and 0.4 % of the β-isomer) were excreted unchanged in the faeces.

No attempt was made to identify the other metabolites

B.6.1.2 Dermal absorption studies

B.6.1.2.1 Rats

Craine, E. M. (1986) (AgrEvo: IIA, 5.1.3.1.1)

Date of experimental work: From April 1986 to July 1986.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (Endosulfan), with 94.6% of purity.

104 male rats (Charles River Breeding Lab., Portage, MI, divided in 3 groups) with 6-7 weeks old and weighing 203-288 g, received 0.1, 1.0 or 10 mg/kg b.w. to groups of 24 animals, applied to the shaved intact dorsal skin and covered until sacrifice. The animals were individually housed in metabolism cages in air-conditioned rooms (72 ± 3 ºF, 40 % or more of relative humidity, 12-h light 12-h dark). Fed (Purina Ceritified Rodento Chow #5002) and tap water *ad libitum*. 4 animals were sacrificed after 0.5, 1, 2, 4, 10 and 24 h; blood, urine from the bladder, skin of the application site, remaining skin and carcass were separated. Liver, kidney and fat tissues sampled, and urine and faeces excreted between treatment and sacrifice collected. Observation of the treated skin area after sacrifice for symptoms of irritation were carried out, and application sites were examined for erythema, oedema and other findings prior to application of dose and at sacrifice. All analysis of ^{14}C involved measurement by LSC.

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.

Craine, E. M. (1988) (AgrEvo: IIA,5.1.3.1.1)

Date of experimental work: From June 16th 1986 to January 1987.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (Endosulfan), with 94.6% of purity.

3 groups of 16 animals each (Charles River rats) with 7-10 weeks old and weighing 211-262 g. Housing, treatment, samples and analytical method are the same of Craine, E. M. (1986), but the animals were sacrificed at 24-, 48-, 72- and 168-h.

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 6.1.2.1-1. Two third of the elimination was via faeces, one third via urine.

Table 6.1.2.1-1: 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.6.1.2.2 Primates

Lachmann, G. & Siegemund (1987) (AgrEvo: IIA, 5.1.3.1.2/1)

Date of experimental work: From February 1987 to May 8th 1987.

The study was performed in accordance with GLP, and the applicant include QA Statement.

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (^{14}C-Thiodan), with at least 98% of purity.

The study is not acceptable. The results cannot be statistically significant because the applicant use two animals only.

Material and methods

2 male Rhesus monkeys (Shamrock Farms, Victoria House, Small Dole, Henfield, Great Britain) weighing 4.5-5 kg, receiving 2.2 and 3 mg/kg b.w. respectively, applied to the shaved intact skin of neck and shoulder. After acclimatisation period (2 weeks), the animals were housed in metabolic cages (22±2 ºC, 55±10 % humidity, 12-15 air changes/h, 12-h light 12-h dark. Fed (Altromin diet 6014 plus 2 bananas, 1 orange and 1 apple per day) 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.

Results

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 6.1.2.2-1.

Table 6.1.2.2-1: 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**

	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 interval									
		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.

B.6.1.2.3 *In vitro* rat: human

Noctor, J. C. & John, S. A. (1995)

Date of experimental work: From June 2nd 1993 to May 8th 1995.

The study was performed in accordance with Hazelton Europe Standard Operating Procedures and the GLP principles. The applicant include QA Statement.

Test substance: ^{14}C-labelled and unlabelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (^{14}C-endosulfan and endosulfan, α- and β-isomers).

The study is acceptable.

Material and methods

Skin preparations of rat and human were used in these assays. The test substance was applied to the epidermal surface of each skin preparation by syringe as indicated Table 6.1.2.3-1.

Table 6.1.2.3-1

dose group	species	nominal mg/cm^2	dose level Kbq/prep*	n° skin preparations
A	rat	1.0	74	12[a]
B	rat	0.1	74	8
C	rat	0.01	74	8
D	human	1.0	74	12[a]
E	human	0.1	74	8
F	human	0.01	74	8

*: Surface area of skin = 2.545 cm^2. [a]: 4 preparations washed at 10 h post-application to remove surface test substance.

The integrity of the epidermal barrier of each skin preparation was assessed prior to application of ^{14}C-endosulfan. Samples were analysed by LSC and metabolite profiling was examined for the presence of metabolite/degradation products using HPLC system.

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 6.1.2.3-2. Mean recovery of radioactivity 72 h after a single application are summarised in Table 6.1.2.3-3.

Table 6.1.2.3-2: 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 6.1.2.3-3: 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.

B.6.1.3 Mammalian metabolism

Summary

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)

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/ B.6.8.1.1). A similar outcome with experiments on rats has been published by Dorough *et al.* (1978/B.6.1.1.2).

In a metabolism experiment Schuphan *et. al.* (1968) 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 hydroxyl-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).

Schuphan *et al* G (1968) (AgrEvo: IIA, 5.1.3.2/1)

Z. Naturforsch vo. 23 b, nº 5: 701-706.

The study was performed prior to GLP regulations.

The study was performed to know the metabolism mechanism in rats and mice

The study is not acceptable. The paper has not sufficient data on number and origin of the animals used, acclimatisation period, housing, collected system of samples, data analysis, etc.

Material and methods

Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3 benzodioxathiepin-3-oxid (Endosulfan). The percentage of purity has been not included in the report.

A number undetermined of Sprague-Dawley rats (240 to 280 g and 110 to 140 g) and mice (no information on mice) were treated according Table 6.1.3-1.

Table 6.1.3-1

SUBSTANCE	ROUTE	DOSE (mg/kg b.w.)
α-endosulfan (αE)	oral intraperitoneal duodenum-fistula	4; 8 4; 8 4
β-endosulfan (βE)	oral intraperitoneal duodenum-fistula	4; 8; 16 4; 8 4
endosulfan sulphate (ES)	oral intraperitoneal	4 4
endosulfan diol (ED)	oral intraperitoneal	0.8; 4 0.8; 4
endosulfan ether (EE)	oral intraperitoneal	4 0.8; 4
endosulfan hydroxyl ether (HE)	oral intraperitoneal	4; 8 4
endosulfan lactone (EL)	oral intraperitoneal	4 4

Mice received labelled αE and βE (4 mg/kg) in oily solution by intraperitoneal injection. The animals were kept in cages that permitted separate collection of faeces and urine. Samples of urine and faeces from all animals were collected, bile only from those dosed by via duodenum-fistula.

Results

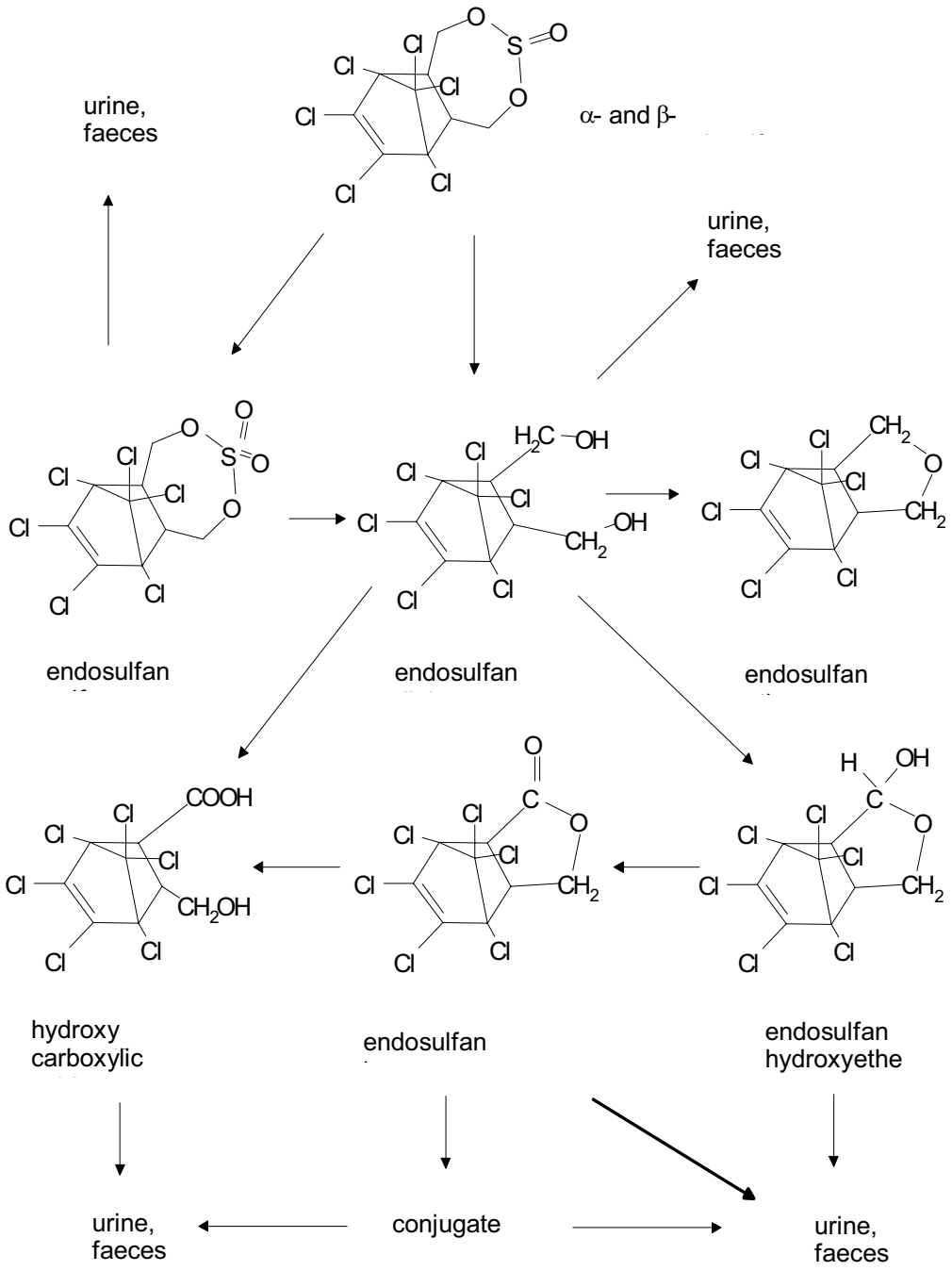
After application of αE and βE, mainly the applied isomers but also ES and HE and especially EL were detected in faeces. After application of any of the metabolites, the dominant excretion products in faeces were the applied molecule, HE and EL. In urine the amount of isomers was rather low after their 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 metabolisation 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.

B.6.1.4 Metabolic pathway of endosulfan in mammalians

Most endosulfan metabolites have been identified (Figure 61.4-1). 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.



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

Summary

Endosulfan has been tested for acute toxicity, primary irritation and sensitisation potential. Three notifier have submitted studies. Besides, AgrEvo has included review document of endosulfan prepared by the Australian National Registration Authority (ANRA) for Agricultural and Veterinary Chemicals, which includes studies previously presented and studies which have not been presented by any applicant. However, additional information to cover acute toxicity has been found from IPCS (1998). Nevertheless, these studies are only a little summaries of the original papers, thus they have been considered only as additional information.

Purity, when reported, range between 96 and 97.3% among all the studies. The followed procedures were in accordance or without significant deviation from USEPA and OECD Guidelines. Not all the studies were performed to GLP.

The LD_{50} of endosulfan varies widely depending on the route of administration, species, vehicle, and sex of the animal. The male rats are clearly more sensitive than male rats, and, on the basis of a single study this sex differences appears apply to mice also (Bremmer & Leist, 1998). The lowest oral LD_{50} value is 9.6 mg/kgbw in female Sprague Dawley rats (Reno, 1975), however, this study is considered as not acceptable due the paucity of information provided.

The acute oral median lethal dose LD_{50} of Endosulfan Technical in rats was calculated to have a range between 48 and 160 mg/kg for male and 10 and 22.7 mg/kg for female rats. These results would require an EEC classification of “T+” (very toxic) for the technical active ingredient, if based on the more sensitive sex alone.

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

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).

Skin and eye irritation studies submitted were considered not acceptable because purity of the technical product was not reported and exposition period after instillation into the eyes was very short. On the basis of the only percutaneous study considered as acceptable (Diehl & Leist,, 1988) plus the additional information (ANRA and IPCS documents reviews) about skin and eye irritation endosulfan could be considered as not irritating to skin and eyes. However, original studies should be provided.

Based on the skin sensitisation studies (Buehler test), there is no evidence that Endosulfan is a contact allergen and it is not classified based on EU criteria. Besides, a summary about skin sensitisation found in IPCS document showed that endosulfan was not sensitising for guinea-pig skin (Arcelin, 1996). However, original studies should be provided.

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 "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.

The results obtained in the studies considered acceptable are summarised in Table 6.2-1.

Table 6.2-1: Summary of acute acceptable 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 198b 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 **1**988a 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 sensitisation Guinea pig, SPF Pirbright-White f	-	Polyethylene glycol 40%	No Sensitizer	Jung 1983 AgrEvo IIA, 5.2.6/1

B.6.2.1 Oral studies

B.6.2.1.1 Rat

Elsea, J.R., 1957 (AgrEvo: IIA, 5.2.1/1)

Report Date: 11 Jan 1957.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is not acceptable. Purity not reported. It was assumed to be 100%.

Materials and methods

Male albino rats, five per dose, were tested. The average weight was 140g. The test substance, Thiodan Technical (purity of the test material was considered to be 100%) was administered orally, by stomach tube, either a 0.1, 1.0 or a 10.0 weight/volume solution of Thiodan in cotton seed oil. The dosage level tested were 10.0, 21.5, 40.0, 100, 215, and 404 mg/kg of bw. Husbandry: groups of five in metal cages with free access to feed and water. Observation for 7 days. Gross autopsies: upon animals that died during the study and those sacrificed after the observation period.

Results

There were no clear symptoms of intoxication at the two lowest doses. At 40.4 mg/kg bw and above the rats exhibited the following symptoms of acute oral intoxication by endosulfan: preening, salivation excessive masticator movements, lacrimation, exophthalmia, and rapid and laboured respiration; in addition prior to death: bloody nasal discharges, ataxia, sprawling, tremors, and pain reflexes. Death was immediately preceded by phonation, tonic and clonic convulsions gasping and coma. Survivor seemed to fully recover within 48 hours. Autopsy of succumbed animals revealed hyperaemic or haemorrhage lungs, irritation of the small intestine, and congested kidneys and adrenals. Surviving animals did not exhibit any gross pathological symptoms upon autopsy.

Conclusions

The study yielded an acute oral LD_{50} of 110 mg/kg (55.0-220) for endosulfan in albino male rats.

Lindquist, D.A.and Dahm, P.A.; 1957 (AgrEvo: IIA, 5.2.1/2)

Report Date: 1957.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is not acceptable. The publication provides little information only. There is no possibility to verify the validity of the results.

Materials and methods

White male Sprague-Dawley rats (3 -8 per group), were tested. Age/Weight: about two months old, 250 to 325 g. The test substance, Endosulfan (Thiodan) Technical, purity unclear, was administered in Corn oil. The dosage level tested were 40, 50, 60, and 70 mg/kg bw. The rats were lightly anaesthetised with diethyl ether to facilitate dosing.

Results

Dose (mg/kg bw)	No of Animals Treated	No of Animals Dead
40	4	0
50	8	5
60	4	4
70	3	3

Death occurred within 24 hours and was preceded by violent convulsions.

Conclusions

The acute oral LD_{50} for endosulfan in male rats would be between 40 and 50 mg/kg bw.

Elsea, J.R., 1958 (AgrEvo: IIA, 5.2.1/3)

Report Date: 28 Feb 1958.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is not acceptable. Purity not reported. It was assumed to be 100%.

Materials and methods

Male Holtzman albino rats, five per dose, were tested. The weight range between 173 to 209g. The test substance, Thiodan Technical (purity of the test material was considered to be 100%), Lot. No. MR6914, was administered orally, by stomach tube, a 1.0% weight/volume solution or a 10.0% weight/volume suspension in corn oil. The dosage level tested were 31.6, 46.4, 68.1, 100, 147 and 215 mg/kg bw. Husbandry: groups in metal cages with free access to feed and water. Observation for 7 days. Gross autopsies: upon animals that died during the study and those sacrificed after the observation period.

Results

Dose (mg/kg bw)	No of Animals Treated	No of Animals Dead
31.6	5	0
46.4	5	1
68.1	5	2
100	5	2
147	5	5
215	5	5

Death occurred within 24 hours.

The following symptoms of intoxication were observed: depressed appearance, lacrimation, salivation exophthalmia, laboured respiration, ataxia, placement, tremors, and convulsions.

Autopsies of animals that died revealed hyperaemic or haemorrhage lungs, irritation of stomach and small intestine, congested kidneys and adrenals. Some of the surviving animals exhibited consolidated areas of the lungs and slightly congested adrenals.

Conclusions

The acute oral LD_{50} of Thiodan (Endosulfan Technical) for male albino rats is 86.6 mg/kg of bw, with confidence limits from 61.6 to 122 mg/Kg.

Kretchmar, B.; Mastri, C. and Keplinger, M.L.; 1971 (AgrEvo: IIA, 5.2.1/6)

Report Date: 13 Dec 1971.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is not acceptable, because the purity is not reported.

Materials and methods

Two acute oral toxicity studies were conducted with two samples identified as Endosulfan Technical (German produced, MRL 815) and Thiodan Technical (Endosulfan code 1318, lot 2-18-G). Purity of the two test material is not reported. The acute oral median lethal dose was determined with each sample in Charles River strain rats. The acute oral LD_{50} of Thiodan Technical (Endosulfan) was then determined in the Sprague Dawley, Holtzman, and Wistar strains. The same procedure was followed with each sample and with each strain of rat. Young male albino rats, 10 per dose, were used. The test material was administered as a 1.0 % (w/v) solution in corn oil. All doses (35.12, 52.67, 79.01, and 118.5 mg/kg) were administered directly into the stomachs of the rats using a hypodermic syringe equipped with a ball-tipped intubating needle. After administration, the rats were housed individually in suspended, wire-mesh cages and observed for the following 14 days. Initial and final body weights, mortalities, and reactions were recorded. A necropsy was conducted on any animal which died during the study and on all animals sacrificed at the end of the 14-day observation period.

Results

Acute Oral LD_{50} (mg/kg)

Test Material	Charles River	Sprague-Dawley	Wistar	Holtzman
MRL 815	66.5	n.d.	n.d.	n.d.
1318; lot 2-18-G	59.0	62.0	94.0	125.0

n.d. = not determined

Death occurred within 24 hours.

Symptoms: Reduced activity, ruffed fur, tremors, diarrhoea, muscular weakness

Necropsy did not reveal any gross pathological findings.

Conclusions

The acute oral LD_{50} values in rats range from 59.0 to 125.0 mg/kg bw, depending on animal strain and origin of Endosulfan.

Scholz and Weigand.;1971a (AgrEvo: IIA, 5.2.1/7)

Report Date: 06 Jul 1971.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is acceptable.

Materials and methods

The acute oral LD_{50} of Thiodan Technical (Endosulfan) was determined in male Sherman rats (175-208 g bodyweight). The active ingredient (Endosulfan; purity 97.2-97.3 % reported by the applicant, but missing in the document) was administered once by gavage in ground-nut oil at various dose levels (20, 32, 50 and 80 mg/kg). The concentrations were always chosen in a way that the animals received 0.5 ml of the solution per 100g bodyweight. Each dosage was tested with 10 rats. The follow-up period after dosing was 14 days. During the test - and follow - up period the rats received LABENA, diet produced by Kraftfutterwerke GmbH Brand- Purina, Krefeld. Food and normal tap water to drink were always provided *ad libitum*.

Results

Dose (mg/kg bw)	No of Animals Treated	No of Animals Dead
20	10	0
32	10	2
50	10	4
80	10	10

Death occurred within 24 hours. Symptoms: desequilibrium, muscular tremors, spasms. Macroscopic findings: Reddening of small intestine.

Conclusions

An acute oral LD_{50} of 48 mg/kg bw was established for male-rats with endosulfan technical.

Scholz and Weigand.; 1971b (AgrEvo: IIA, 5.2.1/8)

Report Date: 06 Jul 1971.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is acceptable.

Materials and methods

The acute oral LD_{50} of Thiodan Technical (Endosulfan) was determined in female Sherman rats (175-200 g bodyweight). The active ingredient (Endosulfan; purity 97.2-97.3 % reported by the applicant, but missing in the document) was administered once by gavage in ground-nut oil at various dose levels (6.3, 8.0, 10.0 and 12.5 mg/kg). The concentrations were always chosen in a way that the animals received 0.5 ml of the solution per 100g bodyweight. Each dosage was tested with 10 rats. The follow-up period after dosing was 14 days. During the test - and follow - up period the rats received LABENA, diet produced by Kraftfutterwerke GmbH Brand- Purina, Krefeld. Food and normal tap water to drink were always provided *ad libitum*.

Results

Dose (mg/kg bw)	No of Animals Treated	No of Animals Dead
6.3	10	0
8.0	10	1
10.0	10	5
12.5	10	10

Death occurred within 24 hours. Symptoms: desequilibrium, muscular tremors, spasms.

Macroscopic findings: reddening of small intestine.

Conclusions

An acute oral LD_{50} of 10 mg/kg bw was established for female-rats with endosulfan technical.

Reno, F E.; 1975c (AgrEvo: IIA, 5.2.1/9)

Report Date: 18 Dec 1975.

The study was conducted according to the Fed. Hazard. Subst. Act, 16 CFR, Part 1500.3. USA.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is not acceptable. Assessment of study results is not possible due to paucity of information provided. Results should be dismissed especially because origin and quality of test material cannot be verified.

Materials and methods

The acute oral LD_{50} of Endosulfan Technical (No purity reported) was determined in male and female Sprague-Dawley albino rats (5 per group). The initial bodyweights range for males from 160 - 200 g and for females from 210 - 241 g. The active ingredient was administered once by oral intubation in Sodium carboxylmethyl cellulose 0.5% suspension to six groups of male rats at graded dosage levels of 15.9, 25.1, 39.8, 63.1, 100.0, 1000.0 mg/kg bw and to the eight groups of female rats at graded dosage levels of 3.98, 6.31, 10.0, 15.9, 25.1, 39.8, 63.1, 100.0 mg/kg bw . No information about husbandry is given. The observation period lasted for 14 days after administration. Autopsy was carried out on animals that died during the study and the remaining after termination.

Results

Dose (mg/kg bw)	No of Males Dead/Treated	No of Females Dead/Treated
3.98	-	0/5
6.31	-	0/5
10.00	-	3/5
15.90	0/5	5/5
25.10	0/5	5/5
39.80	4/5	5/5
63.10	3/5	5/5
100.00	5/5	5/5
1000.00	5/5	-

The animals died within 24 hours. Observations: Listlessness, tremors, increased respiration, prostration. Autopsy revealed reddened intestine for all animals found dead during the study, animals killed at the end showed no gross pathological symptoms.

Conclusions

An acute oral LD_{50} of 40.38 mg/kg bw for male and of 9.58 mg/kg bw for female rats were established with endosulfan technical.

Diehl, K.-H.; Leist, K.-H.; 1988b (AgrEvo: IIA, 5.2.1/10)

Study date: Start: 20 May 1988; End: 29 Jul 1988.

The study was conducted according to the OECD, 401, Update 24 Feb 1987 and EPA Subdiv. F, § 81-1, EPA 540/9-82-025, revised Nov 1984.

GLP Compliance: Yes.

The study is acceptable.

Materials and methods

Male and female Wistar rats, five per sex and dose, were tested. The weight range between 164 to 220 grams (7 to 8 weeks). The test substance, Endosulfan; substance, technical; 96.0 % Code: Hoe 002671 00 ZD96 0002, was administered orally, by stomach tube, a 2 % starch mucilage. The dosage level tested were males: 50, 100, 160, 250, 315 mg/kg bw, females: 12.5, 25, 50 mg/kg bw, by gavage. Husbandry in fully air-conditioned rooms in Makrolon cages on soft wood granulate in groups of 5 animals with free access to feed and water. Withdrawal of food from 16 hours before to 3-4 hours after treatment. Observation for 14 days. Gross autopsies: upon animals that died during the study and those sacrificed, by carbon dioxide asphyxiation after the observation period.

Results

Dose (mg/kg bw)	No of Males Dead/Treated	No of Females Dead/Treated
12.5	-	1/5
25.0	-	2/5
50.0	0/5	5/5
100.0	0/5	-
160.0	5/5	-
250.0	3/5	-
315.0	5/5	-

All deaths occurred within 24 hours.

Clinical signs: Squatting position, high-legged gait, straddling of legs, contracted flanks, prone or lateral position, crawling locomotion, reduced spontaneous activity, spasms, piloerection, myxdriasis, clear, bloody and foamy salivation, increased respiratory rate, and irregular breathing. Symptoms persisted up to the 4th day after treatment at the latest. Autopsy: macroscopic findings of animals found dead during the study: Stomach inflated and filled with light brown mass, small intestine filled with reddish fluid, kidneys with dark patches, liver in light colour. Animals of the 12.5 mg/kg group killed at the end of the observation period exhibited redness of uterus and ovaries.

Conclusions

For endosulfan an acute oral LD_{50} of 100 - 160 mg/kg bw in male and 22.7 mg/kg in female rats was determined.

This result would require an EEC classification of "T+" (very toxic) for the technical active ingredient, if based on the more sensitive sex alone.

Bremmer & Leist, 1998 (IPCS 1998)

There is a little summary about the evaluation of acute oral and dermal toxicity found from IPCS document, thus is considered as only as additional information.

The male rats are clearly more sensitive than male rats, and, on the basis of a single study this sex differences appears apply to mice also.

B.6.2.1.2 Mouse

Wyman Dorough, H., Huhtanen, K., Marshall, T. C., and Bryant, H. E., 1978 (Calliope: 5.2.1/01)
Date of publication: 1978.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is not acceptable. The product utilised in the study is not the Technical ones.

Materials and methods
Female albino mice, source not specified, weighing between 27-30 g, received a and b Endosulfan (sp. act. 0.98 mCi/mmol) by oral administration in a one to one mixture of Tween 80 and water in order to stablish lethal dose.

Results
Approximate lethal dose of α and β Endosulfan

Compound	Lethal dose [mg/kg bw]
α-Endosulfan	11
β-Endosulfan	36

Lethal dose only slightly greater than non-lethal ones; unless death occurred, almost no signs of poisoning were seen; lethal dose caused convulsions and death within 1 h.

Conclusion
The acute oral LD_{50} in female mouse was calculated to be 11 mg/kgbw for α-Endosulfan and 36 mg/kgbw for β-Endosulfan.

B.6.2.1.3 Dog

Keller, J. G.; 1958 (AgrEvo: IIA, 5.2.1/4)
Date of report: 20 Feb 1958.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is not acceptable. The purity is not reported and the number of animals is limited.

Materials and methods
Groups of one male and one female mongrel dog each received Thiodan Technical (purity not reported) by gelatine capsule in a 10% mixture with lactose, at dosage levels of 3, 10, 30 and 100 mg/kg body weight. Age/weight of the animals was not reported. The acute oral LD_{50} could not be determined due to the excessive vomiting noted and the limited number of animals used in the study. Gross autopsies were performed on animals that died during the course of the experiment. At the end of a observation period of 7 days the surviving animals were sacrificed and gross autopsies performed.

Results
Up to 10 mg/kg bw were tolerated by both sexes without symptoms. Excessive vomiting occurred in female dogs at 30 mg/kg bw and more. Male dogs at these higher doses died within hours.

Clinical signs: Symptoms of intoxication prior to death: salivation, convulsions, rasping.

Necropsy: Autopsy of animals dying during the study revealed congestion of lung, liver and kidney as well as irritation of the cardiac portion of the stomach. Animals killed at the end of the study did not show any gross symptoms.

Conclusions
An acute oral LD_{50} for endosulfan technical in dogs could not be established due to excessive vomiting at higher doses.

Author not available; Date not available. (AgrEvo: IIA, 5.2.1/5)
Date of Translation: 16 Mar 1970. Name of translator Nogami, K.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is not acceptable. The purity is not reported.

Materials and methods

Endosulfan Technical (Malix), purity not reported, was administered once orally by gelatine capsules in the doses of 34.0, 39.5, 50.0, 65.0, 84.5, 109.8, 142.7 and 185.5 mg/kg bw to groups of female and male dogs (mixed blood), consisting of 5 dogs per group. The animals were 2-4 yrs. old and weighted between 7.5 - 11.0 kg. During the testing period room temperature range from 19ºC to 25ºC. Animals had free access to food (solid type manufactured by Oriental Co.) and water. Observation period lasted for 7 days after administration. Autopsy was done in animals dying during the experiment.

Results

Dose (mg/kg bw)	No of Animals Treated	No of Animals Dead
34.0	5	0
39.5	5	0
50.0	5	1
65.0	5	3
84.5	5	3
109.8	5	4
142.7	5	5
185.5	5	5

Death occurred within 24 hours.

Clinical signs: Symptoms of intoxication were clonic and tonic convulsions and respiratory paralysis.

Necropsy: Post mortem autopsy revealed congested lung, liver, stomach, and small intestine.

Conclusions

An acute oral LD_{50} of 76.7 mg /kg bw in dogs would have been established for endosulfan if the study could be considered valid.

B.6.2.2 Percutaneous studies

B.6.2.2.1 Rabbit

Elsea, John R.; 1957 (AgrEvo: IIA, 5.2.2/1)

Date of report: 11 Jan 1957.

The study was conducted according to an internal method. No Guideline available.

GLP Compliance: No. The study was conducted prior to implementation of GLP.

The study is not acceptable. Purity not reported. It was assumed to be 100%.

Materials and methods

A total of 20 albino rabbits were used. Animals weight: 1.0 to 2.6 kg. They were housed individually with feed and water available at all times. Survivors were observed daily for a period of 7 days. The test substance, Thiodan Technical (purity was considered to be 100%), was applied as a 10% or 20% w/v solution cotton-seed oil, at dosage levels of: 46.6, 100, 215, 404, and :1000 mg/kg, (four animals per dose) to the clipped intact abdominal skin, covered for 24 hours, thereafter cleaned by rubbing with Fuller´s earth.

Gross autopsies: upon animals that died during the study and upon the survivors after sacrifice at the end of the observation period.

Results

There were no symptoms of intoxication at the two lowest doses.

Clinical signs: At 215 mg/kg bw and above the rabbits exhibited the following symptoms of endosulfan intoxication: diarrhoea, lacrimation, rapid and laboured respiration, and slight sprawling of the limbs. Animals that succumbed showed depression, salivation, excessive masticatory movements, lacrimation, laboured respiration, tremors, movements of the limbs, phonation, and tonic and clonic convulsions prior to death.

The exposed skin showed very slight or mild erythema at termination of the exposure period. This subsided during the following days. The exposed skin of the surviving animals of the higher dosage levels showed slight atonia and/or desquamation towards the end of the observation period.

Necropsy: Gross autopsy of succumbed animals revealed congested lungs containing hemorrhagic areas, granular-appearing livers, irritation of the large intestine, and congested kidneys. Surviving animals did not exhibit any symptoms upon autopsy.

Conclusion

The Study yielded an acute dermal LD_{50} of 359 mg/kg for endosulfan in albino rabbits.

B.6.2.2.2 Rats

Diehl, K.-H.; Leist, K.-H 1988 (AgrEvo:IIA, 5.2.2/2)

Date of report: 01 Sep 1988. Study date: start 05 May 1988, end 22 Aug 1988.

The study was conducted according to: OECD. 402, Guideline, adopted 24 Feb 1987 and EPA . Pestic. Ass. Guidel. F, § 81-2, revised Nov 84.

GLP Compliance: Yes.

The study is acceptable.

Materials and methods

A total of 10 male and 15 female albino rats (Wistar) were used. Animals age/weight: 8 weeks/214 g males and 10 weeks/205 g females. They were housed individually with feed and water available at all times. Survivors were observed daily for a period of 14 days. The moistened test substance, Endosulfan technical; purity of 96.0 %, was applied once as evenly as possible to the shaved and intact dorsal skin., at dosage levels of: 3150 and 4000 mg/kg for the males and 400, 630, and 1000 mg/kg for the females (five animals per dose). The treated area was covered for 24 hours and washed thereafter.

Gross autopsies: upon animals that died during the study and upon the survivors after sacrifice at the end of the observation period.

Results

Dose (mg/kg bw)	No of Males Dead/Treated	No of Females Dead/Treated
400	-	2/5
630	-	3/5
1000	-	4/5
3150	0/5	-
4000	1/5	-

During the first three days after treatment the following symptoms were observed: squatting position, high legged gait, contracted flanks, agitation, increased and/or reduced spontaneous activity, aggressiveness, hypersensitivity to touch, tonic spasms, saltatory and rolling spasms, tonoclonic spasm when touched, piloerection, narrowed palpebral fissures, bloody and foamy salivation, blood-crusted snout, increased respiratory rate, and irregular breathing.

Many rats showed symptoms of irritation on the treated skin areas.

The body weight gain was impaired.

Conclusion

An LD_{50} for endosulfan in male and female Wistar rats of >4000 resp. 500 mg/kg bw was determined. This result would require an EEC classification of "Xn" (harmful) for the technical a.i.

B.6.2.3 Inhalation studies

B.6.2.3.1 Rat

Hollander, H.; Weigand, W.; 1983 (AgrEvo: IIA, 5.2.3/1)
Date of report: 07 Dec 1983.

Study date: Start: 22 Mar 1983; End: 21 Apr 1983.

The study was conducted accordance with EPA Guideline; Pestic. Ass. G. F, § 81-3, Nov 1982

GLP Compliance: Yes.

The study is acceptable.

Materials and methods
Male and female SPF Wistar rats were use to perform an acute nose-only inhalation toxicity test with Endosulfan Technical (purity 97.2%) Ethanol - Polyethylene glycol mixture (50/50) Age/weight: eight to ten weeks, males 168 - 199 g, females 176 - 203 g.

Husbandry: Macrolon cages with groups of 5 animals, free access to feed and water.

Test Concentrations per 5 animals in mg/l air: Males: 0.0123, 0.0288, 0.0401, 0.0658.

Females: 0.0036, 0.0123, 0.0288, 0.0401, 0.0658 Exposure: nose only for four hours.

Observation for 14 days Autopsy was performed on animals dying during the study and the others after the observation period.

Results

Concentration (mg/l air)	No of Males Dead/Treated	No of Females Dead/Treated
0.0036	-	0 / 5
0.0123	0 / 5	3 / 10
0.0288	0 / 5	4 / 10
0.0401	5 / 5	10 / 10
0.0658	5 / 5	10 / 10

Death occurred within 8 hours.

Clinical signs of intoxication were: irregular respiration, passivity, disequilibrium, trembling, tremors, tono-clonic convulsions, and reduced excitability of the tested reflexes.

Autopsy of the animals which died during the study revealed sporadic dark red foci of pinhead size in the lungs. The animals killed at the end of the study revealed no macroscopic abnormalities.

Conclusion

For endosulfan 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.

This result may require an EEC classification of "T+" (very toxic).

B.6.2.4 Skin Irritation

B.6.2.4.1 Rabbit

Reno, F. E.; 1975b (AgrEvo: IIA, 5.2.4/1)

Date of report: 12 Nov 1975.

The study was evaluated according to the criteria of the Fed. Hazard. Subst. Act, 16 CFR, Part 1500.41 USA.

GLP Compliance: NO.

The study is not acceptable. Purity not reported.

Materials and methods

Species/strain/: New Zealand White rabbits (Age/weight are not mentioned). Husbandry: no information is available. Test substance: Endosulfan; technical (purity not reported). Treatment: dorsal skin of 6 rabbits clipped; 0.5 g endosulfan each applied after moistening with water of two areas, one abraded, the other not. The sites were then covered with one inch square gauze patches, the trunk of each rabbit wrapped with a none absorbed binder and the rabbits immobilised in stocks. Treated areas were washed after 24 hours.

Observation and scoring of the treated areas were conducted 24 and 72 hours post application; system of DRAIZE was used for scoring.

Results

Signs of dermal irritation including erythema were observed at the abraded and intact skin areas 24 hours after treatment. Necrosis was observed along the site of an abrasion in one animal. Very little

symptoms were left after 72 hours. The primary irritation score was calculated as 0.9 according to the system of DRAIZE.

Assessment of study results is not possible due to paucity of information provided. Results should be dismissed especially because origin and quality of test material cannot be verified.

Conclusion

Endosulfan would be considered to be not irritating to the skin.

Elsea (1957) (AgrEvo:ANRA)

There is a review of the original report, thus is considered only as additional information.

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

Under the conditions of this study, endosulfan was a slight skin irritancy.

Bracha 1977 (AgrEvo:ANRA)

There is a review of the original report, thus is considered only as additional information.

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

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

Dikshits (1984) (AgrEvo:ANRA)

There is a review of the original report, thus is considered only as additional information.

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

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

Bremmer, 1997b (IPCS 1998)

Guideline: EEC guideline B.4 (Acute toxicity skin irritation of Directive 92/69/CEE)

There is a little summary of the original report, thus is considered only as additional information

The dermal irritancy of technical grade endosulfan (purity, 98.6%) was tested in 3 New Zealand white rabbits by clipping the hair from a dorsal area about 25 cm^2 and 24 h later applying 500 mg endosulfan moistened with deionized water on a 6.25 cm^2 cellullose patch, which was then covered with a semi-occlusive bandage. Exposure was for 4 h, after which the test material was removed of the patch. The overall mean scores for dermal irritancy were 0 for both erythema and scare formation and oedema formation. No signs of systemic toxicity were observed.

B.6.2.5 Eye Irritation

B.6.2.5.1 Rabbit

Reno, F. E.; 1975a (AgrEvo: IIA, 5.2.5/1)
Date of report: 22 Dec 1975.

The study was evaluated according to Draize. An internal method was used as test method.

GLP Compliance: NO. The study was performed prior to GLP.

The study is not acceptable. Purity of the test substance unknown. Exposition period after the instillation was very short.

Materials and methods
New Zealand White rabbits were used (Age/weight are not mentioned). Husbandry: no information is available. Test substance: Endosulfan; technical (purity not reported). Dosing: 3 animals received 0.1 ml (84 mg) into the conjunctival sac of the left eye. The right eyes served as control. The treated eyes were washed 20 seconds after instillation. Observation 24, 48, 72 hours post instillation.

Results
There was no evidence of eye irritation or any corneal damage following instillation of 0.1 ml (84 mg) endosulfan into the conjunctival sac of rabbit eyes.

Assessment of study results is not possible due to paucity of information provided. Results should be dismissed especially because origin and quality of test material can not be verified.

Conclusion
Endosulfan technical would be considered as not irritating to the eye.

Elsea (1957) (AgrEvo:ANRA)

There is a review of the original report, thus is only considered as additional information.

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

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

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

Bracha (1977) (AgrEvo:ANRA)
There is a review of the original report, thus is only considered as additional information.

Endosulfan (100 mg) was instilled in one eye of each o f6 New Zealand white rabbits; the untreated eye served as a control. Reaction to test material was noted on instillation and at 24, 48 and 72h.

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

Bremmer, 1997b (IPCS 1998)
Guidelines: EEC guideline B.5 (Acute toxicity eye irritation of Directive 92/69/CEE)

There is a little summary of the original report, thus is only considered as additional information.

The ocular irritancy of technical grade endosulfan (purity 98.6%) was tested in 3 New Zealand rabbits by applying 100 mg to the conjunctival sac of one eye of each rabbit; the other, untreated eye served as the control. The eyes were exposed for 24 h, after which the endosulfan was washed out, and the eyes examined for ocular lesions 1, 24, 48 and 72 h later The overall means scores for irritation were 0.66 for redness of conjunctiva, 0 for chemosis of conjunctiva, 0 for opacity of cornea, and 0.11 for irritation of the iris. No signs of systemic toxicity were observed.

Endosulfan was not irritating to the eye.

B.6.2.6 Skin sensitisation

B.6.2.6.1 Guinea Pig (Buehler test)

Jung and Weigand 1983 8AgrEvo: IIA, 5.2.6/1)

Dates: start 21 Mar 1983; end 22 Jun 1983.

The study was designed to meet the requirements of the OECD (406, 12 May 81) and EPA (Pestic. Ass. G F, § 81-6, Nov).

GLP Compliance: NO. The study was performed prior to GLP.

The study is acceptable.

Materials and methods

The skin sensitising study was done according to method of Buehler. 30 female Pirbright-White. guinea pigs were use and obtained from Hoechst AG, Kastengrund, SPF Bread, 8-10 weeks old and weighing between 309 and 378 g. Husbandry: Makrolon cages with groups of 5 animals, free access to feed and water. Number of animals: 20 for treatment, 10 for control. Endosulfan; substance, technical; Hoe 002671, purity 97.2% was used as test substance. Test concentration: 40 % in polythene glycol. Number of applications: 3 per week over three weeks challenge treatments: 2 within 48 hours Evaluation: 24 and 48 hours after challenge treatment. Autopsy was performed on one animal dying during the study and the others after the observation period.

Results

Preliminary studies gave no indication of irritating effects in 40% dilutions of endosulfan. This concentration was chosen for the main study, therefore. In the main study one animal died without signs of intoxication on day 11. Two other animals of the treatment group showed inhibited bodyweight gains. There were no other symptoms observed.

There was no occurrence of erythema or oedema in any animal.

Autopsy at the end of the study revealed no macroscopic abnormalities.

Conclusion

Endosulfan gave no signs of sensitising properties in this BUEHLER test.

B.6.2.6.2 Guinea Pig (Maximisation test)

Arcelin, 1996 (IPCS 1998)

Guidelines: EEC guideline B.6 (Acute toxicity-Skin sensitisation of Directive 92/69 % CEE)

There is a little summary of the original report, thus is only considered as additional information.

The cutaneous allergenic potential of endosulfan (purity 98.6%) was examined in 20 treated and 10 control male albino guinea pigs. 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.

None of the treated guinea-pig developed skin reactions. Endosulfan was therefore considered to be non-sensitizing for guinea-pig skin.

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

Summary

Subacute studies

An oral study in rats conformed to GLP regulations and OECD guidelines was conducted (Leist & Mayer, 1987).

In addition, a review about oral subacute toxicity in rats was provided by Australian monograph (ANRA), however only can be considered as additional information even not provide the original study (Nath *et al*., 1978).

Subchronic studies

Data from three oral subchronic toxicity studies were presented: one of 13-week diet in rat (Barnard *et al.*, 1985) ,three on mouse: 13-week oral study (Barnard et al 1984) and 42-day oral study (Donabauer *et al*., 1985).All these studies were performed according to GLPs compliance.

A little summary about an new oral subchronic study in rats was found from IPCS document, thus only could be considered as additional information.. (Leist & Bremmer, 1998).

Other routes

Three 28-day dermal studies in rat (Ebert *et al* 1985a; 1985b; Dikshith *et al*. 1988) and one nose-only inhalation study in rats (Hollander *et al* 1984) were conducted. All these studies was performed according the GLPs compliance minus the last dermal study (Dikshith *et al*. 1988) where the information about GLP compliance is not provided.

The results of these studies are summarised in Table 6.3-1 .

Table 6.3-1: Summary of short term toxicity studies.

Study	NOAEL (mg/kg bw/day)	Main adverse effect	LOAEL (mg/kg bw/day)	Reference and year
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: 0, 10, 30, 60 and 360 mg/kg feed.(equal to 0, 0.64, 1.9, 3.8 and 23 mg/kg/day for males and 0.75, 2.3, 4.6 and 27 mg7kg/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 Concentration 0, 2, 6, 18, and 54 mg/kg feed. (equal to 0, 0.24, 0.74, 2.13 or 7.3 mg/kg/day for males and 0, 0.27, 0.80, 2.39,0r 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 levels 0, 18 ppm				Donaubauer *et al* 1985 AgrEvo IIA, 5.3.2.5/1
Other routes				
28-day dermal, rat 0, 1, 3, 9, 27 and 81 mg/kg bw/day				Ebert *et al* 1985a AgrEvo IIA, 5.3.3.1/1
28-day dermal, rat 0, 12, 48, 96, 192 mg/kg bw/day in males and 0, 3, 6, 12, 40 mg7kg7day in females				Ebert *et al* 1985b AgrEvo IIA, 5.3.3.1/1
28-day dermal, rat (males 0, 18.75, 37.50, 62.50 mg/kg bw/day, females 0, 9.83, 19.66, 32.00 mg/kg).		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 0.0005, 0.0010, 0.0020 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 levels: 0, 11 mg/kg bw/day	Not identified			Nath et al., (1978) (AgrEvo:ANRA)
Subchronic oral toxicity study in rats. Dose levels: 0, 10, 30, 60, 360 ppm (equal to 0, 0.64, 1.9, 3.8 and 23 mg/kgbw/day for males and 0, 0.75, 2.3, 4.6 and 27 mg/kgbw/day	10 ppm (0.64 mg/kgbw/day)	LOAEL: based on haematological changes	30 ppm (1.9 mg/kgbw/day)	Leist & Bremmer (1998) IPCS

The subchronic oral toxicity study in rat revealed a NOAEL of 3.85 mg/kg bw/day (m), and a NOAEL of 2.3 mg/kg bw/day (m/f) in mice . Nevertheless a study of 90 days in dogs is required.

B.6.3.1 Subacute toxicity

B.6.3.1.1 Oral in rats

Leist, K. H. & Mayer, D. (1987) (AgrEvo: IIA, 5.1.2.2./1)
Date of experimental work: From September 17th 1984 to November 15th 1984.

The study was conformed to GLP regulations (OECD principles of GLP, annex 2 of OECD guidelines for testing of chemicals, 1981) and the applicant include QA Statement.

The study is acceptable.

Material and methods
Test substance: ^{14}C-labelled 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (Endosulfan) with 97.9% of purity.

220 male Wistar rats (Hoechst AG, Pharma Research Toxicology, Kastengrund) weighing 138-168 g and divided in 3 groups receiving (20 animals), 360 (100 animals) or 720 (100 animals) mg/kg b.w. fed (equivalent to 34 and 67.8 mg7kg7day) for 4 weeks. The animals were housed in metabolic cages (22± 3 ºC, 50±20 % humidity, 12-12 light-dark, air circulation 6 times/h; diet: Altromin 1321 rat diet and tap water *ad libitum*). Half the animals of each group were sacrificed at termination of treatment, the other half after 4 weeks recovery. 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.

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.

Nath *et al*., (1978) (AgrEvo: ANRA)

This study is a review of the original report, thus is considered only as additional information.

Material and Methods

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

Results

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

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

Conclusion

No NOAEL was established.

B.6.3.2 Subchronic studies

B.6.3.2.1 Rats

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.

Leist & Bremmer (1998) (IPCS 1998)

This study is a little summary of the original report, thus is considered only as additional information.

Material and Methods

Groups of 25 CD Sprague-Dawley rats of each sex were fed diets containing technical-grade endosulfan (purity 97.9%) at concentrations of 0, 10, 30, 60 or 360 ppm equal to 0, 0,64, 1.9, 3.8 and 23 mg/kgbw/day for males and 0, 0.75, 2.3, 4.6 and 27 mg/kgbw/day for females, for 3-months. Five animals of each sex per group were maintained for an additional four-week recovery period.

Results

Three females died, one of each at 0, 60 and 360 ppm. Slight but statistically significant, dose-related reductions in erythrocyte counts and haemoglobin concentrations were seen in males at ≥ 30 ppm in females at ≥ 60 ppm, but were within the reported normal range for this strain and age of rat. Increased mean corpuscular volume was also seen at these doses. Females at 360 ppm had statistically significant decreases in plasma and erythrocyte cholinesterase activities at week 12 (by 41 and 12% respectively), while increased brain acetylcholinesterase activity was observed at 60 and 360 ppm (19 and 20%, respectively). In males at 360 ppm, urinalysis showed a number of reversible changes, including increased urine volume and urinary protein concentrations and decreased specific gravity. Gross examination revealed enlargement of the liver in males at 360 ppm and of the kidneys at 60 and 360 ppm; increases in the absolute weights of the liver (18%) , Kidneys (10%) in females. The kidney weights remained significantly elevated in male rats at 360 ppm (15%) at the end of the withdrawal period.

B.6.3.2.2 Mouse

Barnard, A. V.; Atkinson, J. S.; Heywood, R. *et al*., 1984 (AgrEvo: IIA, 5.3.2.4/1)

Study date: Start: 11 Jul 1983-End: 13 Oct 1983.

Date of report: 25 Sep 1984.

Test method: Internal method. Conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid.

GLP: Yes.

The study is acceptable.

Material and methods

5 groups of 20 male and 20 female CD-1 mice, aged about 4 weeks, source Charles River, received endosulfan; substance, technical; 97.2 %, Code: Hoe 002671 00 ZD97 0003; at dose level of 0, 2, 6, 18,

54 mg substance /kg feed, vehicle acetone and corn oil for incorporation into diet, for 3 months. These concentrations equivalent are equivalent to doses of 0, 0.24, 0.74, 2.13 or 7.3 mg/kg/day for males and 0, 0.27, 0.80, 2.39 or 7.5 mg/kg/day for females.

Husbandry: housing in groups of 4 of same dose and sex in polypropylene cages in air-conditioned rooms, feed and water *ad libitum* with regular checks on consumption.

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; after 6 and 12 (13) weeks haematological and biochemical investigations were performed on the blood of 10 males and 10 females from each group.

Necropsy: After terminal sacrifice all animals were thoroughly examined for external and internal abnormalities, weight of the following organs was determined: adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, uterus; histological study on a very wide range of tissues. Same exercise for animals that died during the study or were killed *in extremis*.

Results

This short term study in mice was conducted to determine dosing levels for endosulfan technical in an oncogenicity study. Up to and including 18 mg substance /kg feed no effects were observed that could be attributed to the substance uptake.

At 54 mg/kg mortality increased significantly for both sexes. At this dose the only clinical sign of intoxication were convulsive episodes. Furthermore feed intake and weight gain were impaired at this dose.

Blood parameter, organ weight, macroscopic and microscopic examinations revealed no treatment related effects.

Conclusion

The NOAEL for oral treatment of CD-1 mice with endosulfan technical over 90 days is 18 mg substance /kg feed. This is equivalent to 2.13 mg/kg bw /day for male and 2.39 mg/kg bw /day for the female mice (2.3 mg/kg bw /day m/f) .

Donaubauer, H.H.; Leist, K.-H.; Kramer, M., 1985 (AgrEvo: IIA, 5.3.2.5/1)

Date of report: 21 Jan 1985.

Test method: Internal method. This is a modified oral dose finding study to check older data It was conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid.

GLP: Yes.

The study is acceptable.

Material and methods

10 male and 10 female mouse, HOE: NMRKf.(SPF71), weighting, not yet fully grown, males mean 20.5 g, females 19 g, source unknown, received endosulfan; substance, technical; 97.9 %, in sesame oil for incorporation into pre-mix of feed, at dose level of 0, and 18 mg endosulfan technical /kg feed (equivalent to 0 or 3.7 mg/kgbw/day for males and 0 or 4.6 mg/kgbw/day for females) for 42-days.

Husbandry: individual housing in Macrolon cages in air conditioned rooms, feed and water *ad libitum*.

Observations: Behaviour / general condition / survival twice daily; body weight / feed consumption / ocular examination / dental examination / visible mucosa weekly; neurological status monthly.

Necropsy: after 42 days the animals were killed, dissected and organs macroscopically examined.

The following organs were weighted: heart, lungs, liver, kidneys, spleen, testes/ovaries, brain. No histology.

Results

The only toxic effect observed was an increase in absolute and relative liver weight for both sexes at this dose. All other parameters showed no response.

Conclusion

Based on the results of this study, it is not possible to established a NOAEL.

B.6.3.3 Other routes

B.6.3.3.1 Rat, 28-day dermal toxicity study

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. There were no other unequivocal symptoms attributable to treatment.

Conclusion

The short term dermal study with endosulfan technical in Wistar rats yielded no clear cut results. A justification concerning the deaths at 9 mg/kgbw/day should be submitted in order to establish a NOAEL.

Ebert, E.; Leist, K.-H.; Kramer, M., 1985b (AgrEvo: IIA, 5.3.3.1/2)

Study date :Start: 17 Aug. 1983-End: 05 Oct. 1983

Date of report: 11 Mar 1985.

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

GLP: Yes.

The study is not acceptable due to a technical application error (excessive constriction of the animals with the elastic occlusive bandage).

Material and methods

Tests groups of Wistar rat, Hoe WISKf (SPF71), aged 8 - 10 weeks, weighting males 176 - 194 g and females 171 - 187 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. Groups of 11 males received 0, 12, 48, 96, 192 mg/kg bw., females 0, 3, 6, 12, 48 mg/kg. 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: The day after the last treatment 6 males and 6 females were sacrificed. The remaining animals were kept for a recovery period of 14 days and sacrificed thereafter. After sacrifice all animals were examined for organ changes; weight of main organs was determined; histological examinations was restricted to liver and kidney. Haematology and clinico-chemical analysis were performed.

Results

This short term dermal study with endosulfan technical in Wistar rats became necessary as a previous investigation yielded no clear cut results. Treatment related mortalities were recorded for female rats at 48 mg/kg bw and for males at 192 mg/kg. Females from 12 mg/kg upwards and males of the highest dose group (192 mg/kg) exhibited typical symptoms of endosulfan intoxication. All other parameters checked in this study revealed no symptoms attributable to treatment.

Conclusion

A “No toxic effect level” was not established from the results of this subchronic dermal toxicity study.

Dikshith, T.S.S.; Raizada, R.B.; Kumar, S.N., 1988 (AgrEvo: IIA, 5.3.3.1/4)

Date of report: 15 Feb. 1988.

Test method: No guideline reported. Information on method is scanty, but test would possibly fulfil today's requirements.

GLP: No information provided.

The study is acceptable.

Material and methods

Tests groups of male and female Wistar rat, age/weight: not recorded, were exposed dermally to the shaved lateral abdominal skin daily for 30 days to endosulfan substance, technical; purity not reported, in ethanol-acetone vehicle. Groups of 6 males received 0, 18.75, 37.50, 62.50 mg/kg bw/day, females 0, 9.83, 19.66, 32.00 mg/kg.

Observations: for symptoms of intoxication during treatment period.

Necropsy: sacrifice of all animals on day after last application; major organs weighed and investigated microscopically; biochemistry of liver and serum; haematology; residue analysis in major organs

Results

In this short term dermal study with endosulfan technical in Wistar rats no mortalities occurred. Typical symptoms of intoxication like hyperexcitation, tremor, dyspnea and salivation were observed during the first days, but disappeared after one week. Neither organ weights nor histopathology revealed treatment related symptoms.

A range of biochemical changes (GOT, GPT etc.) were detected, some of which were statistically highly significant when compared to control. However, as a rule there were no dose relations, thus casting doubts on the biological significance of these results.

Residues (mainly α-endosulfan) were much higher in female rats as compared to males (see table 6.3.3.1-1).

Table 6.3.3.1-1: Residues of total (α + β) endosulfan in organs of treated rats (mg/kg)

	female			**male**		
mg/kg/day	9.83	19.66	32.00	18.75	31.50	62.50
Liver	1.67	8.49	7.75	0.03	0.30	0.09
kidney	1.17	3.16	3.64	0.17	0.24	0.31
brain	0.21	0.61	0.86	0.03	0.15	0.09
testis				0.08	0.19	0.28
fatty tissue	8.20	12.61	16.13	0.42	0.44	0.62
blood	1.80	3.63	5.47	0.43	0.05	0.16

Conclusion

A NO TOXIC EFFECT LEVEL was not determined in this short term dermal toxicity study with endosulfan technical in rats, as transient clinical symptoms were observed in all treated groups.

B.6.3.3.2 Rat, inhalation exposure studies.

Hollander, H., Weigand, W., Kramer, M., 1984 (AgrEvo: IIA, 5.3.3.2/1)

Study date: Start: 08 Jun 1983-End: 08 Jul 1983.

Date of report: 15 Aug 1984.

Test method: US-EPA F, 82-4, Nov 1982.

GLP: Yes.

The study is acceptable.

Material and methods

5 groups of 15 male and 15 female Wistar rat, Hoe WISKf (SPF71), aged 4 to 6 weeks, weighting approximately, males 114 - 135 g, females 114 - 130 g, were exposed, head/nose-only, to endosulfan; substance, technical; 97.2 % (Code: Hoe 002671 00 ZD97 0003) in Ethanol/polyethyleneglycol (1:1) as vehicle, for 6 hours on 5 days a week (21 expos. over 29 days); each receiving the following concentrations: 0 (air only), 0(vehicle), 0.0005, 0.0010, 0.0020 mg endosulfan/litre air. Gravimetric and chemical determination of concentrations were performed at intervals and determination of aerosol particle size was done once every hour. Husbandry: between expositions animals were kept in groups of 5 (same sex and dose) in Macrolon cages in air-conditioned rooms; feed and water *ad libitum* except during exposition.

Observations: prior to, during and at the end of each exposure the animals were observed for signs of intoxication; twice a week weight control and feed consumption, once a week water consumption, check for neurological disturbances, eyes, teeth, and oral mucosa.

One day after the last exposure haematological and chemical parameters of the blood were examined on 10 males and 10 females from each group; blood of the remaining animals were examined 28 days later. Necropsy: Right after blood sampling the animals were sacrificed and macroscopic and microscopic examination performed; the weight of the following organs was recorded adrenals, brain, heart, kidneys, liver, lung, ovaries, pituitary gland, seminal vesica, spleen, testes, thyroid gland, uterus.

Results

This short term inhalation study (head/nose-only-exposition) with endosulfan technical in Wistar rats yielded no symptoms up to the highest dose tested (0.002 mg endosulfan/litre air).

Conclusion

According to the results of this study, it is not possible to establish a NOAEL for endosulfan technical short term inhalation in rats. The author determined the NOAEL based in a preliminary study that was not submitted. This preliminary study or additional information should be submitted.

B.6.4 Genotoxicity (IIA, 5.4)

Summary

Endosulfan has been tested in a wide variety of genetic toxicology assay systems. These consisted of studies presented by AgrEvo, Excel Industries Ltd, and Calliope S.A. Besides, AgrEvo has presented a review document of endosulfan prepared by the Australian National Registration Authority (ANRA) for Agricultural and Veterinary Chemicals, which includes studies previously presented by AgrEvo, Excel or Calliope, and studies which have not been presented by any applicant. The presented studies have employed a variety of endpoints in well-validated test systems as well as some endpoints from test systems which have a severely limited amount of validation data. They include *in vitro* and *in vivo* genotoxicity testing. *In vitro* studies consisted of gene mutation assays (in bacteria, yeast and mammalian cells), chromosomal aberration assays (in yeast and mammalian cells) and assays on DNA effects (the rec-assay in *Bacillus subtilis*, the mitotic recombination assay in *Saccharomyces cerevisiae*, the SCE assay and the UDS assay, both in mammalian cells). *In vivo* studies included somatic and germ cell genotoxicity testing. In somatic cells studies consisted of assays on clastogenic effects in rodents (chromosomal aberration assay and micronucleus test). In germ cells studies included gene mutation and chromosomal aberration assays in *Drosophila melanogaster*, assays on clastogenic effects in rodents (chromosomal aberration assay and dominant lethal test) and mouse sperm abnormality tests. However, evaluation of the mutagenicity is confined to tests using technical endosulfan of clearly defined specifications. These studies are summarised in Table 6.4-1.

Three bacterial gene mutation studies have been reported over the period from 1978 to 1995. The study corresponding to Dighe (1995a) was the only performed according to a specific test guideline and GLPs compliant; however, the purity of the test compound was not reported and TA1535 strain was not included. In the study corresponding to Shirasu, Moriya and Ohta (1978), results should be confirmed in an independent experiment. In the study study corresponding to Pednekar, Gandhi and Netrawali (1987), only two concentrations were tested and TA1535 strain was not included. Nevertheless, negative results were obtained in all cases.

Two gene mutation studies in yeast have been reported over the period from 1982 to 1984. They did not claim adherence to a specific test guideline and GLPs were only applied in the study corresponding to Mellano and Millone (1984a). However, negative results obtained by Mellano and Millone (1984a) are questionable because of the highest concentration tested, the exposure time used, and the performance of a single experiment. On the other hand, in the study from Yadav, Vashishat and Kakar (1982) the test substance was technical endosulfan but its purity was not reported, therefore positive results obtained cannot be considered as acceptable in evaluating the mutagenicity of endosulfan.

One *in vitro* mammalian gene mutation study, corresponding to Cifone and Myhr (1984b), has been presented. It was GLPs compliant and its conduct according to published methods was considered suitable. Negative results were obtained.

One chromosomal aberration assay in *Saccharomyces cerevisiae,* corresponding to Yadav, Vashishat and Kakar (1982), has been presented. The test substance was technical endosulfan but its purity was not reported, therefore positive results obtained cannot be considered as acceptable in evaluating the mutagenicity of endosulfan. Besides, the study is not required and there are not available test guidelines for its conduct.

Two *in vitro* mammalian chromosome aberration studies (Pirovano and Millone, 1986; Asquith and Baillie, 1989) have been reported over the period from 1986 to 1989. Studies did not claim adherence to a specific test guideline but were GLPs compliant. In both studies, endosulfan was not clastogenic with and without metabolic activation to human lymphocytes following a short treatment but a continuous treatment in the absence of S9 was not carried out. Besides, in both cases, a higher concentration that produced a reduction in mitotic index greater than 50% should have been tested.
In vitro DNA effects studies have been reported over the period from 1978 to 1988. They did not claim adherence to a specific test guideline excepting the UDS assay in a human cell line which was performed according to OECD test guideline 482. GLPs were only applied in one mitotic recombination assay and in both UDS assays. Endosulfan was negative in the rec-assay and in both UDS assays. The rec-assay (Shirasu, Moriya and Ohta, 1978) could be only used as an additional information because is neither required nor validated and gives only a qualitative information; in the UDS assay with rat hepatocytes (Cifone and Myhr, 1984a), results were not confirmed in an independent experiment; and in the UDS assay with a human cell line (Müller, 1988b), the exposure time of only 3 hours should be justified. Both negative and positive results have been reported for mitotic gene conversion induction: positive results, from Yadav, Vashishat and Kakar (1982), cannot be considered as acceptable in evaluating the genotoxicity of endosulfan because the purity of the test compound was not reported; and negative results, from Mellano and Milone (1984b), are questionable because of the highest concentration tested, the exposure time used, and the performance of a single experiment. Finally, endosulfan was shown to be a potent inducer of SCE on human lymphoid cells (Sobti, Krishan and Davies, 1983); however, only the top concentration induced double SCE frequency over that of control, after 48 h incubation period without metabolic activation; besides, this study cannot be considered as acceptable in evaluating the genotoxicity of endosulfan because the purity of the test compound was not reported.

Six *in vivo* clastogenic studies in mammalian somatic cells have been reported over the period from 1978 to 1995. Studies included rodent chromosomal aberration assays and mouse micronucleus tests. The two chromosomal aberration assays corresponded to studies published in the scientific literature (Dzwonkowska & Hübner, 1986; Dikshith & Datta, 1978). Endosulfan induced structural chromosome aberrations in hamster but not in rat bone marrow cells. Nevertheless, these results cannot be

considered as acceptable in evaluating the clastogenicity of endosulfan because of the test substance, a commercial preparation with a content of 35% endosulfan in studies carried out with hamsters, and endosulfan assumed as technical but with no reported purity, in studies carried out with rats. Four mouse micronucleus studies have been presented but only two were GLPs compliant and performed according to a specific test guideline (Muller, 1988a; Dighe, 1995b). The two remaining studies corresponded to a published paper (Rani, Reddi and Reddy, 1980) and to a summary of a report from Jung, Weigand and Kramer, 1983, included in the document prepared by ANRA. Negative results were obtained in all cases. Nevertheless, although the test substance was assumed as technical endosulfan, the purity was only reported in one study. Therefore, this study (Muller, 1988a) was the only considered as acceptable in evaluating the clastogenicity of endosulfan. However, a higher dose of endosulfan should be tested in order to give a definitive conclusion.

One study in germ cells of *Drosophila melanogaster* (Velázquez *et al,* 1984) has been presented. Endosulfan was shown to be both mutagenic and clastogenic in *Drosophila melanogaster* SLRL and SCL tests, respectively. However, results cannot be considered as acceptable in evaluating the mutagenicity of endosulfan because the test substance was a commercial preparation with a 50% of ingredient active in both assays.

Four *in vivo* chromosomal aberration studies in rodent germ cells, published over the period from 1978 to 1991, have been presented. Endosulfan induced structural (translocations) and numerical chromosome aberrations in mouse spermatocytes (Rani and Reddy, 1986) but not in rat spermatogonial cells (Dikshith and Datta, 1978). However, in both studies the purity of the test substance and other relevant information were not reported. With respect to the mouse dominant lethal assay, both positive and negative results have been reported. Negative results, corresponding to a summary of a published paper (Dzwonkowska and Hübner, 1991) which is included in the document prepared by ANRA, cannot be considered as acceptable in evaluating the mutagenicity of endosulfan because the test substance was a commercial preparation (Thiodan 35), and some relevant information was not included in the summary. On the basis of results from the study corresponding to Pandey *et al.* (1990), it appears that endosulfan has a damaging effect on mice spermatogonial cells. However, the induction of dominant lethal effects occurred only at the highest dose of endosulfan (16.6 mg/kg/day, equivalent to a total dose of 83 mg/kg). The lack of detail in the reporting of this study makes the significance of the isolated finding questionable. The fact that an increase in dominant lethal mutations was seen only in a single mating interval, with no adverse effects on implants or fertility seen at other intervals, suggests that the result may be an artefact, and no related to treatment. There is no individual animal data to determine if there was large intra group variation in the sixth mating interval, and if a single outlying result led to a statistically significant outcome for this mating interval. The test was not reproduced, and so it is difficult to determine whether the effects seen from the sixth mating interval were spontaneous, or related to endosulfan administration.

Three studies corresponding to the mouse sperm abnormality test, published over the period from 1990 to 1996, have been presented. However, two studies (Sinha *et al,* 1995; Khan and Sinha, 1996)

correspond really to summaries of published papers which were included in the document prepared by ANRA, thus the information given by both studies is insufficient. Positive results were obtained in all cases. According to Pandey *et al* (1990), endosulfan induced increases in sperm head abnormalities, decreases in testis weight and decreases in sperm count. Nevertheless, it is unclear whether these effects are biologically significant. It is unlikely that this increase in sperm abnormalities is causally related to any adverse effects on fertility or other reproductive parameters but the reporting of this study is not adequate to definitely discount the possibility. According to Sinha *et al* (1995), endosulfan appears to impair testicular functions by increasing the enzyme activities responsible for spermatogenesis, thus influencing intratesticular spermatid count, and resulting in low sperm production and increased sperm abnormalities. However, the increase in sperm abnormalities was very slight and it is considered that this effect is not biologically significant. On the other hand, endosulfan was a commercial preparation in the study corresponding to Khan and Sinha (1996); therefore it is unclear whether the increase in abnormal sperm and the reduction in sperm count are related to endosulfan or non active constituents. It should be noted that the sperm abnormality test is not required in evaluating the mutagenicity of endosulfan and there are not available guidelines for its conduct.

The conclusions about the mutagenicity of endosulfan, based in data from studies carried out with technical material of clearly defined specifications, 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 *Saccharomyces cerevisiae* mitotic gene conversion assay, cannot be considered conclusive because of its conduct.

4. Endosulfan appears to be non-clastogenic in mammalian somatic cells *in vivo.* Nevertheless, in the only study, considered acceptable in evaluating the mutagenicity of endosulfan, a micronucleus test, a dose greater than 10 mg/kg should have been tested. On the other hand, Thiodan 35 induced chromosomal aberrations in hamster; although any mutagenic activity may have resulted from non active constituents included in the formulation, it could be advisable to performed one study on chromosomal aberration induction with technical endosulfan.

5. The information given by the two presented chromosome aberration studies precludes any conclusion on the endosulfan clastogenicity for rodent germ cells, because in both studies the purity of the test substance was not reported. On the other hand, it is unlikely that a single isolated increase in dominant lethal mutations at the high dose is related with endosulfan administration; the lack of detail in the published study makes the significance of the isolated finding questionable.

6. Endosulfan induced sperm abnormalities in rodents. Nevertheless, it is unclear whether this effect is biologically significant.

Table 6.4-1: Summary of genotoxicity studies

Test	System	Dosage	Results	Comments	Reference
Bacterial/ mammalian microsome plate incorporation assay	*Salmonella typhimurium* TA1535,TA1537, TA1538,TA98, and TA100. *Escherichia coli* WP2 *hcr*	5, 10, 50, 100, 500, 1000 and 5000 µg/plate (±S9)	Negative	Single experiment.	Shirasu, Moriya & Ohta, 1978 (AgrEvo: IIA, 5.4.1.1/1) (AgrEvo: ANRA) No published
Ames test : plate incorporation test for the non toxic concentration, and preincubation assay for the 90% toxic concentration	*Salmonella typhimurium* TA100, TA98 and TA97a.	41 and 3256 mg/L (±S9) 41 and 3256 mg/L (±CEE)	Negative	At least 5 concentrations should have been tested. TA1535 strain not included.	Pednekar, Gandhi and Netrawali, 1987 (Calliope: IIA, 5.4.1/02) Published
Salmonella/ mammalian microsome plate incorporation assay	*Salmonella typhimurium* TA97a, TA98, TA100 and TA102.	0.05, 0.5, 5, 50 and 500 µ g/plate (±S9)	Negative	Purity of the test substance not given. TA1535 strain not included. Toxicity observed at 500 µg/plate.	Dighe, 1995a (Excel: IIA, 5.4/01) No published
Schizosaccha-romyces pombe gene mutation assay	*Schizosaccharomy-ces pombe* (SP *ade* 6-60/*rad* 10-198, *h*-)	62.5, 125, 250 & 500 µg/mL (±S9)	Negative	At least 5 concentrations should have been tested. At the top concentration survival rates were greater than 10%. Exposure time: 4 h. Single experiment.	Mellano and Millone, 1984a (AgrEvo: IIA, 5.4.1.3/2) (AgrEvo: ANRA) No published
Saccharomyces cerevisiae gene mutation assay	*Saccharomyces cerevisiae* strain D_7 (*ade*2-40/*ade*2-119; *trp*5-12/*trp*5-27; *ilv*1-92/*ilv*1-92)	Cells treated for 10, 20 and 30 min with 1% endosulfan	Positive. The effect was more pronounced at 30 min exposure time.	Purity of the test substance not given. At least 5 concentrations should have been tested. No S9. Maximum exposure time of 30 min. Single experiment. 82% killing at 30 min exposure time.	Yadav, Vashishat and Kakar, 1982 (Excel: IIA, 5.4/05) Published
In vitro mammalian cell forward gene mutation assay	Fischer mouse lymphoma cells (L5178Y $TK^{+/-}$ - 3.7.2C)	6.25, 12.5, 18, 25, 37.5, 50 and 75 µg/mL (-S9) 6.25, 12.5, 25, 50, 75 and 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) (AgrEvo: ANRA) No published

Test	System	Dosage	Results	Comments	Reference
In vitro chromosomal aberration assay in yeast	*Saccharomyces cerevisiae strain* D_7 (*ade*2-40/*ade*2-119; *trp*5-12/*trp*5-27; *ilv*1-92/*ilv*1-92)	Cells treated for 10, 20 and 30 min with 1% endosulfan	Positive. The effect was more pronounced at 30 min exposure time.	Purity of the test substance not given. Not available test guidelines. 82% killing at 30 min exposure time.	Yadav, Vashishat and Kakar, 1982 (Excel: IIA, 5.4/05) Published
In vitro chromosomal aberration assay with mammalian somatic cells	Human lymphocytes from a healthy male volunteer	1, 10 and 100 µg/mL (±S9)	Negative	200 µg/mL (±S9) was toxic. The reduction in mitotic index at 100 µ g/mL was 39% (-S9) and 30% (+S9). Exposure time was only 4 h (±S9). 100 metaphases per treatment scored.	Pirovano & Millone, 1986 (AgrEvo: IIA, 5.4.1.2/1) (AgrEvo: ANRA) No published
In vitro chromosomal aberration assay with mammalian somatic cells	Human lymphocytes from two healthy volunteers	10, 20 and 40 µg/mL (±S9)	Negative	The reduction in mitotic index was 50% at 40 µg/mL (±S9). Exposure time was only 3 h (±S9).	Asquith & Baillie, 1989 (AgrEvo: IIA, 5.4.1.2/2) (AgrEvo: ANRA) No published
Rec assay	*Bacillus subtilis* H17 Rec^+/ M45 Rec^-	20, 100, 200, 500, 1000, 2000 µg/disc	Negative	Not available OECD test guidelines. Qualitative test.	Shirasu, Moriya & Ohta, 1978 (AgrEvo: IIA, 5.4.1.1/1) (AgrEvo: ANRA) No published
Saccharomyces cerevisiae mitotic re-combination assay	*Saccharomyces cerevisiae* D_7 (*ade*2-40/*ade*2-119; *trp*5-12/*trp*5-27; *ilv*1-92/*ilv*1-92)	Cells treated for 10, 20 and 30 min with 1% endosulfan	Negative for crossing-over. Positive for gene conversion. The effect was more pronounced at 30 min exposure time.	Purity of the test substance not given. At least 5 concentrations should have been tested. No S9. Maximum exposure time of 30 min. Single experiment. 82% killing at 30 min exposure time.	Yadav, Vashishat and Kakar, 1982 (Excel: IIA, 5.4/05) Published
Saccharomyces cerevisiae mitotic gene conversion assay	*Saccharomyces cerevisiae* D_4 (heteroallelic at *ade 2* and *trp 5)*	100, 500, 1000, 5000 µ g/mL (±S9)	Negative	At least 5 concentrations should have been tested. At the top concentration survival rates were 52% (-S9) and 100% (+S9). Exposure time was only 4 h. A single experiment.	Mellano and Millone, 1984b (AgrEvo: IIA, 5.4.2.2/2) (AgrEvo: ANRA) No published

Test	System	Dosage	Results	Comments	Reference
In vitro SCE assay with mammalian somatic cells	Human lymphoid cell line LAZ-007	10^{-6}, 10^{-5} and 10^{-4} M for 48 h (-S9) for 1 h (±S9)	Positive for all concentrations but double SCE frequency over that of control only at 10^{-4} M after 48 h incubation.	Purity of the test substance not given. 66% viables in cultures incubated for 48 h with 10^{-4} M.	Sobti, Krishan & Davies, 1983 (Calliope: IIA, 5.4.1/01) (Excel: IIA, 5.4/09) Published
In vitro UDS assay with mammalian somatic cells	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 was 31.5% at 25.5 µg/mL. A single experiment.	Cifone & Myhr, 1984a (AgrEvo: IIA, 5.4.2.2/1) (AgrEvo: ANRA) No published
In vitro UDS assay with mammalian somatic cells	Human cell line A 549	Experiment 1: 1, 3, 10, 30, 100, 300 and 1000 µg/mL (-S9) Experiments 2, 3: 0.1, 0.3, 1, 3, 10, 30 and 100 µg/mL (±S9)	Negative	100, 300 and 1000 µg/mL produced alterations of cell morphology. It should be justified the treatment time of 3 hours.	Muller, 1988b (AgrEvo: IIA, 5.4.2.2/3) (AgrEvo: ANRA) No published
In vivo chromosomal aberration assay with somatic cells	Bone marrow cells from male albino rats.	11, 22, 36.6 and 55 mg/kg administered by oral intubation for 5 consecutive days.	Negative	All rats dosed at 36.6 and 55 mg/kg died before 24 h. No significant mitotic inhibition. Purity of the test substance not given. Required information not included. 50 cells/dose level counted.	Dikshith & Datta, 1978 (Calliope: IIA, 5.4.2/01) (AgrEvo: ANRA) Published
In vivo chromosomal aberration assay with somatic cells	Bone marrow cells from female Syrian golden hamster (*Mesocricetus auratus)*	A single i.p. injection of endosulfan at 8, 16, 40 and 80 mg/kg. Animals were sacrificed 24 h after dosing.	Positive for aberrations excluding gaps at 8 & 80 mg/kg, and for total aberrations at all doses.	Endosulfan was a commercial preparation (Thiodan 35). Only one sample time at 24h. Except for 8 mg/kg, less than 500 cells/dose level were counted. LD_{50} (80 mg/kg) was tested but any toxic signs for bone marrow or animals were reported.	Dzwonkowska & Hübner, 1986 (Excel: IIA, 5.4/07) (AgrEvo: ANRA) Published

Test	System	Dosage	Results	Comments	Reference
In vivo micronucleus test in somatic cells	Bone marrow cells from male Swiss albino mice	Mice orally fed with the dose of 43.3 mg/kg, in two equal instalments separated by 24 h, and sacrificed 6 h after last dose.	Negative	Purity of the test substance not given. Required information not included. 4 mice/dose group. Only one sample time 6 h after last dose. No information about toxicity for mice. No toxicity for bone marrow.	Rani, Reddi and Reddy, 1980 (Calliope: IIA, 5.4.2/02) (Excel: IIA, 5.4/06) Published
In vivo micronucleus test in soma-tic cells	Bone marrow cells from male and female NMRI mice, strain NMRKf (SPF71).	A single oral gavage dose at 2.5, 5 and 10 mg/kg	Negative	Although the top dose was about 50% of the calculated LD_{50}, 6 h post-treatment clinical signs observed in mice disappeared. Besides, no indication of toxicity was observed in the bone marrow .	Muller, 1988a (AgrEvo: IIA, 5.4.2.1/1) (AgrEvo: ANRA) No published
In vivo micronucleus test in somatic cells.	Bone marrow cells from male and female Swiss albino mice	A single oral gavage dose of 6 mg/kg for males, and 8 mg/kg for females. Mice sacrificed at 18, 24 & 30 h after dosing.	Negative	Purity of the test substance not given. The MTD was tested. However, no toxicity was observed in the bone marrow and no information was given about toxicity for mice.	Dighe, 1995b (Excel: IIA, 5.4/02) No published
In vivo micronucleus test in somatic cells.	Bone marrow cells from male and female NMRI mice	A single oral gavage dose at 0.2, 1 and 5 mg/kg	Negative	Only a summary from the report has been presented, thus the information given is considered insufficient.	Jung, Weigand & Kramer 1983 (AgrEvo: ANRA) No published

Test	System	Dosage	Results	Comments	Reference
In vivo gene mutation test in germ cells from insects (SLRL test)	*Drosophila melanogaster* (*Berlin-K* wild-type males and *Basc* females)	Males as larvae were treated with 50 and 100 ppm of ingredient active. Males as adults were treated with 150 and 200 ppm of ingredient active. A 3-2-2 mating scheme for males treated as adults.	Positive after the larval stage treatment at 100 ppm, and after adults treatment at 200 ppm. No stage-specific response.	Endosulfan was a commercial preparation. Required information not included. A detailed description of the test procedure not reported. 3 exposure levels should have been tested. Single experiment. No toxicity data.	Velázquez *et al,* 1984 (Calliope: IIA, 5.4.3/02) (Excel: IIA, 5.4/04) Published
In vivo chromosomal aberration test in germ cells from insects (SCL test)	*Drosophila melanogaster* (*Ring-X* males of the genotype *R1(2), yB/B^{s}Yy^{+}*) (*y sp* females of the genotype *y w spl sn^{3}*; *bw sp^{2})*	Males were treated with 50, 100 & 200 ppm of ingredient active. Three broods.	Positive for all dose levels. No detected partial Y chromosome losses. No dose- effect relationship. The results suggest a pronounced clastogenic effect in sperm.	Endosulfan was a commercial preparation. Required information not included. A detailed description of the test procedure not reported. Single experiment. No toxicity data.	Velázquez *et al,* 1984 (Calliope: IIA, 5.4.3/03 or IIA, 5.4.3/02) (Excel: IIA, 5.4/04) Published
In vivo chromosomal aberration assay with mammalian germ cells	Spermatogonial cells from male albino rats	11, 22, 36.6 and 55 mg/kg administered by oral intubation for 5 consecutive days.	Negative	All rats dosed at 36.6 and 55 mg/kg died before 24 h. No significant mitotic inhibition. Purity of the test substance not given. Required information not included. 50 cells/dose level counted.	Dikshith & Datta, 1978 (Calliope: IIA, 5.4.3/01 or IIA, 5.4.2/01) (AgrEvo: ANRA) Published
In vivo chromosomal aberration assay with mammalian germ cells	Spermatocytes from male Swiss mice	22, 32 and 42 mg/kg administered orally for 5 consecutive days.	Positive for polyploids, autosomal and sex univalents at 32 and 42 mg/kg; for aneuploids at all doses; for translocations at 22 and 42 mg/kg; for total chromosomal aberrations at 32 and 42 mg/kg.	Purity of the test substance not given. Required information not included. No toxicity data.	Rani & Reddy, 1986 (Calliope: IIA, 5.4.3/04) Published

Test	System	Dosage	Results	Comments	Reference
In vivo mammalian chromosome aberration test in germ cells (dominant lethal test)	Swiss albino mice	Males were treated i.p. for 5 consecutive days with 9.8, 12.7 and 16.6 mg/kg. 7-day sequential mating procedure (8 mating intervals).	Positive at 16.6 mg/kg during the mating interval of 36-42 days.	Damage induced specifically in spermatogonia. Fertility was only affected during the mating interval of 36-42 days. The lack of detail in the reporting of this study makes the significance of the isolated finding questionable.	Pandey *et al,* 1990 (Excel: IIA, 5.4/03) (AgrEvo: ANRA) Published
In vivo mammalian chromosome aberration test in germ cells (dominant lethal test)	Balb/c mice	Males were treated i.p, once or for 5 days, with a dose of 0.64 mg/kg. (twelve 4-day mating intervals).	Negative	Only a summary from the published paper has been presented, thus the in-formation given is considered insufficient.	Dzwonkowska & Hübner, 1991 (AgrEvo: ANRA) Published
In vivo mammalian sperm abnormality test	Swiss albino mice	Males were treated i.p. for 5 consecutive days with 9.8, 12.7, 16.6 and 21.6 mg/kg.	Positive at 16.6 & 21.6 mg/kg for sperm head abnormalities. Significant dose-dependent increase.	Significant decrease in testis weight at 21.6 mg/kg. Significant dose-dependent decrease in sperm count at 16.6 & 21.6 mg/kg. Sperm motility remained unaffected. It is unclear whether these effects are biologically significant. Not available test guidelines.	Pandey *et al,* 1990 (Excel: IIA, 5.4/03) (AgrEvo: ANRA) Published

Test	System	Dosage	Results	Comments	Reference
In vivo mammalian sperm abnormality test	Swiss albino mice	A single oral gavage dose of 3 mg/kg/day for 35 consecutive days. One group of treated mice received endosulfan only; three other groups of mice received endosulfan via gavage plus i.v. injection of vitamin C at 10, 20 or 40 mg/kg/day.	Positive. 14% abnormal sperm in treated mice com-pared with 5% abnormal sperm in controls. Vitamin C reduced the sperm abnormalities.	Endosulfan induced significant decreases in sperm count, but this effect was lessened in mice also administered vitamin C. Endosulfan was a commercial preparation. Thus, it is unclear whether these findings are related to endosulfan or non active constituents. Not available test guidelines. Only a summary from the published paper has been presented, thus the information given is considered insufficient.	Khan and Sinha, 1996 (AgrEvo: ANRA) Published
In vivo mammalian sperm abnormality test	Druckrey rats	A single oral gavage dose of 2.5, 5 and 10 mg/kg/day, on 5 days/week for 70 days	Slightly positive at 5 and 10 mg/kg/day. 7% abnormalities at the high dose com-pared with 6% abnormalities in controls.	At 10 mg/kg/day, 2 rats died during the study. Not available test guidelines. Only a summary from the published paper has been presented, thus the information given is considered insufficient. Endosulfan appears to impair testicular functions by increasing the enzyme activities responsible for spermatogenesis, thus influencing intratesticular spermatid count, and resulting in low sperm production and increased sperm deformities.	Sinha *et al.*, 1995 (AgrEvo: ANRA) Published

Additional information to cover this item has been presented by Calliope. Data were taken from IPCS 40 (1984). According to this document, endosulfan was not mutagenic in *Escherichia coli* or *Salmonella typhimurium* (Fahrig, 1974; Moriya *et al,* 1982). It did not induce mitotic conversion in *Saccharomyces cerevisiae* (Fahrig, 1974). However, in one study, technical grade endosulfan was

reported to induce reverse mutations, cross over, and mitotic gene conversion in *Saccharomyces cerevisiae* (Yadav *et al*, 1982). Negative results were observed in a mouse dominant lethal test (Canada, National Research Council, 1975). Nevertheless, this information is considered insufficient for evaluating the genotoxicity of endosulfan. Original papers should be enclosed. Besides, the review document, IPCS 40 (1984), has not been submitted.

B.6.4.1 *In vitro* studies

B.6.4.1.1 Gene Mutation

B.6.4.1.1.1 Bacterial Gene Mutation

Shirasu, Y., Moriya, M. and Ohta, T., 1978 (AgrEvo: IIA, 5.4.1.1/1) (AgrEvo: Australian National Registration Authority)

Dates of experimental work: The study was performed between 13 September and 12 October, 1978.

The objective of the study was testing of endosulfan for mutagenic effects using microbial systems. This summary only shows endosulfan data corresponding to reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli*.

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

GLP: No (the study was performed prior to GLP regulations).

The study is considered acceptable with some reservations.

Material and methods together with findings:

Endosulfan test substance was Code Hoe 02671 0 I AT101, with purity 98.8-98.9%. It was dissolved in DMSO. The study was conducted using five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) and one strain of *Escherichia coli* (WP2 *hcr*). S9 was derived from the liver of male Sprague-Dawley rats induced with Aroclor 1254. Appropriate positive controls (AF-2, β-propiolactone, 2-NF, 9-AA and 2-AA) were included.

The plate incorporation assay was performed according to published methods (Ames, McCann and Yamasaki, 1975). Endosulfan was tested at concentrations of 5, 10, 50, 100, 500, 1000 and 5000 μg/plate with and without S9 metabolic activation with all strains along with concurrent negative and positive controls.

Endosulfan did not induce any significant increase in the numbers of revertant colonies of any strain over the control values, whether the S9 mix was added or not. Positive controls gave a satisfactory response.

Conclusion

Endosulfan did not induce gene mutation in any of the bacterial tester strains, under the conditions of this study although there are some reservations about its conduct. Thus, endosulfan stability in DMSO was not stated, the titre of bacterial cultures was not reported, toxicity data were not showed, only one experiment was carried out, only two plates per concentration were done, untreated or historical control data were not included, the mean number of revertants per plate and the standard deviation were not showed, the criterion used to evaluate results was not defined, and finally, statistical methods supporting the significance of results were not applied.

Pednekar, M. D., Gandhi, S. R. and Netrawali, M. S., 1987 (Calliope S.A.: IIA, 5.4.1/02)

The study has been published in Bull. Environ. Contam. Toxicol, 38: 925-933.

The objective of this study was to evaluate the mutagenic activities of commonly used insecticides before and after metabolic activation in the Ames *Salmonella* test. This summary describes that part of the publication referring to experiments with endosulfan.

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

GLP: No (at the time of the study, GLP was not required yet by the National Authority).

The study is considered acceptable with some reservations.

Material and methods together with findings:

Endosulfan test substance, with purity 90-94%, was obtained from Excel Industries, Bombay, India. It was dissolved in DMSO. The study was conducted using three strains of *Salmonella typhimurium* (TA100, TA98 and TA97a). Two kinds of metabolic activation were used, a cecal cell-free extract (CCE) of micro-organisms located in the rat cecum, prepared by the procedure of Brown and Dietrich (1979), or S9 from Aroclor 1254 induced male Wistar rat liver. Appropriate positive controls, routine for TA98 (± CCE) and benzo(α)pyrene for TA100 (± S9) were used to show the effectivity in metabolic activation of CCE and S9, respectively.

Two concentrations of endosulfan were tested: A non-toxic (41 mg/L) and a 90% toxic (3256 mg/L). These concentrations were previously determined by measuring the growth (18 h, 37^{o}C) of tester strains. The testing for the mutagenicity of endosulfan at non-toxic concentration was carried out by means the plate incorporation assay (Ames, McCann and Yamasaki, 1975), whereas, the testing at the 90% toxic concentration was performed by using the preincubation method (Maron and Ames, 1983). In both cases, plates were incubated for 48 h and revertant colonies were counted. Values for each treatment level were expressed as the average of six replicates of seven independent experiments ± standard error of the mean.

Results demonstrated non-mutagenicity of endosulfan (at non-toxic and 90% toxic concentrations) either before or after the activation with CCE or S9. Positive controls gave a satisfactory response.

Conclusion

Endosulfan did not induce gene mutation in any of the bacterial tester strains, under the conditions of this study although there are some reservations about its conduct. Thus, OECD Guideline 471 recommends to test at least five concentrations with a minimum of four strains, but in this study only two concentrations were tested in three strains. Positive controls should have been included in each experiment. No individual plate counts were given. The criterion used to evaluate results was not defined. Finally, the regular control of genetic identity was not mentioned.

Dighe, R. P., 1995a (Excel Industries Ltd.: IIA, 5.4/01)

Dates of experimental work: The study was performed between 22 February and 20 March, 1995. Date of report: 5 April, 1995.

The objective of the study was to evaluate the ability of endosulfan and/or its metabolites to induce reverse mutations at the histidine locus in the genome of several strains of *Salmonella typhimurium,* in the presence and absence of mammalian microsomal enzymes (S9).

The study was performed in compliance with the OECD Guideline Test No. 471, although some deviations were observed.

GLP: Yes.

The study is only considered acceptable as an additional information because the purity of the test compound was not given.

Material and methods together with findings

The test substance was endosulfan technical of Excel Industries Ltd, Bombay. It was dissolved in DMSO. The study was conducted using four strains of *Salmonella typhimurium* (TA97a, TA98, TA100 and TA102). S9 was derived from the liver of male Sprague-Dawley rats induced with sodium phenobarbital. Positive controls (MMS, 4-NQO, 2-AF and Danthron) were included.

The dose range finding was performed using the tester strain TA100, both with and without metabolic activation, upto a concentration of 5000 μg/plate. The concentrations of 500 and 5000 μg/plate were found to be equally cytotoxic. Therefore, endosulfan concentrations of 0.05, 0.5, 5, 50 and 500 μg/plate were selected for the plate incorporation mutation assay. Each concentration was tested in triplicate and each experiment was repeated once to check the reproducibility of the results. The mean number of revertants for all the treatment groups was compared with the number in the solvent control group. A test chemical is considered mutagenic if it produces an increase in the number of revertant colonies at least twice the concurrent solvent control group with evidence of positive dose response relationship, in two separate experiments, with any bacterial strain either with or without S9 mix.

Endosulfan did not induce any increase in the number of histidine revertants in any of the tester strains over the solvent control values, whether the S9 mix was added or not. Also there was no dose response relationship. Positive controls gave a satisfactory response.

Conclusion

Endosulfan did not induce gene mutation in any of the bacterial tester strains, under the conditions of this study. Nevertheless, there are some reservations about its conduct with respect to recommendations given by OECD Guideline 471. Thus, the purity of the test substance was not specified, the stability in DMSO was not stated, and TA1535 strain was not included.

B.6.4.1.1.2 Gene mutation in Yeast

Mellano, D. and Milone, M. F., 1984a (AgrEvo: IIA, 5.4.1.3/2) (AgrEvo: Australian National Registration Authority)

Dates of experimental work: The study was performed between 12 and 16 April, 1984. Date of report: 18 June, 1984.

The objective of the study was to determine the mutagenicity of endosulfan in *Schizosaccharomyces pombe*.

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

GLP: Yes.

The study is not required. Thus, it is considered acceptable only as an additional information, and with some reservations.

Material and methods together with findings:

Endosulfan test substance was Code Hoe 002671 0I ZD97 0003, with purity 97.2%. It was dissolved in DMSO. The test article was reported to be stable during the experiment and under the conditions of use. Appropriate positive controls (MMS and DMNA) were included. The test organism was *Schizosaccharomyces pombe*, haploid strain SP *ade* 6-60/*rad* 10-198, *h*-. In this strain colonies are red as a result of a mutation at the sixth of the ten genes that control the adenine biosynthetic pathway. If this base is lacking in the growth medium, the strain accumulates red pigment. When a forward mutation is induced by a test substance in one of the five genes preceding the sixth, pigment fails to accumulate, so the colonies are white. S9 was derived from the liver of male Sprague-Dawley rats induced with Aroclor 1254.

A colony was inoculated into liquid growth medium. Following incubation at 32°C with shaking for 48 hours, the cell concentration was determined by microscopic counting in Thoma chamber. The incubation mixtures were prepared with 2.9 mL of phosphate buffer (- S9) or 1.9 mL of phosphate buffer + 1 mL of S9 mix (+ S9), 0.1 mL of the test article solution and 1 mL of the cell suspension ($550x10^6$ cells/mL). The endosulfan concentrations were chosen on the basis of the preliminary toxicity test being the maximum that produced a survival rate of at least 30%. Endosulfan was tested, with and without S9, at 62.5, 125, 250 and 500 µg/mL (expressed as concentration in the incubation mixture) along with concurrent negative and positive controls. All the incubation mixtures were incubated at 35°C with shaking for 4 hours. Afterwards, an aliquot of the mixture was diluted to give a concentration of about 50,000 cells/mL. An aliquot of this suspension was further diluted (1:10). 0.1 mL of the most concentrated suspension was poured into a test tube containing soft agar, the contents of which were shaken and plated (P), and 14 plates per determination were prepared. Aliquots of the tenfold solution were also plated (Px), and 4 plates were used. All plates were incubated at 32°C for 4 days. Thereafter, the mutated colonies and the total colonies grown were counted respectively in plates P and Px. The total colonies grown for each concentration were assessed by multiplying the mean number of colonies grown on the Px plates by the dilution factor 10, and by the number of P plates. The ratio of mutated colonies to total colonies grown gave the mutation frequency. Survival was calculated by multiplying the total number of colonies grown by 100 and dividing this by the number of cell plated. The relative survival was calculated by multiplying the survival at each concentration by 100 and dividing by the control survival. Comparison of the spontaneous mutant frequency in the negative control incubations with that of the test article incubations and with that of the positive control incubations was done by the χ^2 method ($P < 0.05$).

Up to the highest concentration of endosulfan tested (500 µg/mL) mutation frequency did not differ significantly from the negative control. At this concentration the survival rate of cells was 46% without and 75% with metabolic activation. Positive controls gave a satisfactory response.

Conclusion

Endosulfan did not induce gene mutation in the strain of *Schizosaccharomyces pombe*, under the conditions of this study although there are some reservations about its conduct. The OECD Guideline 480 recommends that at least five concentrations of the test chemical should be used, the highest concentration tested should not reduce survival below 5-10%, the exposure time should be for up to 18 hours, and results should be confirmed in an independent experiment. In this study, only four endosulfan concentrations were tested, at the maximum concentration assayed survival rates were greater than 10%, exposure time was 4 hours, and a single experiment was performed.

Yadav, A. S., Vashishat, R. K. and Kakar, S. N., 1982 (Excel Industries Ltd.: IIA, 5.4/05)
The study has been published in Mutat. Res., 105: 403-407.

The objective of the study was testing two insecticides for their ability to induce genotoxicity in *Saccharomyces cerevisiae.* This summary describes that part of the publication referring only to gene mutation assay with endosulfan.

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

GLP: No

The study is not required. Thus, it is considered acceptable only as an additional information, and with some reservations. Besides, the purity of the test compound was not given.

Material and methods together with findings:
Technical grade endosulfan was obtained from Dr. T. S. Kathpal, Department of Entomology, H.A.U., Hissar (India). It was dissolved in acetone. The study was conducted using *Saccharomyces cerevisiae* strain D_7 (*ade*2-40 / *ade*2-119; *trp*5-12 / *trp*5-27; *ilv*1-92 / *ilv*1-92). This strain is homozigous for *ilv*1-92 and this gene locus will be used for the detection of reverse mutants.

Cells were grown on plates of solid YEPD medium at 30^{o} C for 48 h, suspended in sterile water, washed twice and resuspended in 0.1 M phosphate buffer, pH 7. About 5 x 10^{8} cells/mL were treated with 1% endosulfan because concentrations below this did not cause appreciable lethality. At different times (10, 20 and 30 min), samples were taken. About 300 cells were plated onto each plate of complete medium to score survivors. There were 20 plates for each treatment time. About 10^{7} cells were plated onto of each plate of isoleucineless medium to score revertants. There were 5 plates for each time of treatment. Revertants were scored after 7 days of incubation at 30^{o} C. The detailed method of scoring revertants has been described by Zimmermann (1975) and Vashishat *et al*. (1980).

Endosulfan reduced survival and increased the frequency of revertants over the controls where the cells were treated with 10% acetone. Moreover, acetone was without effect on survival and frequency of revertants over the buffer controls. It is apparent from the results that endosulfan is not only toxic to yeast cells but also genetically effective without any activation. The genotoxic effects of endosulfan became more pronounced as the time of exposure was increased. The highest exposure time (30 min) resulted in 82% killing and the frequency of revertants showed an increase of 6 times over the controls.

Conclusion

Endosulfan induced reverse mutation in *Saccharomyces cerevisiae* strain D_7, under the conditions of this study. Nevertheless, there are some reservations about its conduct with respect to recommmendations given by OECD Guideline 480. Thus, the purity of the test substance was not specified, only one concentration of endosulfan was tested instead of the five recommended, cells were not exposed to endosulfan in the presence of a mammalian metabolic activation system, no positive control was included, the maximum exposure time was 30 min when the recommended is up to 18 h, results were not confirmed in an independent experiment, and no statistical treatment was performed.

B.6.4.1.1.3 Mammalian Gene Mutation

Cifone, M. A. and Myhr, B. C., 1984b (AgrEvo: IIA, 5.4.1.3/1) (AgrEvo: Australian National Registration Authority)

Dates of experimental work: The study was performed between 13 August and 4 September, 1984. Date of report: November, 1984.

The objective of the study was to determine the ability of endosulfan to induce forward mutations at the thymidine kinase (TK) locus as assayed by colony growth of L5178Y $TK^{+/-}$ mouse lymphoma cells in the presence of 5-trifluorothymidine (TFT).

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

GLP: Yes.

The study is acceptable.

Material and methods together with findings

Endosulfan test substance was Code Hoe 002671 0I ZD97 0003, with purity 97.2%. It was dissolved in DMSO. Appropriate positive controls (EMS and MCA) were included. The study was conducted using the mouse lymphoma cell line L5178Y $TK^{+/-}$-3.7.2C derived from the Fischer L5178Y line of Dr. Clive. S9 was commercial and derived from the liver of adult male rats induced with Aroclor 1254.

The assay procedure was based on reports by Clive and Spector (1975) and Clive *et al.* (1979). A preliminary cytotoxicity experiment was performed with a wide range of endosulfan concentrations, starting with 1 mg/ml and followed by two-fold dilution steps. After an exposure of 4 hours, cells were

resuspended in growth medium and incubated for 24 hours. A cell count was determined then to measure the reduction in cell growth relative to the solvent control cell cultures. Endosulfan precipitated at 500-1000 μg/mL, and it was lethal at 250 μg/mL (± S9). For mutation assays, cells obtained from logarithmically growing stock cultures were seeded at $6x10^6$ cells per tube. Endosulfan was tested, in duplicate, at concentrations of 6.25, 12.5, 18, 25, 37.5, 50 and 75 μg/mL (-S9), and at concentrations of 6.25, 12.5, 25, 50, 75 and 100 μg/mL (+S9) along with concurrent negative (solvent) and positive controls. After test material exposure (4 hours), cells were washed, resuspended in growth medium and incubated for 2 days in order to allow recovery, growth and expression of the $TK^{-/-}$ phenotype. Cell densities were determined on Days 1 and 2 (tubes with less than $3x10^5$ cells/ml were not considered). The suspension growth was calculated as (Day 1 cell count/ $3x10^5$) x (Day 2 cell count/ $3x10^5$) for solvent and positive controls. The relative suspension growth for endosulfan-treated cultures was expressed as the percentage of the average solvent control suspension growth. Appropriate cultures were selected for cloning and mutant selection. The total number of mutant colonies was obtained, after 10-14 days of incubation, from $3x10^6$ cells sampled from one culture, seeded into selection medium and divided among 3 culture dishes ($1x10^6$ cells per dish). The total viable colonies was obtained, after 10-14 days of incubation, from 600 cells sampled from one culture, seeded into nonselective medium and divided among 3 culture dishes (200 cells per dish). The cloning efficiency was calculated as (total number of viable colonies/600) x 100, for solvent and positive controls. The relative cloning efficiency of the endosulfan-treated cultures was expressed as the percentage of the average solvent control cloning efficiency. The measurement of the toxicity of each treatment (percent relative growth) was obtained by multiplying the relative suspension growth by the relative cloning efficiency and dividing by 100 that is by definition the percent relative growth corresponding to the solvent control. The ratio of cells seeded for mutant selection to cells seeded for cloning efficiency was 10^4 /2. Therefore, the mutant frequency was calculated by dividing the total mutant colonies by the total viable colonies and multiplying by $2x10^{-4}$, and it was expressed in units of 10^{-6}. The minimum criterion considered necessary to demonstrate mutagenesis for any treatment was a mutant frequency that is at least 150% of the concurrent background frequency plus $10x10^{-6}$. A dose-related or toxicity-related increase in mutant frequency should be observed over at least 3 concentrations, but this depends on the concentrations chosen for the assay and the toxicity at which mutagenic activity appears.

Under nonactivation conditions, the percent relative growths ranged from 99% to 32.1%, without taking account the top concentration (75 μg/mL) because it was lethal. The minimum criterion for mutagenesis in this assay was a mutant frequency exceeding $50.7x10^{-6}$, but the mutant frequencies ranged from $21x10^{-6}$ to $41.6x10^{-6}$. Under activation conditions, the percent relative growths ranged from 85% to 8.6%. The minimum criterion for mutagenesis in this assay was a mutant frequency exceeding $70x10^{-6}$. One of the 100 μg/mL treatments induced a mutant frequency that exceeded the minimum criterion. However, this increase was caused by a sharp decrease in the cloning efficiency due to the very high toxicity (8.6% relative growth) rather than to an increase in total mutant colonies. Besides, the duplicate treatment was nonmutagenic. Positive controls gave a satisfactory response.

Conclusion

Endosulfan was not mutagenic in the mouse lymphoma forward mutation assay, under the conditions of this study. The conduct of the study is considered suitable although it does not meet a specific test guideline and some criteria used to evaluate results may be different from those recommended. The study is acceptable although untreated controls should have been included in each experiment. Finally, the ability to recover small colonies must be convincingly demonstrated when using the L5178Y $TK^{+/-}$ mouse lymphoma assay. The positive control substance should be one capable of producing both small and large colonies. Thus, colonies in positive controls should have been sized to validate the conduct of the assay.

B.6.4.1.2 Chromosome aberration

B.6.4.1.2.1 Chromosome aberration in Yeast

Yadav, A. S., Vashishat, R. K. and Kakar, S. N., 1982 (Excel Industries Ltd.: IIA, 5.4/05)
The study has been published in Mutat. Res., 105: 403-407.

The objective of the study was testing two insecticides for their ability to induce genotoxicity in *Saccharomyces cerevisiae.* This summary describes that part of the publication referring only to the detection of aberrant colonies induced by endosulfan.

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

GLP: No

The study is not required. Thus, it is considered acceptable only as an additional information. Besides, the purity of the test compound was not given.

Material and methods together with findings:
Technical grade endosulfan was obtained from Dr. T. S. Kathpal, Department of Entomology, H.A.U., Hissar (India). It was dissolved in acetone. The study was conducted using *Saccharomyces cerevisiae* strain D_7 (*ade*2-40 / *ade*2-119; *trp*5-12 / *trp*5-27; *ilv*1-92 / *ilv*1-92). This strain combines 2 alleles of the gene locus *ade*2 for monitoring induction of aberrant colonies.

Cells were grown on plates of solid YEPD medium at 30^{o} C for 48 h, suspended in sterile water, washed twice and resuspended in 0.1 M phosphate buffer, pH 7. About 5 x 10^{8} cells/mL were treated with 1% endosulfan because concentrations below this did not cause appreciable lethality. At different times (10, 20 and 30 min), samples were taken. About 300 cells were plated onto each plate of complete medium to score survivors and aberrant colonies. The detailed method of scoring aberrant colonies has been described by Zimmermann (1975) and Vashishat *et al*. (1980).

Endosulfan reduced survival and increased the percentage of aberrant colonies over the controls where the cells were treated with 10% acetone. Moreover, acetone was without effect on survival and frequency of aberrant colonies over the buffer controls. It is apparent from the results that endosulfan is not only toxic to yeast cells but also genetically effective without any activation. The genotoxic effects of endosulfan became more pronounced as the time of exposure was increased. The highest exposure time (30 min) resulted in 82% killing and a 9-fold increase in the percentage of aberrant colonies. Formation of these aberrant colonies at the *ade*2 locus is due to a variety of genetic events such as mutation, gene conversion, chromosome breakage and loss (Zimmermann *et al.,* 1975). The observed increase in the frequency of aberrant colonies by endosulfan treatment indicates that it is capable of inducing chromosome breakage and loss; thus it is clastogenic.

Conclusion

Endosulfan was shown to be clastogenic in *Saccharomyces cerevisiae* strain D_7, under the conditions of this study. This study is not required in evaluating the genotoxic potential of endosulfan. Besides, there are not available guidelines for this assay and the purity of the test substance was not specified.

B.6.4.1.2.2 Mammalian Chromosome aberration

Pirovano, R. and Millone, M. F., 1986 (AgrEvo: IIA, 5.4.1.2/1) (AgrEvo: Australian National Registration Authority)

Dates of experimental work: The study was performed between 9 May, 1985 and 17 January, 1986. Date of report: 20 March, 1986.

The objective of this study was to evaluate the clastogenic potential of endosulfan in cultured human lymphocytes.

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

GLP: Yes.

The study is acceptable with some reservations.

Material and methods together with findings

Endosulfan test substance was Code Hoe 002671 0I ZD97 0003, with purity 97.9%. It was dissolved in DMSO. The test article was reported to be stable until 29 August, 1986. The study was conducted using fresh human blood from a healthy male volunteer. S9 was derived from the liver of male Sprague-Dawley rats induced with Aroclor 1254. Appropriate positive controls (MMC and CP) were included. The dose levels of endosulfan were chosen on the basis of a preliminary toxicity test. The highest dose selected was that produced a suppresion of the mitotic activity of about 50%. Whole blood cultures were set up and cultured with PHA for 48 hours. Then, cells were treated with endosulfan at concentrations of 1, 10, 100, and 200 μg/mL, with and without S9, along with concurrent negative (solvent) and positive controls, for 4 hours. After removal of the chemical, a growth period of 23 hours was used. Colchicine was added 3 hours before the cells were harvested. Mitotic indices were determined as the number of cells in metaphase among 1000 cells. At least 100 metaphases were examined at each endosulfan treatment for structural chromosome aberrations (CA). The classification of CA followed that described by Evans and O'Riordan (1977), and Killian *et al* (1977). The χ^2 method ($P < 0.05$) was used to compare the incidence of aberrations among the groups (Snedecor, 1979).

Endosulfan was toxic at the maximum concentration assayed (200 μg/mL) both with and without metabolic activation. At the next higher concentration (100 μg/mL) the mitotic suppression was 39% and 30% respectively in the absence and in the presence of S9. Up to and including this concentration there were no signs of chromosome aberration, wether the S9 was added or not. Positive controls gave a satisfactory response.

Conclusion

Endosulfan did not exhibit clastogenic activity in cultured human lymphocytes, under the conditions of this study. Nevertheless, there are some reservations about its conduct. The solvent final volume in the culture medium should be less than 1% of the total, but in this study it was about 2%. The OECD Guideline 473 recommends that at least three concentrations of the test chemical should be scored for aberrations, covering a toxicity range spanning > 50% to little or no toxicity; this consideration was not taken into account because the highest concentration selected for determining the incidence of CA (100 μg/mL) resulted in a reduction in mitotic index < 50%. If the test was negative both with and without S9 following the treatment short (4 hours), a continuous treatment without S9 should be tested, i.e., treatment for about 1.5 cell cycle lengths; in this study results were negative but a continuous treatment was not carried out. On the other hand, only 100 metaphases per treatment were scored when the recommended number to be analysed is 200 metaphases. Finally, untreated controls were not included.

Asquith, J. C. and Baillie, J. H., 1989 (AgrEvo: IIA, 5.4.1.2/2) (AgrEvo: Australian National Registration Authority)

Date of report: 16 March, 1989.

The objective of this study was to evaluate the clastogenic potential of endosulfan in cultured human lymphocytes.

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

GLP: Yes.

The study is acceptable with some reservations.

Material and methods together with findings

Endosulfan test substance was Code Hoe 002671 0I ZD95 0005 (Batch C0233/2230), with purity 95.4%. It was dissolved in DMSO. The study was conducted using fresh human blood from two healthy volunteers. S9 was derived from the liver of Fischer 344 rats induced with Aroclor 1254. Appropriate positive controls (MMS and CP) were included.

Two dose rangefinder experiments were carried out, both with and without S9, the first at concentrations up to 1000 μg/mL, the second at concentrations up to 40 μg/mL. Based on the results obtained, concentrations of endosulfan of 10, 20 and 40 μg/mL were selected for the cytogenetic assay. The highest concentration used was that produced a suppression of the mitotic activity of about 50%. Whole blood cultures were set up and cultured with PHA for 44 hours. Then, cells were treated for 3 hours, in the presence and absence of S9, with endosulfan along with concurrent negative (untreated and solvent) and positive controls. All cultures received demecolcine 3 hours before harvesting at 71 hours. The mitotic index for each culture was determined, based on a total of 500 cells per culture. 200 metaphases (100 from each of 2 donors) were examined at each level of treatment for structural chromosome aberrations. The classification system used in scoring aberrations is given in Appendix 1 of this report. The χ^2 method ($P < 0.05$) was used to compare the incidence of aberrations among the groups.

Results for the inhibition of mitosis in the cytogenetic assay are in good agreement with results from the dose rangefinder experiments. Thus, it can be said that endosulfan at the highest concentration (40 μ g/mL) caused about 50% inhibition of mitosis in both the absence and the presence of S9. All cultures treated with endosulfan had more aberrations scored than the solvent controls. However, no dose response relationships were seen and only one point (10 μg/mL, with S9) gave a significant increase in aberrations compared to the control. Nevertheless, the total number of aberrations in this point was low (3%) and within the accepted range for aberrations in solvent controls (0-5%), the solvent control showed no aberrations, and when the data were reanalysed statistically against the untreated control, in

which 1 aberration per 100 cells was found, no statistical difference was seen. Positive controls gave a satisfactory response.

Conclusion

Endosulfan did not exhibit clastogenic activity in cultured human lymphocytes, under the conditions of this study. Nevertheless, there are some reservations about its conduct. The OECD Guideline 473 recommends that the highest concentration selected for determining the incidence of CA should cause a reduction in mitotic index greater than 50%, and in this study the highest concentration tested produced a borderline effect. If the test was negative both with and without S9 following the treatment short (3 hours), a continuous treatment without S9 should be tested, i.e., treatment for about 1.5 cell cycle lengths; in this study results were negative but a continuous treatment was not carried out.

B.6.4.1.3 DNA effects

Shirasu, Y., Moriya, M. and Ohta, T., 1978 (AgrEvo: IIA, 5.4.1.1/1) (AgrEvo: Australian National Registration Authority)

Dates of experimental work: The study was performed between 13 September and 12 October, 1978.

The objective of the study was testing of endosulfan for mutagenic effects using microbial systems. This summary only shows endosulfan data corresponding to the rec-assay with *Bacillus subtilis.*

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

GLP: No (the study was performed prior to GLP regulations).

This study is not required. Thus, it is considered acceptable only as an additional information.

Material and methods together with findings:

Endosulfan test substance was Code Hoe 02671 0 I AT101, with purity 98.8-98.9%. It was dissolved in DMSO. Kanamycin and mitomycin C were used as a negative and a positive control, respectively. The rec-assay was conducted using two strains of *Bacillus subtilis*: H17 Rec^+, the repair-proficient strain, and M45 Rec^-, the repair-deficient strain.

The assay was performed according to published methods (Kada, 1973; Shirasu *et al*, 1976). Cultures of the two strains were streaked on the surface agar and the starting points were covered with a paper disk. Then, 0.02 ml solution of the sample, containing 20, 100, 200, 500, 1000 and 2000 μg of endosulfan, were dropped on disc paper. The length of the inhibitory zone of each streak was measured after overnight incubation at 37^o C.

Endosulfan did not cause difference in growth inhibition between H17 Rec^+ and M45 Rec^-. Positive control gave a satisfactory response.

Conclusion

Endosulfan was not genotoxic under the conditions of this study. Nevertheless, this study is not required in evaluating the genotoxic potential of endosulfan. Besides, there are not available guidelines for this assay and the information given was only qualitative.

Yadav, A. S., Vashishat, R. K. and Kakar, S. N., 1982 (Excel Industries Ltd.: IIA, 5.4/05)

The study has been published in Mutat. Res., 105: 403-407.

The objective of the study was testing two insecticides for their ability to induce genotoxicity in *Saccharomyces cerevisiae.* This summary describes that part of the publication referring only to mitotic recombination assay with endosulfan.

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

GLP: No.

The study is not required. Thus, it is considered acceptable only as an additional information, and with some reservations. Besides, the purity of the test compound was not given.

Material and methods together with findings:

Technical grade endosulfan was obtained from Dr. T. S. Kathpal, Department of Entomology, H.A.U., Hissar (India). It was dissolved in acetone. The study was conducted using *Saccharomyces cerevisiae* strain D_7 (*ade*2-40 / *ade*2-119; *trp*5-12 / *trp*5-27; *ilv*1-92 / *ilv*1-92). This strain combines 2 alleles of the gene locus *ade*2 for monitoring mitotic crossing-over and 2 alleles of the gene locus *trp*5 for scoring mitotic gene convertants.

Cells were grown on plates of solid YEPD medium at 30^{o} C for 48 h, suspended in sterile water, washed twice and resuspended in 0.1 M phosphate buffer, pH 7. About 5 x 10^{8} cells/mL were treated with 1% endosulfan because concentrations below this did not cause appreciable lethality. At different times (10, 20 and 30 min), samples were taken. About 300 cells were plated onto each plate of complete medium to score survivors and mitotic cross-overs. There were 20 plates for each treatment time. About 10^{5} cells were plated onto of each plate of tryptophanless medium to score gene convertants. There were 5 plates for each time of treatment. Mitotic cross-overs and gene convertants were scored after 5 days of incubation at 30^{o} C. The detailed method of scoring mitotic cross-overs and gene convertants has been described by Zimmermann (1975) and Vashishat *et al*. (1980).

Endosulfan did not induce mitotic cross-over but reduced survival and increased the frequency of gene convertants over the controls where the cells were treated with 10% acetone. Moreover, acetone was without effect on survival and frequency of mitotic cross-over and gene convertants over the buffer controls. It is apparent from the results that endosulfan is not only toxic to yeast cells but also

genetically effective without any activation. The genotoxic effects of endosulfan became more pronounced as the time of exposure was increased. The highest exposure time (30 min) resulted in 82% killing and the frequency of convertants showed an increase of 4 times over the controls.

Conclusion

Endosulfan induced mitotic gene conversion but not mitotic crossing-over in *Saccharomyces cerevisiae* strain D_7, under the conditions of this study. This study is not required in evaluating the genotoxic potential of endosulfan and besides, there are some reservations about its conduct with respect to recommendations given by OECD Guideline 481. Thus, the purity of the test substance was not specified, only one concentration of endosulfan was tested instead of the five recommended, cells were not exposed to endosulfan in the presence of a mammalian metabolic activation system, no positive control was included, the maximum exposure time was 30 min when the recommended is up to 18 h, results were not confirmed in an independent experiment, and no statistical treatment was performed.

Mellano, D. and Milone, M. F., 1984b (AgrEvo: IIA, 5.4.2.2/2) (AgrEvo: Australian National Registration Authority)

Dates of experimental work: The study was performed between 13 and 17 April, 1984. Date of report: 18 June, 1984.

The objective of the study was to determine the genotoxic potential of endosulfan by means the mitotic gene conversion assay in *Saccharomyces cerevisiae*. The detection of DNA-damaging agents by this assay is based on the production of prototrophic revertants in an auxotrophic heteroallelic strain carrying two different defective alleles of the same gene.

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

GLP: Yes.

The study is not required. Thus, it is considered acceptable only as an additional information, and with some reservations.

Material and methods together with findings

Endosulfan test substance was Code Hoe 002671 0I ZD97 0003, with purity 97.2%. It was dissolved in DMSO. The test article was reported to be stable during the experiment and under the conditions of use. Appropriate positive controls (MMS and CP) were included. The study was conducted using *Saccharomyces cerevisiae* D_4 (heteroallelic at *ade 2* and *trp5*). S9 was derived from the liver of male Sprague-Dawley rats induced with Aroclor 1254.

The endosulfan concentrations were chosen on the basis of the preliminary toxicity test being the maximum that produced a survival rate of at least 50%. Endosulfan was tested, with and without S9, at 100, 500, 1000 and 5000 μg/mL (expressed as concentration in the incubation mixture) along with

concurrent negative and positive controls. The incubation mixtures were prepared with 2.9 mL of phosphate buffer (- S9) or 1.9 mL of phosphate buffer + 1 mL of S9 mix (+ S9), 0.1 mL of the test article solution and 1 mL of the cell suspension ($500x10^6$ cells/mL). All the incubation mixtures were incubated at 35°C with shaking for 4 hours. Afterwards, aliquots of the mixture were diluted 10- and 10^5-fold. Aliquots of 0.1 mL of the 10^5-fold diluted suspension were deposited on each 4 plates containing complete medium and 0.1 mL aliquots of the 10-fold diluted suspension were deposited on 4 plates containing selective medium with adenine and 4 containing selective medium with tryptophan. All plates were incubated at 32°C for 4 days. Following incubation, the total number of colonies plated on complete medium and the number of convertants in the plates with selective medium were counted. The convertant frequency for the *trp5* gene is the ratio of the mean number of colonies growing on selective medium with adenine to the mean number per 10^4 colonies growing on complete medium. Similarly, the convertant frequency for the *ade 2* gene is the ratio of the mean number of colonies growing on selective medium with tryptophan to the mean number per 10^4 colonies growing on complete medium. Comparison of the spontaneous mutant frequency in the negative control incubations with that of the test article incubations and with that of the positive control incubations was done by the χ^2 method ($P < 0.05$).

Up to the highest concentration of endosulfan tested (5000 μg/mL) covertant frequency did not differ significantly from the negative control. At this concentration the survival rate of cells was 52% without but still 100% with metabolic activation. Positive controls gave a satisfactory response.

Conclusion

Endosulfan did not induce mitotic gene conversion in *Saccharomyces cerevisiae*, under the conditions of this study although there are some reservations about its conduct. The OECD Guideline 481 recommends that at least five concentrations of the test chemical should be used, the highest concentration tested should not reduce survival below 5-10%, the exposure time should be for up to 18 hours, and results should be confirmed in an independent experiment. In this study, only four endosulfan concentrations were tested, at the maximum concentration assayed survival rates were greater than 10%, exposure time was 4 hours, and a single experiment was performed.

Sobti, R. C., Krishan, A. and Davies, J., 1983 (Calliope S.A.: IIA, 5.4.1/01) (Excel Industries Ltd.: IIA, 5.4/09)

The study has been published in Arch. Toxicol, 52: 221-231.

The objective of this study was to evaluate the cytogenetic effect of eight different organochlorine pesticides using the *in vitro* SCE test with a human lymphoid cell line. This summary describes that part of the publication referring to experiments with endosulfan.

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

GLP: No (at the time of the study, GLP was not required yet by the National Authority).

The study is considered acceptable only as an additional information because it is not required. Besides, the purity of the test compound was not given.

Material and methods together with findings:

Endosulfan test substance was obtained from Dr. Carl D. Pfaffenberg, Division of Chemical Epidemiology, University of Miami Medical School, FL, USA. It was dissolved in absolute ethyl alcohol (ETOH). The study was conducted using the human lymphoid cell line of B cell origin, LAZ-007, stablished by Dr. H. Lazarus of Sidney Farber Cancer Center, Boston, Mass., USA. Rat liver microsomal S9 was obtained from Litton Bionetics Inc., Kensington, MD, USA. Cyclophosphamide was only used to test the metabolic activating potential of the S9 mix.

Cultures in triplicate of human lymphoid LAZ-007 cells (0.4 x 10^{-6} cells/mL) were treated with different concentrations of endosulfan (10^{-4}, 10^{-5} and 10^{-6} M) for 48 h without S9 mix, and for 1 h with and without S9 mix. The control cultures were treated with 0.1% of ETOH. Cultures were incubated in dark for 48 h, in the presence of BrdU. Colchicine was added 4 h before the cells were harvested. Chromosome preparations were made and stained for SCE analysis. A minimum of 25 metaphases were scored for each dose level and the data were analysed by using two sample t-test at 1% significance level (Colton, 1980). 25 metaphases were also scored to determine the percentage of first (M_1), second (M_2) and third (M_3) mitoses. Viable cell counts (dye-excluding) were taken in a hemocytometer after staining cells with trypan blue.

In cultures incubated with endosulfan for 48 h, viable count was 66% (10^{-4} M), M_1's ranged from 1% (10^{-6} M) to 7% (10^{-4} M) as compared to 0% in control cultures, M_3's ranged from 15% (10^{-6} M) to 10% (10^{-4} M) as compared to 17% in control cultures, and the frequency of SCEs increased significantly at the three concentrations tested over that of the control. In cultures incubated with endosulfan and S9 mix for 1 h no significant increase in SCE frequency values with activation was seen at any concentration tested over that of non-activated cultures. The gene-tox program committee (Latt *et al*, 1981) has recommended that only those chemicals which at least double the SCE frequency over that of control should be considered positive. According to these guidelines, endosulfan was only positive at 10^{-4} M after 48 h incubation.

Conclusion

Endosulfan was shown to be a potent inducer of SCE as well as an inhibitor of cell cycle traverse, under the conditions of this study. Metabolic activation did not enhance the potency of endosulfan. Nevertheless, there are some reservations about its conduct with respect to recommendations given by OECD Guideline 479. Thus, the purity of the test substance was not specified, positive controls were not included in each experiment, the mean number of SCEs per chromosome was not given, and the criterion used for scoring SCEs was not defined.

Cifone M. A. and Myhr, B. C., 1984a (AgrEvo: IIA, 5.4.2.2/1) (AgrEvo: Australian National Registration Authority)

Dates of experimental work: The study was performed between 17 July and 26 September, 1984. Date of report: November, 1984.

The objective of this study was to assess the potential of endosulfan to induce unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes.

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

GLP: Yes.

The study is considered acceptable with some reservations.

Material and methods together with findings

Endosulfan test substance was Code Hoe 002671 0I ZD97 0003, with purity 97.2%. It was dissolved in DMSO. The positive control was 2-AAF. Hepatocytes were isolated from a single male Fischer 344 rat.

Primary cultures of rat hepatocytes were prepared according to the method described by Williams (1977, 1980). Viability of hepatocytes was measured by tripan blue exclusion. The hepatocytes for the UDS assay were collected at approximately 86.8% viability. Then, culture dishes containing plastic coverslips were inoculated with approximately 0.5 x 10^6 viable cells in 3 mL of WME plus dexamethasone and 5% serum per dish. About 78.8% of the viable cells attached to the culture dishes during the 1.5 hour settling period. The treatments were initiated approximately 3 hours later with cell monolayers that were about 91.6% viable. Fifteen endosulfan concentrations from 0.026 to 1020 µg/mL were prepared. The test material appeared soluble up to 51µg/mL. Thus, for UDS assay cells were exposed to endosulfan at selected concentrations from 0.102 to 51 µg/mL for 18 hours in the presence of ^{3}H-thymidine. Negative (solvent) and positive (2-AAF) controls were also run. Five cultures were prepared per treatment group. Two of them were used for cytotoxicity measurements, these were refed with WME medium and at 22 hours after the initiation of treatments, viable cell counts were determined to estimate cell survival relative to the negative control that was approximately 98% of the viable count at the beginning of the treatments. The resting three cultures were used for UDS assay, they were washed and fixed. The coverslips were mounted on microscope slides and prepared to autoradiography (exposure time was 7-10 days). The slides were stained with hematoxylin-eosin. UDS was measured by autoradiography and quantified by a nuclear increase of silver grains. Nuclear grains were counted in 50 cells on each of 3 slides per treatment. The net nuclear count was determined by substracting the mean of three adjacent cytoplasmatic counts from the nuclear count. Those cells undergoing semiconservative DNA synthesis (DNA replication) were easily visualised because of their completely blackened nuclei and were not counted. The minimum criteria for UDS in this trial were a mean net nuclear grain count exceeding 6.69 or at least 10% of the nuclei containing 6 or more net

grains, or at least 2% of the nuclei containing 20 or more net grains. Any one of the criteria and the existence of a dose-related response are sufficient evidence for UDS.

Endosulfan was lethal at 51 μg/mL. Survival increased to 31.5% at 25.5 μg/mL and cells were enlarged and rounded indicating toxicity. Cells returned to normal appearance and number at 2.55μg/mL (101.3% survival). Seven treatments were selected for analysing UDS activity (0.102, 0.255, 0.51, 1.02, 5.1, 10.2 and 25.5 μg/mL). Endosulfan did not induce changes in the nuclear labelling and no evidence for a dose-related response was obtained. Positive control gave a satisfactory response.

Conclusion

Endosulfan was not genotoxic under the conditions of this study. Nevertheless, the OCDE Guideline 482 recommends that data should be evaluated using appropriate statistical methods, in this study no statistical treatment was applied. Besides, negative and equivocal results should be confirmed in an independent experiment.

Müller, W., 1988b (AgrEvo: IIA, 5.4.2.2/3) (AgrEvo: Australian National Registration Authority)

Dates of experimental work: The study was performed between 14 and 28 January, 1988. Date of report: 2 February, 1988.

The objective of this study was to assess the potential of endosulfan to induce unscheduled DNA synthesis (UDS) in the human cell line A 549.

The study was performed according to the OECD-guideline (Gen 85.4, Genetic Toxicology: DNA Damage and Repair / Unscheduled DNA Synthesis in Mammalian Cells *in vitro*, 1986) and the EPA-guideline (Unscheduled DNA Synthesis in Mammalian Cells in Culture, HG-DNA-Unsched., August 1982).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

Endosulfan test substance was Code Hoe 002671 0I ZD95 0005 (Batch C0233/2330), with purity 95.5%. It was dissolved in DMSO. The study was conducted using the permanent human cell line A 549 (American Type Culture Collection no CCL 185). S9 was derived from the liver of male Sprague Dawley rats induced with Aroclor 1254. Appropriate positive controls (NQO and BP) were included.

$4x10^5$ cells/35 mm culture dish were seeded and cultured. Six cultures were used for each experimental point. Two days before the start of the experiment the medium was replaced by an arginine-deficient medium which contains 10 mM hydroxyurea to reduce or inhibit semi-conservative DNA replication. The incubation of the test substance at various concentrations, with and without S9, was performed at

37° C for 3 hours. Tritiated thymidine was added to the cell culture immediately after the test compound. Three experiments were carried out. In Experiment 1, cells were exposed to endosulfan at 1, 3, 10, 30, 100, 300 and 1000 µg/mL (-S9). In Experiments 2 and 3, cells were exposed to endosulfan at 0.1, 0.3, 1, 3, 10, 30 and 100 µg/mL (± S9). Concurrent negative (untreated and solvent) and positive controls were included in each experiment. At the end of the incubation period DNA was extracted from the cells. The DNA concentration was determined colorimetrically using the diphenylamine reaction of deoxyribonucleic acid (Burton, 1956). The incorporation of radiolabel into DNA was determined by liquid scintillation counting. Results were given as dpm/µg DNA and data were statistically evaluated using Student's t-test.

Visible microscopic alterations of the cell morphology were observed with endosulfan at 100, 300 and 1000 µg/mL indicating toxicity. No relevant reproducible increase in the rate of UDS was observed at any concentration of endosulfan. Positive controls gave a satisfactory response.

Conclusion

Endosulfan was not genotoxic under the conditions of this study. The study is considered acceptable. Nevertheless, it should be advisable to justify the treatment time of 3 hours and to report the cell density obtained at time of treatment.

B.6.4.2 *In vivo* studies in mammalian somatic cells

B.4.4.2.1 Chromosome aberration

Dikshith, T. S. S. and Datta, K. K., 1978 (Calliope S.A.: IIA, 5.4.2/01) (AgrEvo: Australian National Registration Authority)

The study has been published in Bull. Environ. Contam. Toxicol., 20: 826-833.

The objective of this study was to evaluate the ability of endosulfan to induce chromosome aberrations in somatic and germinal cells of male rats. This summary describes only that part of the publication referring to experiments with somatic cells.

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

GLP: No (at the time of the study, GLP was not required yet by the National Authority).

The study is considered acceptable only as an additional information. The main reason is that purity of the test compound was not given. Besides, there are some reservations about its conduct.

Material and methods together with findings:

Endosulfan test substance was obtained from National Chemical Laboratory, Poona, India. It was suspended in peanut oil. The study was conducted using male albino rats.

Animals were divided into five groups, with 8 animals in each group. Rats of group 1, 2, 3 and 4 were administered 11, 22, 36.6 and 55 mg/kg of endosulfan respectively, and rats of group 5 (control) were given peanut oil alone. The administration was by oral intubation for 5 consecutive days. Colchicine was administered to each rat by i. p. injection 4 h before killing by decapitation. Bone marrow cells were collected and stained. Scoring was made to determine mitotic index and chromosome damage. The mitotic index was calculated as % cells at metaphase after scoring a total of 100 cells per slide. Chromosome damage was analysed for chromatid breaks, chromosome breaks and exchange figures scoring 50 metaphases per treatment.

All animals dosed at 36.6 and 55 mg/kg died before 24 h; two animals dosed with 22 mg/kg died after 72 h. There were no death in the control and lowest dose group. No major chromosomal aberrations were observed, only chromatid breaks and 1 to 2 exchange figures. There was no chromosomal deletion nor formation of large number of fragments. No significant mitotic inhibition was seen in any of the treated groups.

Conclusion

Endosulfan did not induce any significant chromosome damage in rat bone marrow cells, under the conditions of this study. Nevertheless, the scientific validity of the study is questionable for several reasons. OECD Guideline 475 gives recommendations which have not been observed in this study. Thus, purity of the test substance was not specified; the volume of liquid administered was not given; the strain, age and source of animals used were not reported; only male animals were employed; no positive control was included; the time of sacrifice after administration of the last dose was not given; at least 100 cells should have been analysed for each animal (500 cells per treatment) but only 50 cells per treatment were analysed for chromosomal aberrations; the criterion for scoring aberrations was not defined; mitotic indices and frequencies of aberrations were not reported; no information was given about the number of cells analysed per animal, the type and number of aberrations (given separately for each animal), the total number of aberrations per group and the number of cells with aberrations per group; and, finally the significance of results was not justified because data were not analysed statistically.

Dzwonkowska, A. and Hübner, H., 1986 (Excel Industries Ltd.: IIA, 5.4/07) (AgrEvo: Australian National Registration Authority)

The study has been published in Arch. Toxicol., 58: 152-156.

The objective of this study was to determine the potential clastogenicity of different insecticides. This summary describes that part of the publication referring to experiments with endosulfan only.

No reference to a specific test guideline was made.

GLP: No.

The study is considered acceptable as an additional information. The main reason is that the test compound was a commercial preparation. Besides, there are some reservations about its conduct.

Material and methods together with findings

Endosulfan test substance was Thiodan 35, a commercial preparation with a content of 35% endosulfan, obtained from Farbwerke Hoeschst AG, FRG. It was dissolved in water. The study was conducted using female Syrian golden hamsters (*Mesocricetus auratus)*.

The LD_{50} dose was established for Syrian hamsters after a single i.p. injection of the compound for 24 h (LD_{50} = 80 mg/kg). Endosulfan was administered by i.p. injection at doses of 80, 40, 16 and 8 mg/kg in aliquots of 1 mL/100 g. Dose groups consisted of 6 animals. 8 hamsters which had not been treated with any compound constituted negative controls. A positive control was also obtained by injecting i.p. 6 animals with cyclophosphamide. Animals were sacrificed at 24 h. Colchicine was administered to each animal by i. p. injection 2 h before bone marrow was isolated. Chromosome preparations were made according to the method of Killian *et al.* (1977) by the ignition-drying technique. Staining was performed with Giemsa. Average mitotic index was calculated for each dose. At least 50 metaphases obtained from the bone marrow of each hamster were analyzed, though in some cases, due to a low mitotic index, this was not possible. Chromosome damage was analysed for chromatid breaks, chromosome breaks and exchange figures. The activity of endosulfan was compared with results of negative and positive controls, using a proportions difference test, which is an alternative to the χ^2 test (Blalock, 1960).

Endosulfan induced a statistically-significant increase in the number of total aberrations at all doses tested. A statistically-significant increase in the number of aberrations were also observed at doses of 8 and 80 mg/kg when gaps were excluding, and the number of aberrations did not differ from the positive control at the highest dose.

Conclusion

Endosulfan was shown to be clastogenic in hamster bone marrow cells, under the conditions of this study. Nevertheless, there are some reservations for accepting this information as appropriate to evaluate the clastogenicity of endosulfan. The test substance was thiodan 35 (commercial endosulfan) and OECD Guideline 475 gives recommendations which have not been observed in this study. Thus, only females were used without justification for this election, samples should have been taken at two separate times following treatment but only one sample was taken 24 h after treatment; 500 cells per treatment (100 cells for each animal) should have been analysed for chromosomal aberrations but in some cases this was not possible (535, 241, 466 and 100 cells were analyzed per each treatment of 8, 16, 40 and 80 mg/kg, respectively); the type and number of aberrations were not reported separately for each animal; although the highest dose tested was the LD_{50} no information was given about deaths or signs of toxicity for animals and the mitotic index at all doses of endosulfan tested was higher than the mitotic index corresponding to the negative control.

Rani, M. V. U., Reddi, O. S. and Reddy, P. P., 1980 (Calliope S.A.: IIA, 5.4.2/02) (Excel Industries Ltd.: IIA, 5.4/06)

The study has been published in Bull. Environ. Contam. Toxicol., 25: 277-282.

The objective of this study was to determine the potential genotoxicity of different pesticides. This summary describes that part of the publication referring to the micronucleus test with endosulfan.

No reference to a specific test guideline was made.

GLP: No (at the time of the study, GLP was not required yet by the National Authority).

The study is considered acceptable only as an additional information. The main reason is that purity of the test compound was not given. Besides, there are some reservations about its conduct.

Material and methods together with findings

Endosulfan test substance was dissolved in sterile distilled water. The study was conducted using Swiss albino male mice.

4 mice were orally fed with endosulfan at 43.3 mg/kg. Each animal received the dose in two equal instalments separated by an interval of 24 h. Control animals (6 mice) were treated in an identical manner with distilled water. Mice were sacrificed by cervical dislocation 6 h after administration of the second dose. Air-dried smears of the bone marrow were prepared by the method of Schmid (1975). From each animal 2000 polychromatic erythrocytes (PCE) and corresponding number of normochromatic erythrocytes (NCE) were enumerated and examined microscopically for micronuclei. In addition, the ratio of PCE:NCE was determined.

Endosulfan showed no significant differences from control in the % of micronucleated PCE, the % of micronucleated NCE and the ratio PCE:NCE ($P > 0.05$).

Conclusion

Endosulfan was considered negative in the mouse bone marrow micronucleus test, under the conditions of this study. Nevertheless, the scientific validity of the study is questionable for several reasons. OECD Guideline 474 gives recommendations which have not been observed in this study. Thus, purity of the test substance was not specified; the volume of liquid administered was not given; the age, source, housing conditions and diet of animals used were not reported; only male animals were employed; only 4 animals were used in the endosulfan group instead of the minimum of 5/group required; no positive control was included; animals were killed 6 h after the second dose, whereas sampling times should be not earlier than 12 h after treatment; only one dose was tested; no indication of toxicity of the applied dose was given; the criterion for scoring micronuclei was not defined; the number of micronucleated PCE was not given separately for each animal; the number of

micronucleated PCE per group was not expressed as mean ± standard deviation; the applied statistical analysis was not indicated.

Müller, W., 1988a (AgrEvo: IIA, 5.4.2.1/1) (AgrEvo: Australian National Registration Authority)
Dates of experimental work: The study was performed between 9 and 12 November, 1987. Date of report: 2 February, 1988.

The objective of this study was to determine the potential of endosulfan to induce micronuclei in the bone marrow polychromatic erytrocytes of NMRI mice.

The study was performed according to the EPA-guideline, *In vivo* mammalian bone marrow cytogenetic tests: micronucleus assay, HG-Chromo-Micronuc, August 1982, and took into consideration the proposals and recommendations given in the EPA Gene-Tox Program, 1983.

GLP: Yes.

The study is considered acceptable with some reservations derived from the highest dose tested.

Material and methods together with findings
Endosulfan test substance was Code Hoe 002671 0I ZD95 0005 (Batch C0233/2230), with purity 95.5%. It was dissolved in sesame oil. The test compound dilutions were prepared fresh each day. The study was conducted using male and female NMRI mice, strain NMRKf (SPF71).

The dose levels for micronucleus testing were selected on the basis of a preliminary study to determine the acute toxicity and the maximal applicable dose. Oral administration of endosulfan at 15 and 20 mg/kg caused partial lethality in male and female mice. The highest sublethal dose of 10 mg/kg was selected for the main study. Endosulfan was administered by single oral gavage to mice at 0 (sesame oil control), 2.5, 5 and 10 mg/kg in aliquots of 10 mL/kg. Dose groups consisted of 5 males and 5 females for each sacrifice time (24, 48 and 72 hours post treatment). Animals treated with Endoxan^{R} and sacrificed at 24 hours served as positive controls. Bone marrow cells were collected and stained. 1000 immature polychromatic erythrocytes (PCE) were counted from each animal and examined microscopically for micronuclei. As a control measure 1000 mature normochromatic erythrocytes (NCE) were also counted and examined for micronuclei. In addition, the ratio of PCE:NCE was determined. The results of the treatment group in the micronucleus test at each dose and killing time were compared with corresponding control values according to Wilcoxon (paired, one-sided, increase). The ratio of PCE:NCE was also statistically evaluated by the method of Wilcoxon (paired, two-sided). The statistical evaluations were performed using the “Diamant” computer program, version 2.0. All statistical results were based on a 95% level of significance. Actual data were also compared with historical controls.

All animals survived after application endosulfan at 10 mg/kg. The following signs of toxicity were observed: increased spontaneous of activity, stilted gait, clonic convulsions, trembling, back-arched position, narrowed palpebral fissures, forward movement in crawling posture. Six hours after application all animals were free of clinical signs of toxicity. The dissection of the animals revealed no macroscopic findings. The incidence of both micronucleated PCE and micronucleated NCE in animals treated with endosulfan was within the normal range of the negative control groups. Because of individual variations, the ratio of PCE:NCE lied outside of the normal range after 24, 48 and 72 hours in the male control groups and after 48 hours in the female treatment group of 5 mg/kg. This effect was considered as of no toxicological significance. The positive control gave a satisfactory response.

Conclusion

Endosulfan was considered negative in the mouse bone marrow micronucleus test, under the conditions of this study. Nevertheless, the OECD Guideline 474 recommends that the highest dose tested should be the maximum tolerated dose based on mortality or bone marrow cell toxicity. Although the highest dose tested was about 50% of calculated in the preliminary study LD_{50}, 6 hours post-treatment clinical signs observed in mice disappeared and any indication of toxicity in the bone marrow was observed.

Dighe, R. P., 1995b (Excel Industries Ltd.: IIA, 5.4/02)

Dates of experimental work: The study was performed between 18 March and 15 June, 1995. Date of report: 30 June, 1995.

The objective of this study was to determine the potential clastogenicity of endosulfan, measured as the frequency of micronucleated polychromatic erythrocytes.

The study was performed in compliance with the OECD Guideline Test No. 474, although some deviations were observed.

GLP: Yes.

The study is considered acceptable only as an additional information because the purity of the test compound was not given. Besides, there are some reservations derived from the dose tested.

Material and methods together with findings

The test substance was endosulfan technical of Excel Industries Ltd, Bombay. It was suspended in corn oil. The positive control was MMS. The study was conducted using Swiss albino male and female mice.

The dose selected was the maximum tolerated dose which was ascertained by the dose range finding of acute toxicity studies. Each animal was administered 0.2 mL of endosulfan suspension, by gavage, at the dose of 6 and 8 mg/kg to male and female animals respectively. The positive and vehicle controls were administered by the same route. Each group included 10 animals (5 males and 5 females). Mice were sacrificed by cervical dislocation at 18, 24 and 30 h after treatment. Bone marrow was aspirated and smears were made. The slides were air-dried, and stained with May-Grünwald and Giemsa. From each animal about 1000 polychromatic erythrocytes (PCEs) and the number of normochromatic erythrocytes (NCEs) observed in the same field were counted and recorded. The percent of micronucleated PCEs and the ratio of PCE to NCE were calculated. Data was evaluated for statistical significance by using χ^2 and Student's t-test.

Endosulfan showed no significant differences from control in the % of micronucleated PCE and the ratio PCE:NCE ($p > 0.05$).

Conclusion

Endosulfan was considered negative in the mouse bone marrow micronucleus test, under the conditions of this study. Nevertheless, the scientific validity of the study is questionable for several reasons. OECD Guideline 474 gives recommendations which have not been observed in this study. Thus, purity of the test substance was not specified; stability in vehicle was not indicated; although in 1983 OECD Guideline 474 recommended to use only the maximum tolerated dose for a initial assessment of genotoxicity, at present this guideline recommends to use three dose levels and defines the highest dose tested as the dose producing some clinical signs in the animals or some indication of toxicity in the bone marrow; the criterion for scoring micronuclei was not defined; the number of micronucleated PCE per group was not expressed as mean ± standard deviation.

Jung, Weigand and Kramer, 1983 (AgrEvo: Australian National Registration Authority)

Date of report: 3 October 1983.

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

No reference to a specific test guideline.

No reference to GLPs.

The study is considered acceptable only as an additional information because it is summary of a report which does not include the required information.

Material and methods together with findings

NMRI mice (5/sex/group) were administered endosulfan technical, by gavage, at single doses of 0, 0.2, 1 and 5 mg/kg. The doses were repeated after an interval of 24 h. The animals were killed 6 h after the second dose and bone marrow smears prepared from each animal. The number of cells with micronuclei was recorded out of 2000 polychromatic erythrocytes counted for each animal.

The positive control (cyclophosphamide) induced a marked and significant increase in the number of cells with micronuclei in both sexes; endosulfan was without cytogenic activity at all doses tested.

Conclusion

Endosulfan was considered negative in the mouse bone marrow micronucleus test, under the conditions of this study. However, the information given is only a summary which is considered insufficient for evaluating the mutagenicity of endosulfan. The full report should be enclosed.

B.6.4.3 *In vivo* studies in germ cells from *Drosophila Melanogaster*

B.6.4.3.1 Gene Mutation

Velázquez, A., Creus, A., Xamena, N. and Marcos, R. 1984 (Calliope S. A.: IIA, 5.4.3/02) (Excel Industries Ltd.: IIA, 5.4/04)

The study has been published in Mutat. Res., 136: 115-118.

The objective of this study was to determine the genetic damage produced by endosulfan in germ cells using the sex-linked recessive lethal (SLRL) and the sex-chromosome loss (SCL) tests in *Drosophila melanogaster*. This summary describes only that part of the publication referring to the evaluation of induction of sex-linked recessive lethal.

No reference to a specific test guideline was made.

GLP: No (at the time of the study, GLP was not required yet by the National Authority).

The study is considered acceptable only as an additional information because it is not required. Besides, the test substance was a commercial preparation and there were some reservations about its conduct.

Material and methods together with findings

Endosulfan test substance was 50% wettable power (50% active ingredient, 50% kaolin, dispersing + wetting agents), obtained from Hoeschst Ibérica. It was first dissolved in DMSO and then diluted with a 5% sucrose solution to give a final DMSO concentration of 1% and the desired insecticide concentration (based on the active ingredient). The SLRL test was performed with two strains of *Drosophila melanogaster*. *Berlin-K* wild-type males were treated and crossed to *Basc* females.

Males were exposed to endosulfan during the larval and adult stages by means the following procedures: 1º) Eggs were collected from 4-day-old *Berlin-K* females and put into culture bottles containing standard food medium, and 24 h later, test solution (50 and 100 ppm active ingredient) was dropped on the surface of the food of first-instar larvae. 2º) For adult treatments, *Berlin-K* males 2-3 days old were starved for 4 h and then were fed the test solution (150 and 200 ppm active ingredient) in glass filter special feeding units for 48 h. In both cases, controls were treated with 5% sucrose only. The mutagenicity test was performed as follows: 4-5 day old *Berlin-K* males treated as larvae and as adults (and control males) were crossed individually with 3-4 day old *Basc* females for 3 days. To test the sensitivity of the germ cell stages of the males treated as adults, a 3-2-2 mating scheme (broods) was followed by transferring the males to fresh virgin females. The progeny of individual P males were identified so that clusters of lethals could be detected. The number of lethals per number of chromosomes tested was calculated and expressed as a percentage. The statistical significance was determined by using the Kastenbaum and Bowman (1966) test.

Results from males exposed to endosulfan, during the larval stage, showed that the difference between the control and 100 ppm endosulfan was significant at the 5% level. The increase observed at 50 ppm was not significant because of the small number of chromosomes tested. Results obtained after treatment of adult males showed that 200 ppm endosulfan yielded a significant increase in the 3 broods. The difference between the controls and 200 ppm was also significant at the 5% level if all the data were pooled. There was no stage-specific response.

Conclusion

Endosulfan was shown to be mutagenic in the *Drosophila melanogaster* SLRL test, under the conditions of this study. This study is not required in evaluating the mutagenic potential of endosulfan and besides, there are some reservations about its conduct with respect to recommendations given by OECD Guideline 477. Thus, the number of males treated, sterile males, F_2 cultures established and F_2 cultures without progeny were not reported. There was not a detailed description of the test procedure. Only two exposure levels were used instead of three. No positive control was included. No toxicity data was reported. The criterion used for scoring lethal mutations was not defined. Results should be confirmed in an independent experiment. In addition, the test substance was a commercial preparation.

B.6.4.3.2 Chromosomal aberration

Velázquez, A., Creus, A., Xamena, N. and Marcos, R. 1984 (Calliope S. A.: IIA, 5.4.3/03 or IIA, 5.4.3/02) (Excel Industries Ltd.: IIA, 5.4/04)

The study has been published in Mutat. Res., 136: 115-118.

The objective of this study was to determine the genetic damage produced by endosulfan in germ cells using the sex-linked recessive lethal (SLRL) and the sex-chromosome loss (SCL) tests in *Drosophila melanogaster*. This summary describes only that part of the publication referring to the evaluation of induction of sex-chromosome loss.

No reference to a specific test guideline was made.

GLP: No (at the time of the study, GLP was not required yet by the National Authority).

The study is considered acceptable only as an additional information because it is not required. Besides, the test substance was a commercial preparation and there were some reservations about its conduct.

Material and methods together with findings

Endosulfan test substance was 50% wettable power (50% active ingredient, 50% kaolin, dispersing + wetting agents), obtained from Hoeschst Ibérica. It was first dissolved in DMSO and then diluted with a 5% sucrose solution to give a final DMSO concentration of 1% and the desired insecticide concentration (based on the active ingredient). The SCL test was performed with two strains of *Drosophila melanogaster. Ring-X* males of the genotype *R1(2), yB / $B^S Yy^+$* were treated with endosulfan and crossed to *y sp* females of the genotype *y w spl sn^3*; *bw sp^2*.

Ring-X males 2-3 days old were starved for 4 h and then were fed the test solution (50, 100 and 200 ppm active ingredient) in glass filter special feeding units for 24 h. Controls were treated with 5% sucrose only. The mutagenicity test was performed as follows: 3-4 day old *Ring-X* males (treated and control) were mass-mated in bottles to 3-4 day old *y sp* virgin females in a ratio of 2 females per male for 3 days, followed by two 2-day successive broods. The F_1 offspring were scored and the exceptional phenotypes noted and expressed as a percentage. The statistical significance was determined by the chi-square test.

For the pooled data (total of three broods) all concentrations of endosulfan tested yielded a similar and significant increase of entire sex-chromosome losses. Partial Y chromosome losses were not detected. No concentration-effect relationship was observed. These results suggest a more pronounced clastogenic effect in sperm, since the increase in the frequency of XO exceptional offspring was significant in brood 1 at all concentrations tested.

Conclusion

Endosulfan was shown to be clastogenic in the *Drosophila melanogaster* SCL test, under the conditions of this study. However, this study is not required in evaluating the mutagenic potential of endosulfan and besides, there are some reservations about its conduct. There is not a specific OECD guideline for this test. Nevertheless, taking into account the OECD Guideline 477 (Sex-linked Recessive Lethal Test in *Drosophila melanogaster*) some deviations were observed. The number of males treated, sterile males, F_1 cultures established and F_1 cultures without progeny were not reported. There was not a detailed description of the test procedure. No positive control was included. No toxicity data was reported. The criterion used for scoring sex-chromosome losses was not defined. Results should be confirmed in an independent experiment. In addition, the test substance was a commercial preparation.

B.6.4.4 *In vivo* studies in mammalian germ cells

B.6.4.4.1 Chromosomal aberration

Diskhith, T. S. S. and Datta, K. K., 1978 (Calliope S.A.: IIA, 5.4.3/01 or IIA, 5.4.2/01) (AgrEvo: Australian National Registration Authority)

The study has been published in Bull. Environ. Contam. Toxicol., 20: 826-833.

The objective of this study was to evaluate the ability of endosulfan to induce chromosome aberrations in somatic and germinal cells of male rats. This summary describes only that part of the publication referring to experiments with germ cells.

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

GLP: No (at the time of the study, GLP was not required yet by the National Authority).

The study is considered acceptable only as an additional information. The main reason is that purity of the test compound was not given. Besides, there are some reservations about its conduct.

Material and methods together with findings:

Endosulfan test substance was obtained from National Chemical Laboratory, Poona, India. It was suspended in peanut oil. The study was conducted using male albino rats.

Animals were divided into five groups, with 8 animals in each group. Rats of group 1, 2, 3 and 4 were administered 11, 22, 36.6 and 55 mg/kg of endosulfan respectively, and rats of group 5 (control) were given peanut oil alone. The administration was by oral intubation for 5 consecutive days. Colchicine was administered to each rat by i. p. injection 4 h before killing by decapitation. Slides were prepared from seminiferous tubules cell suspensions. Air dried and flame fixed cells were stained with Giemsa. Scoring was made to determine mitotic index and chromosome damage in spermatogonial cells. The mitotic index was calculated as % cells at metaphase after scoring a total of 100 cells per slide. Chromosome damage was analysed for chromatid breaks, chromosome breaks and exchange figures scoring 50 metaphases per treatment.

All animals dosed at 36.6 and 55 mg/kg died before 24 h; two animals dosed with 22 mg/kg died after 72 h. There were no death in the control and lowest dose group. No major chromosomal aberrations were observed. There was no chromosomal deletion nor formation of large number of fragments. No significant mitotic inhibition was seen in any of the treated groups.

Conclusion

Endosulfan did not induce any significant chromosome damage in rat spermatogonial cells, under the conditions of this study. Nevertheless, the scientific validity of the study is questionable for several reasons. OECD Guideline 483 gives recommendations which have not been observed in this study. Thus, purity of the test substance was not specified; the volume of liquid administered was not given; the strain, age and source of animals used were not reported; no positive control was included; the time of sacrifice after administration of the last dose was not given; at least 100 cells should have been analysed for each animal (500 cells per treatment) but only 50 cells per treatment were analysed for chromosomal aberrations; the criterion for scoring aberrations was not defined; mitotic indices and frequencies of aberrations were not reported; no information was given about the number of cells analysed per animal, the type and number of aberrations (given separately for each animal), the total number of aberrations per group and the number of cells with aberrations per group; and, finally the significance of results was not justified because data were not analysed statistically.

Rani, M. V. U. and Reddy, P. P., 1986 (Calliope S.A.: IIA, 5.4.3/04)

The study has been published in ICRS Med. Sci., 14: 1125-1126.

The objective of this study was to detect the chromosomal breaking ability of the insecticides, aldrin and endosulfan, in male germ cells of Swiss mice. This summary describes only that part of the publication referring to experiments with endosulfan.

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

GLP: No (at the time of the study, GLP was not required yet by the National Authority).

The study is considered acceptable only as an additional information. The main reason is that purity of the test compound was not given. Besides, there are some reservations about its conduct.

Material and methods together with findings:

Endosulfan test substance was obtained from Excel Industries (India) Ltd. It was suspended in distilled water. The study was conducted using male Swiss mice.

Endosulfan suspension was administered orally for 5 consecutive days, in equal parts daily, at doses of 22, 32 and 42 mg/kg. Control animals were given an equal quantity of distilled water. Eight animals were used for each experimental point. Mice were sacrificed by cervical dislocation on the 60th day after the treatment schedule and both testes were dissected out. Spermatocytes at meiotic metaphases were presumed to have been spermatogonia at the time of treatment. Meiotic preparations were made by the air drying technique of Evans *et al,* 1964. A total of 100 spermatocytes were examined from each mouse for structural and numerical chromosomal abnormalities at the diakinesis first metaphase stage of meiosis. To assess the significance of differences in the frequency of chromosomal abnormalities between control and treated groups the data was subjected to the χ^2 test.

Endosulfan, at 32 and 42 mg/kg, resulted in a significant increase in the frequency of polyploids, autosomal univalents and sex univalents. The frequency of aneuploids was significant at all doses, whereas a significant number of translocations was recorded at 22 and 42 mg/kg. The total number of chromosomal aberrations was found to be significantly elevated at 32 and 42 mg/kg.

Conclusion

Endosulfan induced a significant chromosome damage in mouse spermatocytes, under the conditions of this study. Because there were several divisions between treatment and observation, reciprocal translocations were the only structural aberrations which could be observed. However, the scientific validity of the study is questionable for several reasons. Thus, purity of the test substance was not specified; the volume of liquid administered was not given; the source of animals used was not indicated; each testis should be considered separately but it was not specified if the contents of the two testes were mixed or prepared separately; no positive control was included; it was not reported if a spindle inhibitor was used or not; no information about toxicity for animals or cells was given; the criterion for scoring aberrations was not defined; and finally, types and numbers of chromosome aberrations were not given separately for each animal.

Pandey, N., Gundevia, F., Prem, A. S. and Ray, P. K., 1990 (Excel Industries Ltd.: IIA, 5.4/03) (AgrEvo: Australian National Registration Authority)

The study has been published in Mutation Research, 242: 1-7.

The objective of this study was to evaluate the genotoxic properties of endosulfan in germ cells of male mice using the dominant lethal and the sperm shape abnormality tests. This summary describes only that part of the publication referring to the dominant lethal test.

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

GLP: No.

The study is considered acceptable with some reservations due to the lack of detail in the study.

Material and methods together with findings:

The test substance was Thiodan (technical endosulfan with purity 97.03%) purchased from Hoechst Pharmaceuticals Ltd., Bombay, and dissolved in DMSO. The positive control was cyclophosphamide. The study was conducted using Swiss albino mice.

The dominant lethal assay was performed following the described protocol of Bateman (1984). Three groups of male mice (20 animals per group) were treated with endosulfan for 5 consecutive days at dose levels of 9.8, 12.7 and 16.6 mg/kg (i.p.). After the treatment, each male was caged with a virgin female. A 7-day sequential mating procedure was used over a total of 8 mating intervals. The uterine contents were determined 11 days after the day of separation from the males. The frequency of induced dominant lethal mutations was calculated as 1- (live implants per female of the test group/ live implants per female of the control group) x 100. The mean percentages, standard errors and deviations were calculated. The level of significance between the treated and control groups was determined with Student's *t*-test.

The results did not indicate any adverse effect of the compound on fertility in the dose range tested except during 1 post-treatment mating interval. With the lower dose levels (9.8 and 12.7 mg/kg) no induction of dominant lethals could be observed. A dose of 16.6 mg/kg significantly induced dominant lethal mutations during the mating interval of 36-42 days. A marked reduction in total implants was observed with the same dose. The mating interval (sixth week) indicated that damage that resulted in dominant mutation was induced specifically in spermatogonia. The results indicated that the induction of mutation was due to pre-implantation as well as post-implantation loss, the post-implantation loss (50%) being more pronounced than the pre-implantation loss.

Conclusion

On the basis of the present *in vivo* study, it appears that endosulfan has a damaging effect on mice spermatogonial cells. However, the induction of dominant lethal effects occurred only at the highest dose of endosulfan (16.6 mg/kg/day, equivalent to a total dose of 83 mg/kg). The lack of detail in the reporting of this study makes the significance of the isolated finding questionable. The fact that an increase in dominant lethal mutations was seen only in a single mating interval, with no adverse effects on implants or fertility seen at other intervals, suggests that the result may be an artifact, and no related to treatment. There is no individual animal data to determine if there was large intra group variation in the sixth mating interval, and if a single outlying result led to a statistically significant outcome for this mating interval. The test was not reproduced, and so it is difficult to determine whether the effects seen from the sixth mating interval were spontaneous, or related to endosulfan administration.

Dzwonkowska, A. and Hübner, H., 1991 (AgrEvo: Australian National Registration Authority)

The study has been published in Polish Journal of Occupational Medicine and Environmental Health, 4 (1): 43-53.

The objective of this study was to evaluate the genotoxic properties of endosulfan in germ cells of male mice using the dominant lethal test.

No reference to a specific test guideline.

No reference to GLPs.

The study is considered acceptable only as an additional information because it is summary of a published paper which does not include the required information. Besides, the test substance was a commercial preparation.

Material and methods together with findings

Endosulfan (Thiodan 35; Hoechst), along with a number of other chemicals, was administered via intraperitoneal injection to adult male Balb/c mice. Animals received either a single administration (0.64 mg/kg; 17 animals) or daily administrations for 5 days (0.64 mg/kg/day; 20 animals), with the dose estimated as 20% of the LD_{50} value. Vehicle control animals received distilled water, while positive control animals received cyclophosphamide (25 animals per group). Administration of the test material was followed by twelve 4-day mating periods where each male was mated with untreated virgin females. Females were examined for the number of total implantations, the number of live implantations, and the number of dead implantations.

The administration of endosulfan did not result in statistically significant changes in the parameters measured, and did not induce an increase in the dominant lethal index.

Conclusion

Endosulfan was negative for the mouse dominant lethal test, under the conditions of this study. However, the information given is only a summary which is considered insufficient for evaluating the mutagenicity of endosulfan. The original paper should be enclosed. Besides, the test substance was a commercial preparation.

B.6.4.4.2 Sperm Shape Abnormality test

Pandey, N., Gundevia, F., Prem, A. S. and Ray, P. K., 1990 (Excel Industries Ltd.: IIA, 5.4/03) (AgrEvo: Australian National Registration Authority)

The study has been published in Mutation Research, 242: 1-7.

The objective of this study was to evaluate the genotoxic properties of endosulfan in germ cells of male mice using the dominant lethal and the sperm shape abnormality tests. This summary describes only that part of the publication referring to the sperm shape abnormality test.

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

GLP: No.

The study is considered acceptable only as an additional information because it is not required and there are not available test guidelines.

Material and methods together with findings:

The test substance was Thiodan (technical endosulfan with purity 97.03%) purchased from Hoechst Pharmaceuticals Ltd., Bombay, and dissolved in DMSO. The positive control was cyclophosphamide. The study was conducted using Swiss albino mice.

Four groups of male mice (5 animals per group) were treated with endosulfan for 5 consecutive days at dose levels of 9.8, 12.7, 16.6 and 21.6 mg/kg (i.p.). After the treatment, the caudae epididymides were excised, weighed, minced in physiological saline, dispersed and filtered. Smears were prepared on clean slides after staining the sperms with Eosin Y. 200 sperm per animal were examined for morphological abnormalities by light microscopy at 100 x 10 magnification with a green filter, and counts were determined using a haemocytometer. Percentage motility was assessed. The weight of each testis was recorded. The mean percentages, standard errors and deviations were calculated. The level of significance between the treated and control groups was determined with Student's *t*-test.

Endosulfan induced various types of abnormalities in sperm shape. Many aberrant sperms displayed double heads, big heads, amorphous types, coiled tail, etc. A statistically significant dose-dependent increase in sperm abnormalities was observed with endosulfan treatment. The two higher dose levels produced significant increases in sperm head abnormalities. Endosulfan exposure (21.6 mg/kg) caused a significant decrease in testis weight. A statistically significant dose-dependent decrease (34-39%) in sperm count was observed with higher doses (16.6 and 21.6 mg/kg). Sperm motility remained unaffected at all dose levels.

Conclusion

On the basis of the present *in vivo* study, it appears that endosulfan has a damaging effect on mice sperm morphology. However, it is unclear whether the decrease in sperm count and increase in sperm abnormalities are biologically significant. It is unlikely that this increase in sperm abnormalities is causally related to any adverse effects on fertility or other reproductive parameters in this study, but the reporting of this study is not adequate to definitely discount the possibility.

Khan, P. K. and Sinha, S. P., 1996 (AgrEvo: Australian National Registration Authority)

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

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

No reference to a specific test guideline.

No reference to GLPs.

The study is not required. Thus, it is considered acceptable only as an additional information. Besides, the test substance was a commercial preparation. In addition, only a summary of the published paper has been presented and it does not include the required information.

Material and methods together with findings

A number of compounds, including endosulfan, were administered to male Swiss albino mice (6-8 weeks old; Central Drug Research Institute, India). Endosulfan (35% emulsificable concentrate) was

administered to group of 6 animals via oral gavage at a dose of 3 mg/kg/day (estimated to be the maximum tolerated dose) for 35 consecutive days, with a dose volume of 0.2 mL of an aqueous solution of the test material (0.1% v/v). One group of treated animals received endosulfan only, while three other treatment groups received endosulfan via gavage, plus intravenous administration of vitamin C at 10, 20 or 40 mg/kg/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, with sperm collected from the cauda and cauda epididymides of each mouse for counting and morphological analysis.

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

Conclusion

Under the conditions of this study, the administration of endosulfan at a dose of 3 mg/kg/day resulted in an increase in abnormal sperm from 5 to 14%, and this effect was reduced slightly in animals also administered vitamin C. No historical control incidences for abnormal sperm from the testing laboratory were provided, and there is no indication of whether this incidence of 14% was biologically significant, and/or within normal biological variation for this strain of test animal. Significant reductions in sperm count were seen following the administration of endosulfan (80% reduced), and this effect was lessened in animals also administered vitamin C. However, the test material was a 35% emulsifiable concentrate containing solvents, emulsifiers and stabilizers, and it is unclear whether these findings are related to endosulfan or these non active constituents. Finally, it should be noted that this study is not required in evaluating the genotoxicity of endosulfan, and besides, only a summary of the published paper has been presented.

Sinha, N., Narayan, R., Shanker, R. and Saxena, D. X., 1995 (AgrEvo: Australian National Registration Authority)

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.

No reference to a specific test guideline.

No reference to GLPs.

The study is considered acceptable only as an additional information because it is a summary of the published paper. Besides, the sperm abnormality test is not required and there are not available guidelines.

Material and methods together with findings

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

At 10 mg/kg/day, 2 animals died during the study, but no mortality was reported at other dose levels. No change in body weights or testis weigh were seen in treated animals compared with controls. Statistically significant, dose related increase in testicular lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), gamma glutamyl transpeptidase (GGT) and glucose-6-phosphate dehydrogenase (G6PDH) activity were seen at all endosulfan dose levels.

Statistically significant decreases in cauda epididymis sperm counts were seen at all test doses, with reductions of 22%, 43%, and 47% at 2.5, 5, and 10 mg/kg/day, respectively. The reduction in sperm count at 5 and 10 mg/kg/day was also statistically significant when compared with the reduction seen at 2.5 mg/kg/day. The incidence of sperm abnormalities was statistically 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. Statistically significant reductions in spermatid count (about 16%) and sperm production rate (about 22%) were also reported at 5 and 10 mg/kg/day compared with controls, but there is no consistent dependence on endosulfan dose for these 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, thus influencing intratesticular spermatid count, and resulting in low sperm production and increased sperm deformities. The data presented in this study supports the notion that the administration of endosulfan at relatively high doses (2.5 mg/kg/day and above) for several months resulted in an increase in activity of a number of enzymes found in the testes, and that at doses of 5 mg/kg/day and above, there was a marked reduction in sperm count (up to 47%) compared to controls. In the absence of historical control data, it is unclear whether the decrease in sperm count at 2.5 mg/kg/day (22%) was within normal biological ranges for the test animals. The 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. It should be noted that the sperm abnormality test is not required in evaluating the genotoxicity of endosulfan, and besides, only a summary of the published paper has been presented.

B.6.5 Long-term toxicity and carcinogenicity (IIA, 5.5)

AgrEvo, Calliope and Excel presented eight studies. Besides, AgrEvo has included review document of endosulfan prepared by the Australian National Registration Authority (ANRA) for Agricultural and Veterinary Chemicals, which includes studies previously presented by AgrEvo and Calliope, and studies which have not been presented by any applicant.

Four chronic toxicity studies were performed on rats (Keller, 1959c), mice (Arai, 1981) and .dogs (Keller, 1959b and Brunk 1989; 1990).

Chronic toxicity study on rats was carried out prior to GLP regulations and is not considered acceptable because the purity of the test substance was not reported The second study performed on mice is only a review of the original paper, thus only can be considered as additional information .

Finally, two 1-year feeding toxicity studies on dogs were presented by AgrEvo. The first study carried out on Mongrel dogs (Keller, 1959b), was performed prior to GLP regulations and is not considered acceptable for many reasons: the purity of the test substance was not reported, the higher dose level used did not induced any toxic effect and the number of dogs used by group does not permit obtaining significant results . Only, the other study carried out on Beagle dogs was conducted according to OCDE guidelines and GLPs compliance.

The possible carcinogenic effect of Endosulfan was studied in rats and mice by Thomas *et al*., (1978) The study correspond to a published work. in US National Cancer Institute. and it did not claim any adherence to a specific test guideline or GLPs compliance. No treatment-related neoplastic lesions were seen in female rats; owing to the high mortality in rat males, no valid conclusion could be drawn about carcinogenic effects in male rats A NOAEL for rats was not identified, as treatment-related changes occurred in the kidneys and the testis at all doses.

A high early mortality in male mice was observed but due the high mortality in control groups this results were doubtful. As consequence, it could not be established a NOAEL for male mice . The NOAEL for female mice was 0.58 mg/kg/day.

The combined chronic /carcinogenic studies were carried out on Charles River rats (Ruckman *et al*., 1989) and on NMRI mice (Donaubauer 1989a, 1989b).

In the first case, the study was performed according to OECD: “Short-term and Long-term toxicology group guideline” and following the GLP regulations Progressive glomerulonephrosis and aneurysms among in male rats aneurysms were detected. and, both signs were studied with more detail by

histophatology techniques by Gopinath & Cannon, (1990). A second addendum was provided by Leist et al., (1989a) where 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.

In the second combined study was evaluated the chronic oral toxicity and carcinogenic potential of endosulfan in NMRI-mice during two years . The study was conducted according to OECD 451 guideline in compliance with EPA guideline and following the GLP regulations. In support of this study, the residues of α-endosulfan, β-endosulfan, endosulfan-hydroxiether, endosulfan-sulphate, endosulfan-lactone and endosulfan-diol, were determined in the liver and kidneys (Leist, 1989b).

Both combined chronic and carcinogenic studies were summarised by Hack and published in Fd. Chem. Toxic. Vol.33, nº 11, pp: 941-950 (1995)

On the overall of these studies, no carcinogenic effect was observed in rats and mice at any Endosulfan dose tested.

Table: 6.5-1: Summary of Long-Term and Carcinogenicity effect of Endosulfan

Study	NOAEL		LOAEL		Main Adverse Effect	Reference/year
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d		
Chronic toxicity studies						
Wistar rats. Oral. 104weeks. Concentrations: 0, 10, 30 and 100 ppm (equivalent to 1.5 and 5 mg/kg bw/day	30	1.5	100	5	LOAEL based on Kidney effects mainly in male rats.	Keller (1959c) (AgrEvo: IIA, 5.5.1/1) (AgrEvo: ANRA)

	NOAEL		LOAEL			
12-months oral study in mice. Dose levels: 0, 10,30,100 and 300 ppm equivalent to 1.17, 4.08, 15.2, 41.7 mg/kg/bw in males and 1.41, 4,74, 13,5, 42 mg/kg/bw in females	30	4.1 (m) 4.7 (f)		15.2 (m) 13.5 (f)	LOAEL based on histological findings in the liver and lymphatic system	Arai et al.,(1981) AgrEvo:ANRA
1-year toxicity study in Beagle dogs. Oral. 1 year. Dose levels: 0, 3, 10,30 ppm.(equivalent to 0. 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)
1-year toxicity study in Mongrel dogs Oral. 1 year Dose levels: 0, 3, 10, 30 ppm (equivalent to 0, 0.0075, 0.25, 0.75 mg/kgbw/day). Initial high dose of 100 ppm(2.50 mg/kg/day)	30	0.75	100	2.5	LOAEL based on the clinical signs(vomiting , tremors, convulsion, rapid respiration, mydriasis, salivation and tonic-clonic convulsions) at found at the initial high dose tested (2.5 mg/kg/day during the first three days of the study	Keller(1959b) AgrEvo IIA, 5.3.2.3/01

Carcinogenic studies

	NOAEL		LOAEL			
Osborne-Mendel rats Oral. (78 weeks) and average dose levels: 0,220, 410 or 950 ppm for males and 220 and 400 for females males/females;	Not identified				No tumours were found in females; and no valid conclusion can be drawn about carcinogenicty in males	Thomas, LW *et al* (1978) (AgrEvo: IIA, 5.5.1/2) (AgrEvo: ANRA) (Calliope: IIA, 5.5/01)
B6C3F1 mice (78 weeks Oral.)Average dose levels: 3.5 and 6.9 ppm for males and 2 and 3.9 ppm for females	3.9 (f)	0.58 (f)			Owing the high early mortality rates, no conclusion can be drawn about carcinogenicty in males No carcinogenic effects in females.	Thomas, LW *et al* (1978) (AgrEvo: IIA, 5.5.1/2) (AgrEvo: ANRA) (Calliope: IIA, 5.5/01)
Combined chronic/carcinogenic study						
Charles River rats Oral.104 weeks.. Dose levels: 0,3,7.5, 15 and 75 ppm (equivalent to 0, 0.1, 0.3, 0.6 and 2.9 for males and 0, 0.1, 0.4, 0.7 and 3.8 mg/kg/day for females)	15(m/f)	M 0.6 F: 0.7	75(m/f	M 2.9 F 3.8	LOAEL based on low body gain weigh (m/f), low food consumption in females and kidney alterations in both sexes No evidence of increased carcinogenicity findings at any dose tested.	Ruckman SA et al., (1989) (AgrEvo: IIA, 5.5.1/4) (AgrEvo: ANRA) Hack et al., (1995) (Published) (AgrEvo:IIA, 5.5.1/6)
NMRI mice. Oral, 24 months. Dose levels: 0, 2, 6, 18 ppm (equivalent to 0.28, 0.84 and 2.51 for males and 0.32, 0.97 and .2.86 mg/kg/day for females)	6	0.84 (m) 0.97 (f)	18	2.51 m 2.86 f	LOAEL base on decreased body weight in males at 24 months and decreased weight in males at 24 months and decreased weights of the liver, ovaries and lung in males and females at 12 and/or 18 months. No carcinogenic properties in mice	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)

m =male
f = female

B.6.5.1 Chronic toxicity studies

B.6.5.1.1 Rats

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

Dates of experimental work: this report covers the entire two years experimental period and completes, amends, and supersedes the progress report dates July 27, 1957, October 22, 1957, April 30, 1958 and May 28, 1958 (revised April 15, 1958).Date of report: May 22, 1959.

The objective of the study was testing the chronic toxicity of endosulfan technical in exposed rats for two years.

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

The study was performed prior to **GLP** regulations. It provides the core information required in those days.

The study is not considered to be acceptable. The main reason is that the purity of the technical product was not specified.

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), weighing 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. The animals in group Nº 1 were designated as control animals.

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, growth, food and compounds consumption, clinical signs, organ weights and microscopic pathology.

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

There were no treatment-related clinical signs, and body weights were unaffected except for a non significant decrease in body weights and food consumption in the high dose males. Survival of 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. Females form the high dose group had a reduced survival rate after 26 weeks (93% in controls, 74% in high dose) and 104 weeks (88% in controls, 46% in high dose). The deaths were predominantly associated with respiratory infections.

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

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

Histopathologic changes were observed in kidney in male rats treated with 100 ppm Thiodan at 104 weeks consisted of enlarged kidneys, mild-to severe renal tubule dilatation (12/12), mild-to moderate formation of irregular albuminous casts (10/12), pronounced focal interstitial nephritis (7/12) and mild to severe degeneration of the renal tubule epithelium 811/12). At 104 weeks, female rats at the high dose showed some minimal degeneration of renal tubules (2/3) and some focal nephritis (1/3. At 10 and 30 ppm, a number of animals displayed kidney effects also, but these findings were not considered to be related to treatment as the incidence of these findings was similar to controls, and there was no consistent dose response relationship at the lower dose.

Microscopic alteration in the liver were seen in 50% of males at the high dose at week 104, consisting of focal areas of hydropic cells, which were pale swollen, the nuclei wee surrounded by a clear zone, and a few cells appeared to have eosinophilic cytoplasmic inclusions. Few females at the high dose showed changes in liver cells. The tumour incidence is summarised in Table 6.5.1.1-1.

Table 6.5.1.1-1: 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

None of the noted tumours occurred consistently or in a dose related manner and were not considered to be related treatment**.**

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 as the purity the test substance and the maintenance and age of the animals.

B.6.5.1.2 Mouse

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 a report which does not include the required information.

Material and Methods

Endosulfan technical (91.4%) was administered daily to 4 weeks old ddY mice (10/sex/group) at dietary levels of 0, 10, 30 ,100 and 300 ppm for 12 months. The endosulfan was dissolved in corn oil and formulated into the diet; the dietary concentration of corn oil was 2%. Actual achieved doses were 0, 1.17, 4.08, 15.2 and 41.7 mg/kg/day in males and 0, 1.41, 4.74, 13.5 and 42 mg/kg/day in females.

Animals were observed twice daily for clinical signs and morbidity and were palpated for masses monthly. Body weights and food consumption was determined weekly. 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), hematocrit (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 infiltation	M	0/10	0/10	0/9	1/10	6/10
	F	1/10	1/10	1/10	0/10	8/10
Nodular hyperplasia	M	0/10	0/10	0/9	0/10	1/10
Pigmented histocytic 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 al lower dose levels. The histopathological findings in the kidney are summarised in Table 6.5.2-2.

Table 6.5.2-2: Incidence of histopathological findings in kidney

Findings	Sex	Doses (ppm)				
		0	**10**	**30**	**100**	**300**
Intersticial 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 fcortical 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 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.6.5.1.3 Dogs

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 2.3 mg/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 o f3 ppm 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 6.5.1.3-1: 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.

Pathological examination did not reveal any neoplastic or non-neoplastic lesions that were attributed to the administration of endosulfan.

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, and no increase in incidence of neoplastic or non-neoplastic lesions were observed in treated animals.

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

Upon the conditions of this study endosulfan technical is not considered carcinogenic.

Keller (1959b) (AgrEvo: IIA, 5.3.2.3/01)

Date of report: 12 May 1959.

The objective of this report was testing the toxicity of endosulfan in dog for one year.

The report does not claim adherence to a specific guideline.

GLP: No (The study was performed prior to GLP regulations).

The study is considered not acceptable. The main reason is that the purity of endosulfan was not given, besides, the higher dose level used did not induced any toxic effect and the number of dogs used by group does not permit obtaining significant results.

Material and Methods

Endosulfan technical was administered orally, by gelatine capsule, to adult mongrel dogs (2/sex/group) at dose levels of 0, 3, 10 and 30 ppm (0, 0.075, 0.25 and 0.75 mg/kg/day) on 6 days/week for one year. The group receiving 3 ppm originally was treated at 100 ppm (2.50 mg/kg/day)for the firs 3 days of treatment however clinical signs of vomiting, tremors, convulsions, rapid respiration, mydriasis, salivation, tonic-convulsion and rapid respiration in one male and both female dogs led to the dose

being reduced to 3 ppm for the rest of the study. Endosulfan was administered as a weight/weight mixture with lactose. Control dogs received lactose only.

The dogs were weighed on the first day of each week and the dosage were adjusted to the weekly by weight. The animals were observed daily as to appetite, elimination, and gross signs of toxicity or pharmacological effect.

The following clinical tests were performed on all dogs: erythrocyte counts, total and differential leukocyte count, haematocrits, total and differential leukocyte counts, haematocrits, haemoglobin determinations, sedimentation's, sedimentation rates, bromosulphalein liver function tests, and complete urine analysis. At completion of the one-year period all dogs were sacrificed by exanguination and gross autopsies performed. The weights of liver, kidneys, spleen, adrenals, and testes wee recorded.

Results

No clinical signs or treatment related effects on body weight gains were seen, other than one female at 10 ppm exhibited an eczema-like irritation on the nose, another female showed, after 52 weeks, a 12% reduction in body weigh with respect to initial weight, and one male at 30 ppm also showed a 12% reduction in body weight.

In general, the haematological and biochemical values and the results of urine analysis remained within normal limits in all dogs throughout the study. Gross autopsies performed on the four control dogs revealed no gross pathological changes. Gross autopsies performed on the rest dogs which received Thiodan showed no gross pathological changes attributable to the oral administration of the compound. A mild infestation with intestinal parasites was presented among several dogs of all groups. Organ weights and organ-body-weight ratios for experimental dogs of both sexes for each dosage level compare favourably with the respective controls, and are therefore considered to be within normal limits.

Microscopic examination of the tissue sections from the test dogs revealed no significant or consistent pathological changes which could be attributed to the oral administration of Thiodan in doses ranging from 0.075 to 0.75 mg/kg/day for one year, as the tissue sections were comparable to those of the control dogs. Incidental findings were present in both control and test dogs and involved mainly the lungs, liver, kidneys, and spleen. Mild congestion and atelectasias were found in the lungs of some of the dogs. Most all dogs were found to have minimal liver changes consisting of mild cytoplasmic disturbances, chiefly and increase in granularity. Some of the spleen showed free and unphagocytized accumulations of pigment. The kidney of several dogs appeared to be mildly congested and in one dog there was focal interstitial nephritis. The above coincidental findings are not considered uncommon for mongrel dogs.

Conclusions

On the basis of the data presented, and under the experimental conditions of this study it may be stated that the highest dosage level of Thiodan, which does not produce signs or pharmacological effects in the dog is 0.75 mg/kg/day (30 ppm).

Nevertheless, this study is considered as not acceptable because the purity of the test substance is not provided and the highest dose tested of 0.75mg/kg/day did not induce any toxic effect in dogs. Besides, the dogs treated with the dose of 2.5 mg/kg/day only for three days showed toxic signs and this dose was changed by the lowest dose tested (0.075 mg/kg/day). A justification about this fact is necessary.

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

The objective of the study was testing Technical Endosulfan for carcinogenicity in rats.

The study does not claim adherence to a specific test guideline

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

The study is considered to be acceptable.

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*.

The used animals was-Osborne -Mendel rat, (m/f): .Age 7 weeks, body weights: 200g (males) and 150g (females).

Groups of 50 males and 50 females except control group (20 m/20 f). were feed diets containing technical grade Endosulfan at time-weighted average doses of 220, 410 or 950 ppm for males and 220 or 400 ppm for females for 78 weeks. Control group 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.

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

The clinical signs observed as alopecia, reddener or squinted eyes, rough fur, sores bon the body and/or extremities, eye or nasal discharge and swollen areas does not specifically treatment related.

Statistical analysis on survival rates in male rats showed a highly significant ($p<0.001$) morbidity rate in male rats at all doses, and by week 54, 52% of the high dose rats had died. Due the high mortality rates no conclusion could be drawn on analysis of tumour rates in males rats. Survival rates in females wee unaffected, except in the low dose group where (7/50) 14% died in week 21, of which, six were found to have cerebral haemorrhage. These cerebral lesions were not found in any other groups and were not considered related to treatment.

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.

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

The toxic nephropathy observed in animals was characterised as degenerative changes in the proximal convoluted tubules at the junction of the cortex and medulla, and associated cloudy swelling, fatty degeneration, and necrosis of the tubular epithelium. Some tubules had hyalin cats, and infrequent enlarged dark-staining regenerative tubular epithelial cells were observed (at the high stage kidney often had infiltration of inflammatory cells, fibrosis, and focal mineralisation. Parathyroid hyperplasia possibly associated with renal lesions occurred in 0/20 controls, 21/48 (44%) low dose males, 18/47 (38%) high dose males, and 1/49 (2%) low dose females.

Male rats showed medial calcification of the aorta; 29/50 (58%) in the low dose group, 22/49 (45%) in high dose; and medial calcification of the mesenteric artery, 28/50 (56%) in the low dose and 23/49 (47%) in the high dose group. Calcium deposits were noted in the stomach of 31/50 (62%) of low dose, and 21/47 (45%) high dose males. Female rats showed low incidences of arterial 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 aspermatogenesis. No treatment related effects were noted on the reproductive organs in female rats.

Conclusion

A **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.

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 objective of the study was testing Technical Endosulfan for carcinogenicity in mice.

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

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

The study is considered to be acceptable.

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*.

The animal used was -B6C3F1 mice (m/f);Age 6 weeks;. body weights: 15g (females) and 20g (males) .

Groups of 50 male and 50 female mice were fed diets containing Endosulfan at time-weighted average concentrations of 3.5 or 6 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.

Results

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

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

Conclusion

Owing to the high early mortality rates, no conclusion could be drawn about the carcinogenic potential of Endosulfan in males. None of the non-neoplastic changes seen in the kidneys and sex organs of male and female mice could be attributed to treatment.

The **NOAEL** for female mice was 3.9 ppm, equal to 0.58 mg/kg bw/day.

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.

The objective of this report was to assess the toxicity and potential carcinogenicity of Endosulfan to rats by continuous dietary administration (104 weeks).

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

GLP :yes

The study is considered to be acceptable

Material and Methods

The test substance was Endosulfan technical (97.1% purity) Stability was confirmed periodically during study. A pre-mix was prepared each week by first dissolving the test material in acetone then mixing in corn oil.

Test animals were Charles River rats (5-6 weeks old and body weight of about 23 g for males and about 27 g for females) .

Five main groups of 50 males and 50 received Endosulfan diet 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. . In addition to the main group, which was intended primarily for

tumorogenic evaluation, there were satellite groups each consisting of 20 animals/sex/dose, which was intended for blood sampling at intervals and sacrifice after 104 weeks.

Once daily animals were observed for symptoms of ill health and water consumption; weekly detailed observations for health, bodyweight, food consumption; every 6 months blood and urine analysis of 10 animals of each group and sex were performed, 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, and mortality was similar in control and treated groups during the study. The major factors contributing to death included mammary tumours in females, and pituitary tumours in males and females, both of which normally occur at relatively high frequency in aging laboratory rats. The incidence of these as causes of mortality were similar in control and treated animals, and were not considered to be related to the administration of Endosulfan. Another major contributory factor for mortality in main and satellite group males was renal lesions. While renal lesions appeared to contribute more to the mortality of males administered Endosulfan at doses of 7.5 ppm and above than for control animals, there was no dose dependence for this factor, and overall mortality was not increased in treatment groups.

Table 6.5.3.1-1: Factors contributing to death (nº animals with factor/nº animals dead)

Dose	Males					Females				
Ppm	**0**	**3**	**7.5**	**15**	**75**	**0**	**3**	**7.5**	**15**	**75**
Pituitary tomour										
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

Reductions in body weight and body weight gain were observed in males (group mean, 17% at 104 weeks) and females (group mean, 18% at 104 weeks) at 75 ppm but no clinical signs of poisoning were seen at any dose.

Food 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 urianalysis 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: 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 6.5.3-2: 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

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

Statistically significant ($p<0.01$) decreases in group mean 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, typical of tumour types commonly seen in aging laboratory rats.

Progressive glomerulonephrosis (PGN) was a common finding in control and treated animals at histopathological examination. PGN was recorded in 3 grades of severity namely minimal, moderate and marked. Clear-cut quantitative criteria are difficult to prescribe for such a complex lesion. For practical purposes the pathologist has used the following criteria for grading: Minimal, when the lesions characteristic of PGN affected up to 15% of the nephrons with large areas of unaffected renal tissue in the minimal as sections; Moderate, similarly 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 6.5.3.1-3: Non-neoplastic findings in kidneys

Dose	**Males**					**Females**				
ppm	**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

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

Table 6.5.3.1-4: Non-neoplastic findings in other tissues

Dose ppm	Males					Females				
	0	**3**	**7.5**	**15**	**75**	**0**	**3**	**7.5**	**15**	**75**
Incidence of blood vessel aneurysms										
Main[a]	9	4	12	7	13	1	1	4	4	3
Satellite[b]	1	2	2	3	6	0	1	1	0	0
Lumbar lymph nodes										
Main[a]	23	17	20	13	23	2	1	3	2	4
Satellite[b]	4	5	3	5	8	0	1	0	0	0

Main[a:]: Main group (50 animals/sex/dose9
Satellite[b:]: Satellite group (20 animals/sex/dose)

Conclusion

The **NOAEL** was 15 ppm, equivalent to about 0.6 and 0.7 mg/kg/day for males and females respectively, based on low body weight gains in both sexes, low food consumption in females, and kidney alterations in both sexes 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.

Dates of experimental work: the study was performed between 17 October, 1988 and 03 March. 1989.

Date of report: 27 July 1980.

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 to be 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.6.5.3.2 Mouse

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

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

Date of report: 6 April 1988.

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

Amendment to report IIA, 5.5.2/1.

Date of report: 6 April 1988.

The objective of the study was to determine the chronic oral toxicity and potential carcinogenicity of Endosulfan Technical in mice.

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 mucosa) 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 general health were unaffected by administration of endosulfan. Food consumption, haematology and clinical chemistry did not differ between treated and untreated groups. Histopathological examination did not reveal any pathological changes, and non-neoplastic and neoplastic findings did not reveal treatment related effects. At 18 ppm the mortality in females was increased (similar mortality for control group) and the body weight gains in males was retarded.

No statistically significant changes were observed on haematological or clinical chemistry parameters between control and treated animals during the study. Macroscopic examination did not reveal any findings that were related to treatment with Endosulfan.

On occasion, slight but statistically significant changes in organ weights were observed at the 12 or 18 months interim sacrifices (decreased lung and ovary weights in females at 12 months; decreased liver weights in males and ovary weights in females at 18 months) at the high dose level, and while the magnitude of the effects was not great, they are considered to be related to the administration of Endosulfan.

Histopathological examination did not reveal any effects that were related to the administration of Endosulfan in this study, with a range of neoplastic and non-neoplastic lesions seen in control and treated group, consistent with the spontaneous tumours seen in aging laboratory rodents. There was no treatment related increase in the incidence of any tumour type or in the total number of animals with benign or malignant tumours in any group. A slight increase in the incidence of minimal focal epithelial thickening and minimal epithelial thickening was observed in the urinary bladders of treated females. However, as there was no progression to any further proliferation and no clear dose dependence or this effect, is not considered to be toxicologically significant.

After 24 months, endosulfan–sulphate was detected in the liver at about 1 ppm and in the kidney. at 0.2 ppm The other metabolites of endosulfan were at or below the detection limit.

Conclusions

The 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 decrease organ weighs (liver, ovaries, lung) in males and females at the 12 and/or 18 month interim sacrifices at 18 ppm (approximately 2.51mg/kg/day in males and 2.86 mg/kg/day in females). No increase in the incidence of neoplastic or non-neoplastic lesions was observed in mice administered endosulfan at levels up to 18 ppm for 24 months.

Endosulfan exhibits no carcinogenic properties in mice.

Leist, (1989b) (AgrEvo, IIA,5.5.2/4)

Amendment to report IIA, 5.5.2/1.

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

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.6.6 Reproductive toxicity (IIA, 5.6)

Summary

Eight studies have been conducted to evaluate endosulfan toxicity on reproductive system. They include three multigenerational studies on rats and five developmental studies, four on rats and only on rabbits- All these studies are sponsored mainly by AgrEvo company.

Multigenerational toxicity

To establish, the maximum tolerated dosage of endosulfan for use in a multigenerational study in rats was performed a preliminary study by Edwards *et al*., (1982). This study does not claim adherence to specific guidelines and GLP compliance.. Under the conditions of this study, it was concluded that 75 ppm (equivalent to 8.26 mg (kg/day and 8.36 mg/kg/day in males and females respectively), would be suitable fur use as the highest dose level in the subsequent multigeneration studies.

Kennedy *et a*l., (1965) study was conducted prior to the requirement of GLP and did not claim adherence to a specific guideline besides, the purity of the endosulfan was not reported , thus this study is considered as not acceptable. In addition, the dosages employed are referred to mg/kg/diet , thus it has not been possible to relate diet concentration of endosulfan to mass of endosulfan/kg bw animal/day.

In the study carried out by Edwards et al (1984) and Offer (1985) was evaluate endosulfan effects on the reproductive performance and developmental of F0, F1B and F2B generation rats.

Both studies were conducted to GLP compliance. Endosulfan did not affect reproductive performance or the growth or developmental of the offspring of rat over the course of a two generation study. The NOAEL for maternotoxicity was 1 mg/kg bw/day and for reproduction toxicity was 6 mg/kg bw/day. Developmental NOAEL could not be stabilised.

Developmental toxicity studies:

Five studies on developmental toxicity were performed, four of them on rats and one on rabbits:

1.-The first teratology study submitted was performed prior to GLP regulations and no guideline method was available at the time of the study. The study was published in Acta Pharmacol. Toxicol. vol 42: 150-152.by Gupta et al., (1978). The level reporting in this published paper is not adequate for the purposes of defining an NOAEL for developmental toxicity Besides, the paper can not be considered acceptable because the purity of the test substance as the stability of the test substance and strain and age of the animals are not provided.

2.-An other study to determine the potential teratogenic of thiodan upon gravid albino rats was performed prior to GLP regulations and without any guideline specification (Haley, 1972). On the other hand, the dosages used in this study were not sufficiently high to induce any toxicity.

3.-.The only study performed according to OECD guideline referent to Teratogenicity studies and following the GLPs ,was carried out by Albrech and Baeder (1993). The NOAEL for maternotoxicty and for developmental toxicity was 2 mg/kgbw/day.

4.- A last report provide by AgrEvo company to evaluate the embriofetotoxicity in rats was designed by McKenzie et al (1980).The study was performed prior to GLP regulation and no guideline method was available at the time of the study**.** This study is considered as acceptable with some reservation, mainly because the replacement of animals during the study made difficult to interpret the data .

5.- Finally, one year later, the same author studied the embrio-fetal and teratogenic method nor GLP compliance. Besides, the interpretation of data is not clear .because some animals were also replacement during the study .

On the overall of these studies, non critical effect was identified to reproduction after administration of endosulfan and the fetotoxicity effects appear at maternal toxic doses.

Table 6.6-1: Summary o reproduction toxicity studies

Study	NOAEL		LOAEL		Main Adverse Effect	Reference/year
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d		
Preliminary study to determine doses used in two generation study in rats .Dosages: 0, 50, 75, 100 ppm	Maternal.50	M 6.25 F 5.92	Maternal: 75	M 8.26 F 8.36	Maternal: decreased of food consumption and body weights. Litter weights of dams were significantly decreased	Edward et al (1982) AgrEvo: IIA, 5.6.1/2
Two generation reproduction toxicity in rats. dose levels: 2, 50 ppm					No adverse effect in any generation	Kennedy et al., (1965) AgrEvo: IIA, 5.6.1/1
Two generation reproduction toxicity in rats. Dose levels: 0, 3, 15, 75 ppm (0.2,1, 4.99 mg/kg bw/day for males and 0.24, 1.23, 6.18 mg/kg bw/day for females)	Maternal 15 Reprod 75:	Maternal 1 Reprod 6	Maternal:75	Maternal:1	Maternal: Increased relative liver and Kidney weights-	Edwards et al., (1984) AgrEvo:IIA, 5.6.1/1 Offer., (1985) AgrEvo, IIA: 5.61/4
Developmental toxicity studies						
Rats Dose levels: 0, 5, 10 mg/kg bw/day					No maternal toxicity was seen at any dose. Not possible to fix a NOAEL for developmental toxicity	Gupta et al., (1978) AgrEvo: IIA, 5.6.2.1/1 and Calliope: IIA, 5.6.2/01
Rats Dose levels: 0, 0.5 ,1.5 mg/kg bw/day					No significant maternal and developmental toxicity was observed at any dose tested.	Haley (1972) AgrEvo: IIA5.6.2.1/1
Rats Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal:2 Develop:2		Maternal:6 Develop:6	Maternal:. On the basis of the deaths, clinical signs and decreased body weight Develop: increase incidence of fragmented thoracic vertebral centra No teratogenic effects	Albrech & Baeder, 1993 AgrEvo: IIA, 5.6.2.1/4

Study	NOAEL		LOAEL		Main Adverse Effect	Reference/year
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d		
Rats Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal 0.66 Develop:2		Maternal:2 Develop:6	Maternal: decreased body weight gain and clinical signs. Develop: delayed development and a low incidence of isolated skeletal variation No teratogenic effects	McKenzie (1980) AgrEvo: IIA, 5.6.2.1/3)
Rabbits Dose levels: 0, 0.3, 0.7, 1.8 mg/kgbw/day		Maternal 0.7 Develop: 1.8		Maternal:1.8	Maternal: based on Clinical signs (noisy, rapid breathing, hyperactivity and convulsions) No teratogenic effects	McKenzie et al., 1981 AgrEvo: IIA, 5.6.2.2/1

B.6.6.1 Multigeneration reproductive studies

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

Data of report: 2 December 1982.

This report was a preliminary study to determine the maximum tolerated dosage of endosulfan on reproduction on the rats.

The report does not claim adherence to specific guidelines.

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

The study is considered acceptable.

Materials and methods

The test material used in this investigation was technical grade Endosulfan (Batch No. HOE 02671 OI AT 209), 97% purity. The test material as supplied was weighed out, ground to a powder and dissolved in a small volume of acetone, corn oil was then added. The mixture was then mixed with register diet (Spratts Lab. No 2) and stirred. Diets were storage until use in heal-sealed, opaque polythene bags. The stability of Endosulfan in the diet, at ambient temperature in the animal rooms, was confirmed during storage for 18 days.

In this study four groups of 10 males and 10 females each of the F0 and F1 generation of Charles River rats (approximately 7 weeks old, body weigh 263-265 g males and 191-193 g females), were used. The dietary concentrations employed in this study was 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 females food consumption was initially lower among test groups particularly at 70 and 100 ppm, also at these two concentrations lower weekly mean body weight were recorded, initially to a greater extent at 100 ppm than at 75 ppm, and overall weight gain during gestation was lower. At terminal autopsy mean liver weights were significantly higher among females in all test group. In comparison than the control value Litter size 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 insignificantly lower litter weights at 75 ppm and to a greater extent at 100 ppm from day 4-post partum.

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.

No treatment-related abnormalities were found in the young.

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 nor the stability of the thiodan administered to the animals. Furthermore 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.

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.

This report describes the effects of Endosulfan technical grade on the reproductive performance and developmental of multiple generation in the rat. Besides, two addends were performed to determinate of concentrations of Endosulfan in rodent diet and for microscopic observations in F1B generation adults and F2B generation weanling.

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

Addendum to report IIA, 5.5.1/3.

Data of report: 22 March 1985.

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

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 considered acceptable.

Material and Methods

The test substance was endosulfan technical grade (97% purity). It was dissolved in a small volume of acetone and corn oil was then added. The homogeneity and stability of endosulfan in the diet was established in conjunction with the preliminary investigation (IIA, 5.6.1/2).

The test substance 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, 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.

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

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

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

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

Statistically significant ($p<0.01$) increases in kidney weight were seen at 75 ppm in F0 and F1B males. Relative kidney weights in males at 3 and 15 ppm, and in females at all doses, were not increased compared with controls. Statistically significant increases in relative lever weights were observed in F0 males ($p<0.05$) and females ($p<0.01$) at 75 ppm, and in F1B females at 15 ($p<0.01$) and 75 ppm ($p<0.001$). The effect at 15 ppm in F1B was not seen at this dose level in any other matting during the study, and is considered incidental to treatment. On occasion, statistically significant increases in relative organ weights were seen during the study (F0 male hearts, F0 female brains, F0 firs matting female offspring pituitaries, F1B first matting females uteri), but in the absence of any consistent relationship with dose, theses effects are considered to be incidental to treatment with Endosulfan.

On the other hand, hispotathology review carried out by Offer (1985) show occasionally yellowish discoloured and granular pigmentation in proximal convoluted tubules associated with the dietary administration of Endosulfan at levels of 3, 5 and 75 ppm in male F1B rata and at 75 ppm in female

F1B generation. The incidence of and extent of this effect was dose-related. .In addition traces of minimal granular/cumped pigments in cells of proximal convoluted tubules were present in F1B generation adult rats at a dietary level of 75 ppm. These lesions were not found in F2B generation at any dose level.

The increase in incidence and severity of yellowish discoloration of proximal tubular cells in F1B adult rats (table 6.6.1-1) appears to be treatment related, with no control animals displaying these effects. However, this effect does not appear to be toxicologically significant, with no adverse effects associated with these findings at any dose level. At 75 ppm, all animals displayed either trace or minimal discoloration, and at this dose level, signs of granular/cumpled pigment were also detected. No treatment related increase in the incidence of progressive chronic glomerulonephrosis or other kidney related effects were reproted.

Table 6.6.1-1: Renal changes in F1B generation adult rats

Group	**1**		**2**		**3**		**4**	
Compound			**Endosulfan**					
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

While the increase in incidence of the cellular discolouration is related to the administration of Endosulfan, the findings is considered not to be toxicologically significant as no adverse effects were seen on cells and the yellow pigment was considered likely to be due to storage of Endosulfan and its metabolites in lysosomes before excretion. The presence of the pigment is thus an indication of exposure to Endosulfan rather than of toxicity.

Conclusion

Under the conditions of the present study the NOAEL for maternotoxicity was 15 ppm (approximately 1.0 mg/kg/day), based on the increase on liver and kidney weights at 75 ppm (approximately 6

mg/kg/day). The NOAEL for reproductive effects was 75 ppm (approx. 6 mg/kg/day), with no effects on reproductive parameters or treatment related abnormalities being seen at any dose level tested .

B.6.6.2 Developmental studies

B.6.6.2.1 Rats

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. Vol. 42: 150-152.

The objective of the study was to determine the teratogenic and embryotoxic effects of endosulfan in rats.

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 and strain and age of animals were not specified.

Material and methods
Female albino rats (180-200g) 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. The number of females copulates was 20, 26 and 32 females/dose level respectively and the number of pregnants 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).

Using Student's test performed statistical analysis of the data. 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 during 0-6 or 6-15 or 15-21 days of gestation 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.

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.

No abortions were observed in any group, but there was a significant increase in the percent of litters with resorptions (5.5% in controls, compared with 20% at 5 mg/kg/day, and 22.8% at 10 mg/kg/day) and an increase in foetal mortality, though this effect was slight and not dose related (2 foetal deaths at 5mg/kg/day, 1 at 10 mg/kg/day, compared with no foetal deaths in control).

Skeletal abnormalities revealed a statistically significant increase of absent 5 th sternebrae (19.9% in control rats and, 24.9 to 31.0% in treated groups) and in foetuses with incomplete ossification . The missing 5th 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.

No significant change in foetal weight was observed; none of the foetuses observed showed any gross abnormalities.

Table 6.6.2.1-1: Results of embryotoxicity and teratogenicity

Parameter	**Dose mg/kg/bw/day 0**	**Dose mg/kg/bw/day 5.0**	**Dose mg/kg/bw/day 10**
Percent of litters with resorptions	5.5	20*	22.8*
Skeletal abnormalities			
Sternebrae 5th absent	3	5*	6*
Percent of litters with abnormalities	15.7	26.6*	22.2
Percent of foetuses with abnormalities	19.9	31.0*	24.9*

*) The result is significantly different from control

Conclusions

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

However, the study can not considered acceptable because the purity, the stability of endosulfan technical and strain and age of animals were not specified.

Haley *et al* (1972) (AgrEvo, IIA, 5.6.2.1/1)
Data of report: 18th July 1972.

The objective of the study was to determine the potential teratogenicity of thiodan upon gravid albino rats.

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

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 daily from the 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 initial dosing was at 6 day of gestation and the final dosing day 15. 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.

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.

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.

The objective of the study was to evaluate the potential embryofetal toxicity and/or teratogenicity of endosulfan in rats.

No reference to a specific guideline.

GLP: Yes.

The study is acceptable with some reserves.

Material and methods

The test substance was endosulfan (FMC 5462), purity 97.3%, which was dissolved in corn oil (5 ml) and administered via gavage to CD Sprague Dawley albino rats (8-weeks old) at dose levels of 0, 0.66, 2 and 6 mg/kg bw/day on days 6-19 of gestation.

These doses were selected based on the results of a range finding test with FMC 5462 in pregnant rats. Each animal individual daily dosage was determinate by the animal body weight on day 6 of gestation (first day of treatment). Mated rats were obtained by housing each virgin female with one male and were 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. Initially the animals were classified in four groups (25 females per dose level) Later, 5 rats were added to control group (because problems in the staining of bone material) and 10 mated animals in the highest dose group (because mortality of seven animals, probably due, in five animals, to technical factors as indicated by necropsy).

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

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. There was a dose-related decrease in maternal body weight gains at 2 and 6mg/kg/day.

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. Mean foetal weight and shorter crown-rump length were significantly reduced in litters of dams treated with 6 mg/kg/ day.

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.

No statistically significant treatment related effect on sex ratios was observed, with the percentage of males ranging from 50.5% in controls to 45.8% at 6mg/kg/day. No external variations or malformations were seen at 0.66 or 2 mg/kg/day, however, at the high dose, 5 fetuses lordosis and 6 fetuses had oedema. All five of the foetuses with lordosis, and 5/6 of the fetuses with oedema were from a single litter from the one dam (nº 109). One fetus had the skin of the upper forelimb b to the chest.

Common minor skeletal variations were presented in all groups. The incidence of poorly ossified sternebrae (6^{th}) in the high dose group was significantly greater than for the control group. Two fetuses had clubbed left hind limbs in the high dose group. The five fetuses from dam nº 109 which had oedema and lordosis also had shortened, curved and twisted. Four of these fesuses had shortened pubes and two had an unossified hyoid bone. The incidence of these effects was generally <1% , and the effects were largely related to delayed developmental and confined mainly to a single litter from a single dam that showed numerous signs of poisoning related to administration of Endosulfan, including face rubbing, alopecia, flaccidity and hyperactivity. The developmental effects therefore probably related to the maternal toxicity of the high dose.

Conclusions

When endosulfan was administered to pregnant rats during organogenesis at doses up to 6 mg/kg/day, no treatment related birth defects were observed. Signs of maternotoxicity (including decreased body weights, convulsions, hypersalivation, hyperactivity) were observed at doses of 2 and/or 6 mg/kg/day. Accompanying this maternotoxicity was evidence of delayed development and/or an isolated low incidence of skeletal variations at 6.0 mg/kg/day.

Based on these effects, the NOAEL for developmental toxicity was 2 mg/kg/day on the basis of delayed developmental toxicity. The NOAEL for maternotoxicity was 0.66 mg/kg/day based on decreases in body weight gain at 2 mg/kg/day and decreased body weight gain and clinical signs at 6 mg/kg/day.

However, an increased incidence of litters with extra ribs and poorly ossified and unossified sternebrae was observed at the high-dose level. More detailed historical control data may be useful in determining whether the apparent increase in misalign sternebrae is due to an unusually low incidence in the concurrent control group and within the variability observed between studies. In the absence of such information, it is recommended that this finding be considered to be compound-related. 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 NOEL 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 objective of this study was testing endosulfan for embryotoxicity in rats after oral administration.

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 rats (aged about 65-70 days and body weight of 189±19g), at dose levels of 0, 0.66, 2.00 and 6.00 mg/kg once daily from days 7 to 16 gravidity. (Doses based in a range-finding study conducted beforehand were Hoe 002671 were tested with groups of two or three gravid Wistar rats in oral doses of 2, 6, 9 or 18 mg/kg.). The stability and homogeneity of the solutions were guaranteed for 4 hours .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. No statistically significant changes in reproductive or pup parameters were observed at any dose level in this study. The foetal sex ratio was relatively balanced, with a slightly increased percentage of males at 0.66 and 2 mg/kg (55% and 50% males, respectively), and slightly more females than males in controls and 6 mg/kg groups (52% males in both groups). No statistically significant increase in the incidence of abnormalities was observed in foetuses during examination. A single oedematous, retarded foetus in the 6 mg/kg group presented with brachygnathia superior with a relatively small alveolar cavity in the upper jaw combined with cleft palate, bending of both hind feet in the tarsal joint, wavy clavicles, and bent and shortened scapula. These findings were considered to be spontaneous in nature, given that no other limb or head defects were observed in any pup in any of the litters at this dose level.

Skeletal examination revealed a statistically significant increase in fragmented thoracic vertebral centre at 6 mg/kg, with seven foetuses from three litters seen with this effect (6.3% incidence versus previous

control values 3.9% maximum incidence). This effect was considered to be treatment related, and reflects the frank maternotoxicity of endosulfan seen at the high dose level, as no other significant skeletal abnormalities were seen at 6 mg/kg in this study.

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. It is to be noticed that the effect on the foetuses (fragmented sternebrae) observed at 6 mg/kg b. w/day would be considered as a consequence of the toxic effect on dams at this dose level. and not a teratogenic effects it.

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.

The study was designed to evaluate the potential embryofetal toxicity and/or teratogenicity of endosulfan to pregnant rabbits .

No reference to a specific guideline

GLP: yes.

The study is considered as acceptable with some limitations.

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 0.3, 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

There were no changes in mean body weight with endosulfan treatment, no does aborted and no signs of mortality were seen at the lower doses of 0.3 and 0.7 mg/kg/day. 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. The cause of death of the four the animal in the 1.8 mg/kg group was not determined, although histopathologic findings included the vacuolisation of hepatocytes, which is associated with systemic disturbances. This was attributed to bleeding into the bowel.

The number of implantations, litter size, sex ratio, mean foetal weight and length and the number of live and resorbed foetuses were unaffected by endosulfan treatment. There were no death foetuses in any of the treated or control groups. No gross external observations were reported. The only soft tissue anomaly in 6/167 high doses foetuses and consisted of the left carotid arising from the innominate; 1/141 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.

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 (IIA, 5.7)

Summary

Two studies were reported by AgrEvo and Excel companies to evaluate delayed neurotoxicity of endosulfan (Robert & Phillips, 1983 and Gupta, 1976) , nevertheless the second study was considered as not acceptable because any reference about the purity of the test substance was provided.

Robert & Phillips,(1983) designated a study to determine LD_{50} and delayed neurotoxicity of endosulfan in hens 200. birds were used and allocated in three different treatment: LD_{50} determination, protection assessment and neurotoxicity assessment. To determine LD_{50} was developed a preliminary range finding study on 5 groups of 2 birds doses with different concentrations to endosulfan. On the

basis of this results, 30 birds were allocated to 6 treatment groups of 5 birds each, at doses to 0, 40, 60, 90,135 and 110 mg/kg of endosulfan.

A small study was carried out to determine the protective effects of phenobarbitone, diazepam, atropine and 2-PAM when administered prior to dosing with endosulfan.

For neurotoxicity determination were used six groups of 10 birds each (including positive and negative control),treated with 96 mg/kg endosulfan (LD_{50} calculated).Negative control birds were dose only with corn oil and positive control with 500mg/kg TOCP in corn oil Under the conditions of this study, endosulfan did not produce any clinical signs of neurotoxicity at the LD_{50} calculated .

Table 6.7-1: Neurotoxicity studies

Study type/species/ dose levels		Comments	Reference and years
Acute Delayed Neurotoxicity in hens. Dose levels 0,40,60,90,110, 135mg/kg	LD_{50} value of the 96 mg/Kg	Any clinical signs of neurotoxicity at the LD_{50} calculated .	Roberts & Phillipps (1983) AgrEvo: IIA, 5.7/1
Neurotoxicity in Rats and mice		Endosulfan produce toxic effects due to CNS stimulation and the death may be due to direct depressant effect on some vital organ oft he body.	Gupta P(1976) Excel: IIA, 5.7/02)

Roberts, NL. & Phillips, CN (1983) (AgrEvo: IIA, 5.7/1)

Data of report: 22nd December 1983.

The study was designed to determine the acute oral toxicity ($LD_{50)}$ and delayed neurotoxic effects of doses adults hens with endosulfan.

The study was conducted to OCDE guideline “Acute Delayed Neurotoxicity”; Draft guideline may 20, 1981, in compliance EPA 540/9-82-0.25 .

The study is considered to be acceptable.

Materials and Methods

Assays were conducted with endosulfan technical 97.2%, pure, dissolved in corn oil. and administered to test birds via gavage as a single oral dose.

A total of 200 adults domestic hens (Gallus gallus domesticus), all females were obtained fort his study. The birds were a hybrid brown egg laying strains and were 12 months old at time of arrival and body weights range 2040g to 2565 g at the start of the pre-treatment period. A total of 155 birds was used in

the study: 10 for range finding, 30 for the LD_{50} , 25 for the assessment, and 60 for the neurotoxicity determination. An additional 30 birds were maintained during the pre-treatment periods for use as replacements when necessary.

The study was carried out in three parts :

1.- The preliminary range finding was carried out on five group of 1-2 birds dosed with endosulfan at 200, 130, 100, 150, 100, 13, 70 mg/kg/day respectively to established the dose level to be used in the LD_{50} determination (dosing followed by a 14-day observation period) .On the basis of this results, six groups (5 birds each) were endosulfan treated at dose levels to 40, 60, 90, 110, 135 mg/kg /day. (14-day pre-treatment period, followed by 14 day observation period after treatment).

2.- A small study was performed to determine the protective effects of phenobarbitone, atropine and 2-PAM when administered prior to dosing. at 96 mg/kg/kg endosulfan 5groups of 5 birds each.. Dosing was following by a 14-day observation period.

3.- Finally, for neurotoxicity assessment six groups of 10 birds each (including a negative control, only corn oil, and positive control ,only 500mg/kg of TOCP dissolved in corn oil,) were treated.with:96 mg/kg/day. A further group of 10 birds treated with 96 mg/kg endosulfan, was maintained for use as replacements during the pre-treatment period. Positive control was observed by 21-days post-treatment. at the end of which all group were sacrificed .All negative control birds were re-dosed with endosulfan at the same dose level at the end of day 21. A further 21-day observation period followed the second dose.

For range finding, LD_{50} determination and protection assessment bird daily, mortality and body weight were observed. Neurotoxicity was evaluated on basis of bird health, mortality, ataxia, body weight, food consumption and post-mortem and histological examens (brain, spinal cord, peripheral nerve.

Statistical analysis: Finney, (1971)

Results

For range finding study only in the lower dose group did not was mortality.

LD_{50} was determined according to the mortal findings showed in table 6.7-1 All mortalities occurred within 48 hours of dosing. The LD_{50} value was calculated to be 96 mg/kg (table 6.7-1).

Table 6.7-1: Deaths in the LD_{50} study for endosulfan in hen (experimental groups of 5 animals each)

Dose Mg/kg b. w.	Number of animals dead
0	0
40	0
60	1
902	2

110	3
135	4

All groups showed body weight losses during the pre-treatment settling in period. In the protection assessment study phenobarbitone (15 mg/kg/bw), diazepam (2 mg/kg/bw) and atropine plus 2-PAM (10+25 mg/kg b. w) were tested in separate groups. None of the treatments gave a clear reduction of in mortality. Symptoms of intoxication were delayed under protection with atropine+2-PAM.

In the study of neurotoxicity determination, a number of mortalities occurred during the pre-treatment settling-in period (Table 6.7-2).Due the high mortality found in Group 6 it was discarded and substitute by group 7.All mortalities appeared to be related to treatment.

Table 6.7-2: Mortality in Neurotoxicity determination

Group	Treatment	Dose (mg/kg/day)	Group (size)	Mortalities		
				First dose	Re-dose	Total
1	Corn oil	-	10	0	0	0
2	TOCP	500	10	0	0	0
3	Endosulfan	96	10	3	3	6
4	Endosulfan	96	10	6	3	9
5	Endosulfan	96	10	7	1	8
6	Endosulfan	96	10	7	1	8

Signs of intoxication were observed in almost all birds doses with endosulfan (lethargy, unsteadiness, and loss of balance, trembling, wing flapping and leg kicking). The majority of surviving birds recovered within two days of dosing. 7 birds to positive control developed ataxia on or after day 10 , however, three of them did not showed neurotoxicity signs. The body weight changes were variable in all groups to neurotoxicity determination, but generally within normal limit. The majority of birds showed overall bodyweight gains between days 21 and 42.The food consumption were again very variable. This was particularly marked in group 4, in which there was only one surviving bird.

Histopathological and gross macroscopic post mortem examinations revealed no treatment related changes, with the exception of animals dosed TOCP, which showed degeneration in the spinal cord and peripheral nerve sand several pale subcapsular areas on the liver

Conclusions

Under the conditions of this test, oral administration of endosulfan did not produce any clinical signs of neurotoxicity at the unprotected LD_{50} value of the 96 mg/Kg. This results was confirmed by the histological examination of the brain, spinal cord and sciatic nerve.

Gupta PK (1976) (Excel: IIA, 5.7/02)

The study has been published in Bulletin of Environmental Contamination & Toxicology, vol. 15 (No. 6).by Springer-Verlag New York Inc.

The objective was to determine endosulfan-induce neurotoxicity in rats and mice.

No guideline method was specified.

GLP: No

The study is to be considered as not acceptable because the purity of the test substance was not reported.

Material and Methods

Endosulfan dissolved in different vehicles (alcohol and 10% alcohol in ground nut oil) was given introperitoneally to adults rats and mice of either sexes.

LD_{50} was determined according to the method of Weil,(1952) starting from four dose levels (16 animals each) of endosulfan and observing the animals for mortality for a period of 7 days. The table 6.7-3 show the main value of LD_{50} in different sexes and species.

Table 6.7-3: Influence of sex and species on the acute toxicity of endosulfan given intraperitoneally

Species	Sex	Vehicle	LD_{50} (mg/kg)	95% confidence limits
Rats	F	Alcohol	22.1	18.6-26.9
Rats	M	Alcohol	46.7	38.9-55.6
Rats	F	alcohol/ground nut oil	48.6	36.4-51.8
Rats	M	alcohol/ground nut oil	89.4	73-107.4
Mice	F	Alcohol	7.5	5.3-10.1
Mice	M	Alcohol	6.9	5.4-8.9
Mice	F	alcohol/ground nut oil	13.5	10.6-16.8
Mice	M	alcohol/ground nut oil	12.6	9.4-16.8

During the experiment the animals were also observed for cage-sign of toxicity. Cholinesterase activity (AChe) was also estimated .Animals dying during the course of the experiment were subjected to post-mortem examination. The animals were killed by decapitation and brain removed.

Results

Rats were relatively resistant to the toxic effects of endosulfan compared to mice and the sex variations of about the same degree was seen only in rats. Thus the female rats were about twice sensitive to the lethal effects of endosulfan compared to the males irrespective to vehicle. The first apparent signs of intoxication observed were hyperresponsiveness to tactile stimuli and between 2^{nd} and 3^{rd} hours, tremors of fore limbs. Occasionally these tremors were intense. .Initially, respiration was increased followed by depression. The body temperature remained unchanged and sometimes it was even subnormal and difficult None of the animals shower diarrhoea or any discharge from the eyes. At lower doses endosulfan did not produce any significant change in AChe activity of brain;. however, at high dose level (60mg/kg) endosulfan induced a significant decreased of AChee activity in brain.

Conclusion

This study indicated, that endosulfan toxicity was depended on the vehicle used and according to the sexes. Tremors induced by endosulfan could be due to increase in the rat brain Ache. Endosulfan produce its toxic effects due to CNS stimulation and the death may be due to direct depressant effect on some vital organ oft he body.

B.6.8 Further toxicological studies (IIA, 5.8)

B. 6.8.1 Supplemental studies

Three applicants, AgrEvo, Excel and Calliope have provide several supplemental studies about:

1.-Enzyme induction
2 Tumour Promotion
3.-Endocrine system:
4.-Sperm effect
5.-Inmunotoxicity
6.-Neurobehaviour

Besides, AgrEvo has included review document of endosulfan prepared by the Australian National Registration Authority (ANRA) for Agricultural and Veterinary Chemicals, which includes studies previously presented and studies which have not been presented by any applicant.

However, additional information to cover these items has been found from IPCS (1998). Nevertheless, this information is only a little summary of the original papers, thus they have been considered only as additional information within of summary of each item.

Table 6.8.1-1 Summary of supplemental studies

Study	Dose levels	Main Effects	Reference
Enzyme induction			
3-days. Oral gavage in male mice.	5 mg/kg/day	Cytochrome P-450 group of enzymes is not significantly activated.	Robacker et al., (1981) (AgrEvo: IIA, 5.1.3.2/2):
Promotion study			
In vitro metabolic co-operation (V79 cells) and scrape loading/dye transfer (WB cells) assays *Invivo* EAF incidence assay, Oral gavage10-weeks, rats(m),	Doses:1 and 5 mg /Kg/ bw/day	*In vitro*:ENDOαβ, ENDOα , ENDOβ, technical Endosulfan and Endosulfan-sulphate metabolite were potent inhibitors of intracellular communication in both assays in vitro. In addition Endosulfan-ether inhibited transfer in WB cells. *In vivo*: Technical endosulfan produced congestion of the peritoneum and inner organs, and increased liver weights	Flodström et al, (1988) (AgrEvo:
Endocrine system			
In vitro and In vivo studies		Endosulfan does not meet the criteria of a endocrine disrupter	Bremmer & Leist (1998) AgrEvo review
Effects on sperm			
Oral short-term/chronic study in male rats	2.5, 5. 7.5.10 mg/kg	Possible deleterious effects on male reproductive organs (testis) and biosynthesis and secretion of testosterone	Singh & Padney(1989) (Excel, IIA, 5.5/01
Oral subchronic study in male Wistar rats	0, 7.5, 10 mg/kg/day	Testicular testosterone levels remained significantly decreased.	Singh & Padney(1990) (Excel, IIA, 5.5/03)
Immunotoxicity studies			
Oral, six week study in male Wista rrats	0,10,30,50 ppm	Humoral and cellular immunity was depressed at doses of 30 and 50 ppm	Banerjee & Hussain (1987) (AgrEvo: IIA, 5.8.2.1/3)
Oral study in albino rats for up to 22 weeks	0,5,10,20 ppm	Marked suppression of the humoral and CMI responses in rats. Cellular and humoral immune responses were decreased in a dose-time dependent pattern.	Banerjee & Hussain (1986) (AgrEvo: IIA, 5.8.2.1/2)
Oral Wistar rats study	0.5, 1.5, 4.5 mg/kgbw/day		Hack & Leist (1988) (IPCS 1998)
Oral study in Wistar rats (3-weeks)	20, 100, 250 ppm	At 100 ppm: reduction in body weight gain.	Vos et al, (1982) (IPCS 1998)
Neurobehavioral studies			
Oral acute study in rats	25, 50, 100 mg/kg/day (males) 3,6,12 mg/kg/day (females	LOAEL: 50 and 6 mg/kg/bw/day male and female respectively, based on serious neuropharmacological effects.	Bury (1997) (IPCS 1998)

Study	Dose levels	Main Effects	Reference
Rats	10mmol/L	No inhibition of rat brain AchE activity was observed for up to 75 min treatment.	Müller (1989) (IPCS 1998)
30-daysdietary study in Wistar rats	0, 3 and 6 mg/kg/day	A significant dose–related increase in motor activity in both sexes at low and high dose.	Paul , V et al., (1995) (AgrEvo:ANRA)
90-Days oral study in male rats	2 mg/kg/day	Changes in central nervous system, but not impair motor responses	Paul , V et al., (1993) (AgrEvo:ANRA)
90-Days oral study in male rats	2 mg/kg/day		Paul , V et al., (1994) (AgrEvo:ANRA)

B.6.8.1.1 Enzyme induction

Robacker et al E (1981) (AgrEvo: IIA, 5.1.3.2/2)

Date of experimental work; Not provided in the report.

The study was performed prior to GLP regulations.

The study is not acceptable. The applicant does not report groups and number of animals used in this study.

Material and methods

Test substance: 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxid (Endosulfan). The percentage of purity has been not included in the report.

Male mice (Flow Lab., Duplin, Virginia) of 6-7 weeks old were dosed with 5 mg/kg/day by oral gavage during 3 days. For acclimatisation, all 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.

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.6.8.1.2 Promotion study

Summary

Tumour promotion of endosulfan and some of its metabolites was tested in different in vitro and in vivo systems by Flödstrom *et al*., (1988) The study has been published in Pharmacol. Toxicol.,Vol. 62. Pages 230-235**.**. Two in vitro test systems inhibition of intracellular communication. However, endosulfan administered orally (1 or 5 mg/kg/day) did not enhance enzyme altered foci incidence The contradictory opinions on the tumorogenesis of endosulfan could be caused by the different behaviour of compounds in animals and species specificity in tumour promotion effects.

Flodström, *et al.,* (1988). (AgrEvo: IIA,5.5.3/1)

The study has been published in Pharmacol. Toxicol.,Vol.. 62. Pages 230-235**.**

The objective was testing endosulfan and some of its metabolites in different *in vitro* and *in vivo* systems for effects possibly related to tumour promotion.

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

GLP: :No

This study is considered acceptable only as an additional information with some reservations.

Material and Methods

Test substances: Analytical grade Endosulfan (ENDOαβ, ENDOα, ENDOβ with α: β–isomer ratio 70:30, 99.7:0.3 and 0.5:99.5 respectively) Technical grade Endosulfan (96.2% purity, α: β–isomer ratio 70:30) and Endosulfan metabolites (Endosulfan-sulphate, Endosulfan-alcohol, Endosulfan-ether, Endosulfan-lactone).

The solvents were ethanol (V79 cell system) and DMSO (WB cell system). Corn oil was the vehicle in the *in vivo* test Phenobarbital was the positive control in the *in vivo* test.

Two *in vitro* test systems detecting inhibition of intercellular communication were used: the Chinese hamster lung fibroblasts (V79) metabolic co-operation assay (Trosko *et al*., 1981 and Warngard *et al.,* 1987) and a scrape loading/dye transfer assay rat liver WB epithelial cells (El-Fouly *et al*., 1987).

In the metabolic co-operation assay at least 5 concentrations were tested in a range from < 25 to 100μM for Endosulfan ether, from < 20 to 80 μM for Endosulfan-lactone and from 5 to 30 μM for the remaining test substances.

The concentrations selected for testing in the scrape loading/dye transfer assay were: 40 μM for Endosulfan-alcohol, 80 μM for Endosulfan ether and Endosulfan lactone, and 20 μM for the remaining test substances.

Endosulfan was also studied for enhancement of enzyme altered foci (EAF) incidence in rat liver *in vivo* (Flodström *et al*., 1988). Male Sprague Dawley rats, weighing 130-140 g were used. The experimental protocol consisted on eight treatment groups: The groups **1, 2** and **3** (4 rats per group) were partially hepatectomized (PH) and not NDEA-injected but were administered vehicle, technical Endosulfan (1 mg/kg) and technical Endosulfan (5 mg/kg), respectively . Group **4** (5 rats) were neither PH, nor injected with NDEA but treated with technical Endosulfan (5 mg/kg). Group **5** (10 rats) were PH, NDEA-injected and treated with the vehicle. Groups, **6** and **7** (11 rats per group) were PH, NDEA-injected and treated with technical Endosulfan at doses of 1 and 5 mg/kg respectively. Group, **8**, (5 rats) were PH, NDEA-injected and treated with phenobarbital. The vehicle and endosulfan were administered via gavage, and phenobarbital in the drinking water. NDEA was injected 24 hours after hepatectomization; the following day the test substance was administered 5 days a week for 10 weeks. Exposure to test chemicals and vehicle was discontinued two days before sacrifice. Liver slices were prepared and γ-glutamyl transpeptidase (GGT) was determined. Plasma activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and GGT were also determined.

The metabolic data were analysed by the T-method (Sokal & Rohlf, 1981) and the *in vivo* data were evaluated by the GT2-method (Sokal & Rohlf, 1981) .Differences between treatment groups were considered significant at the level $P< 0.05$.

Results

ENDOαβ, ENDOα and technical Endosulfan were cytotoxic to the V79 cells at concentrations above 20-25μM. ENDOβ was slightly more cytotoxic as demonstrated by a decrease in colony size from 20μM. The endosulfan sulphate and alcohol metabolites elicited cytotoxic effects in the same dose range as the parent substances, whereas endosulfan lactone and ether were less cytotoxic. ENDOαβ, ENDOα , ENDOβ, technical Endosulfan and Endosulfan-sulphate all produced a dose-dependent inhibition of metabolic co-operation at non cytotoxic concentrations from 15μM, while the other three metabolites tested were inactive.

The results, only qualitative, from scrape loading/dye transfer assay were in agreement with the outcome of the V79 metabolic co-operation assay. In addition, Endosulfan-ether inhibited dye transfer in this assay.

No clinical signs of toxicity related to drug treatment were observed in rats. Congestion of the peritoneum and inner organs appeared in all rats administered endosulfan. No significant differences between the treated groups and control groups were observed regarding body weight gains, relative liver weights and plasma transaminase activities with the only exception of increased liver weights in the endosulfan treated groups 6 and 7. Endosulfan technical grade was found not to enhance the incidence of GGT-positive hepatocyte foci. .Positive control gave a satisfactory response.

Conclusion

Under the conditions of this study, ENDOαβ, ENDOα , ENDOβ, technical Endosulfan and Endosulfan-sulphate were shown to be inhibitors of intercellular communications in both *in vitro* assays. In addition, Endosulfan-ether inhibited dye-transfer in rat liver WB cells. Nevertheless, No inhibition no to enhance the incidence of GGT-positive hepatocyte in NDEA initiated was found in male rats treated with endosulfan.

These apparently contradictory results with regard to possible tumour promoting activity of endosulfan could be explained in relation to metabolism, systemic toxicity and tissue species specificity in tumour promotion.

However, there were some reservations about the performance of the tests. More details about the experimental protocol are necessary for the *in vitro* assays and a positive control should have been included in them. On the other hand, the highest dose used in the *in vivo* assay was 5 mg/kg/day since higher doses increased mortality in a pilot study; however, Date from the pilot study were not shown and besides, this dose did not produced any signs of toxicity for animals. Therefore, a higher dose should be tested.

B.6.8.1.3 Endocrine system

Summary

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

A revision of possible endocrine effects of endosulfan in mammalian species have been provided by AgrEvo:

Bremmer, JN & Leist KH (1998) (AgrEvo)
Date of report: 18 December, 1998

The possibility of endosulfan to cause endocrine modulation has been evaluated based on the by OECD and EU agreed definition of an endocrine disrupter .

A large number of in *vivo* mammalian toxicity studies on endosulfan together with various uterothropic assays strongly suggest that endosulfan does not elicit endocrine disruption. No endocrine effects were found at doses up to and including toxic levels (Raizada *et al*., 1991; Ashby *et al*., 1997; Shelby *et al* ., 1996; Wade *et al*., 1997).

The in *vitro* studies show that endosulfan has extremely low binding potency to the human oestrogen receptors, 10^5 to 10^6 times smaller than that of the natural hormone 17b-estradiol. The binding potency of endosulfan is also low compared to that of many natural phyto-oestrogens in the human diet. (Soto *et al*., 1995; Wade *et al*., 1997; Arcaro et al., 1998; Shelby *et al.,* 1996; Ashby *et al*., 1997; Ramamoorthy *et al*., 1997).

Published screening studies for androgenic effects are inconclusive. Isolated findings steroid metabolism and reduced sperm count have been claimed. Characterisation of the test substance was not adequately defined.

In contrast, the many in vivo toxicity studies gave no indication of any endocrine organ and/or system. Neither morphological or functional effects on endocrine or reproductive organs , nor any effects on reproductive performance, sexual development, differentiation or maturation, or activity related to any other endocrinological endpoints was found, although in these studies doses were applied in the toxic range.

Synergist effects of endosulfan with other compounds were not found. In vitro studies unequivocally indicate that endosulfan does not enhance oestrogenic or endocrine endpoints of other pesticides.

It is concluded that endosulfan does not meet the criteria of an endocrine disrupter. No effects were found on endocrine, reproductive or sexually regulated systems in vivo at doses causing clear toxicity.

B.6.8.1.4 Effects on sperm

Summary

There were two published studies provided by Excel company (Singh & Pandey 1989; 1990). However, both studies have been considered as not acceptable mainly, because the purity of the test substance was not provided.

Singh, & Pandey, (1989) (Excel; IIA, 5.5/01)

This work has been published in Indian Journal of Experimental Biology; vol. 27:341-346.

The purpose of this study was to evaluate the effect of short-term chronic exposure of endosulfan in male rats on testosterone biosynthesis and its secretion to serum.

The paper does not claim adherence to a specific guideline.

GLP: was not indicated.

The study is considered as not acceptable. There is neither indication on the purity of the product (technical grade) and on its stability**.** Nevertheless, in spite of its technical unacceptability, the below-described findings should be taken into account as a warning red light for likely effects of endosulfan on male reproductive organs, especially testis and its endocrine function.

Materials and Methods

The purity and stability of the Endosulfan (Technical grade endosulfan from M/s Hoechst Bombay, India) is not indicated.

A total of 60 adult male Wistar rats (bodyweight: 200-250g) were divided into 5 groups containing 12 animals in each and given the treatments as shown below.

Group A: this group was kept as control and animals were given only vehicle solution of ground nut oil.
Group B: this group was given Endosulfan dissolved in ground nut oil at 2.5 mg/kg/bw daily oral through a cannula.

Group C: Received Endosulfan at 5.0 mg/kg dose level.

Group D: Received Endosulfan at 7.5 mg/kg dose level.

Group E: Received Endosulfan at 10 mg/kg dose level.

Body weights of individual animals were recorded every alternate day. Six rats from each treatment group were killed after 7 and 15 days treatment along with their respective controls. The animals were fasted overnight, sacrificed by decapitation and blood was collected from individual animals through heart puncture.

Body and organ weight was measured. The effects on testicular microsomal and cytosolic protein contents, on steroidogenic enzymes 3β-hydroxysteroid dehydrogenase, on cytosolic conjugation activity, on testosterone levels were studied.

Statistical analyses: Student's test.

Results

Organ and body weight of the treated animals did not change significantly; however, an appreciable decrease was noticed at 2.5 and 5.0 mg/kg dose levels after 15 days treatment. The testicular protein content was found to be increased appreciably at 2.5, 5.0 and 10.0 mg/kg after 7 days treatment. The activity profile of cytosolic conjugation enzyme remained low during 7 days treatment at all the dose levels (2.5, 5.0, 7.5 and 10.0 mg/kg), however, the two steroidogenic enzymes showed much individual variations in response to endosulfan treatments. An overall varied response with respect to testosterone biosynthesis and its secretion to serum was observed suggesting nevertheless, a profound hormonal imbalance caused by this insecticide to male gonads on short term chronic exposures.

Conclusions

Under the experimental conditions described, technical grade endosulfan produced possible deleterious effects on male reproductive organs, especially testis and its important endocrine functions viz., biosynthesis and secretion of testosterone.

Singh & Pandey, (1990) (Excel: IIA, 5.5/03)

The study has been published in Indian Journal of Experimental Biology, vol. 28, pp: 953-956.

The biochemistry toxicity of sub-chronic endosulfan treatments in relation to gonadal hormones was investigated in rats, via plasma and testicular testosterone, plasma gonadothrophins enzymes, FSH, LH and 3B-hydroxisteroid dehydrogenase (3B-HSD) and 17-hydroxisteroid dehydrogenase (17B-HSD) .

The paper does not claim adherence to a specific guideline.

GLP: No

The study is considered not acceptable because the purity of the test substance was not specified.

Material and Methods

Technical endosulfan (α- and β–isomers in the ratio 70:30) was administrated to male Wistar strain rats (150-200g) in the diet.

A lot of 48 rats were divided into three groups:

Group A: rats were further divided into three subgroups of six tats each and received treatments as control (only vehicle solution of ground nut oil); endosulfan at 7.5 mg/kg/bw dissolved in ground nut oil, orally (through a steel cannula) for 15 days and, endosulfan at 10mg/Kg/bw daily for 15 days.

Group B: (18 rats), received identically treatments as group A, daily for 30 days

Group C: was divided in into two subgroups of six rats each, 3 as control and the other 3 rats received endosulfan at 10mg/kg/bw dose level for 30 days and were kept for 7 days on normal dietary regime.

The animals were sacrificed by decapitation on 16th, 31st and 38th day respectively.

In this study, testis were removed and decapsulated and was measured steroidogenic enzymes content. By radioimmunoassay hormones (plasma gonathrophines and testosterone), plasma concentrations o FSH and LH and levels of plasma and testicular testosterone was determinate.

The results were statistically analysed using Student's t test and the values were considered significant at $P< 0.05$.

Results

A significant inhibition of 3B—and 17-BHSD in the testes of treated animals occurred at 30 days of treatment. The plasma FSH, LH and testosterone levels were significantly reduced in rats treated for 15 and 30 days at both dose levels. Plasma testosterone and testicular testosterone levels at the lower dose of 7.5 mg/kg were not significantly reduced after 15 days of treatment. A significant decrease in the contents/activities of microsomal cytochrome P-450 and related mixed function oxidases (MFOs) in the testes of the treated animals was observed, along with a marked inhibition in the activity of the glutathione-S-transferase at both dose levels. These changes were reversed when endosulfan was with drawn, however the testicular testosterone levels remained significantly reduced.

These observations clearly demonstrated the inhibitory effects of endosulfan, on the secretion of pituitary gonadotrophines (FSH and LH) and in turn on the testosterone biosynthesis in testis of rats.

Most of biochemical alterations were reversible after endosulfan withdrawal. However, the testicular testosterone levels remained significantly decreased suggesting a comparatively slow recovery in the internal reorganisation of microsomal membrane system responsible for the biosynthesis of testosterone, possible due to long biological half-life of this insecticide in animals systems.

However, this paper does not given information about purity and stability of endosulfan as well the housing conditions and years old to the rats. This information should be incorporated to considered the validation study.

B.6.8.1.5 Immunotoxicity studies

Summary

Two immunotoxicity published studies have been provide by AgrEvo (Banerjee & Hussain 1986;1987) Both clearly indicated reduced ability to mount an immune response to an antigen following subchronic exposure to endosulfan. It should be noted that the methodology used to asses cellular immunity in these studies is far from ideal as it is flawed by large inherent errors, lack of objectivity and, except in very experienced hands, lack of accuracy. Less subjective tests of cellular immunity eg. Cytotoxic T cell response to a virus would have provided more reliable results.

Other immunotoxicity studies corresponding to Hack & Leist (1988) and Vos *et al*., (1982) were summarised in IPCS. Both studies, suggest that it does not have any adverse effect on the immune function of laboratory animals

Banerjee & Hussain (1987) (AgrEvo: IIA, 5.8.2.1/3)

The study was published in Bull Environ Contam Toxicol. 38: 438-441.

The present study was designed to evaluate the humoral and cell-mediated immune (CMI) responses of endosulfan in albino rats.

The study is considered as not acceptable because the purity of the test substance is not provided.

Material and Methods

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

Results

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

A significant decrease in total serum antibody titre to tetanus toxoid occurred at 30 and 50 ppm endosulfan with a slight decrease (not statistically significant) at 10 ppm. The decrease was observed in

both IgM and IgG levels at 50 ppm. Measurement of total gamma globulin content of rat serum again indicated suppression at 50 ppm. Rats exposed to endosulfan and subsequently immunised with tetanus toxoid showed a significant decrease in LMI and MMI responses in a dose-dependent pattern, the decrease becoming statistically significant at the 3 0 and 50 ppm level.

Conclusion

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

Banerjee & Hussain (1986) (AgrEvo: IIA, 5.8.2.1/2)

The study was published in Arch Toxicol. 59: 279-284.

The present study was designed to evaluate the effect of sub-chronic doses of endosulfan on humoral and cell-mediated immune (CMI) responses in albino rats.

The study is considered as not acceptable because the purity of the test substance is not provided.

Material and Methods

Endosulfan was administered to male Wistar albino rats (50-60/group) in their diets at concentrations of 0, 5, 1 0 or 20 ppm for up to 22 weeks. At 20 days before the end of exposure, rats were immunised with tetanus toxoid in complete Freund's adjuvant. Interim kills (10-12 animals) were carried out at 8, 12, 18 or 22 weeks of study. Total Ig, IgM and IgG levels were estimated. Leukocyte migration inhibition test and macrophage migration inhibition test were also carried out at 8, 12, 18 or 22 weeks of exposure.

Results

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

The total Ig levels were increased in all stimulated groups as expected, except in the 20 ppm dose group at weeks 12, 18 and 22 and in the 10 ppm dose group at week 22. A significantly lower increase in serum IgG was seen in rats exposed to 1 0 or 20 ppm endosulfan at weeks 12, 18 and 22 as compared to antigen-stimulated control. IgM levels were unaffected by the endosulfan treatment. The specific response (serum antibody titre to tetanus toxoid) showed a marked decrease in rats exposed to 10 or 20 ppm endosulfan throughout the experiment in a dose-time dependent pattern. Cellular immunity was assayed by measuring migration inhibition of activated leukocytes and macrophages. Endosulfan treatment diminished migration inhibition responses of both leukocytes and macrophages at the 10 and 20 ppm dose level throughout the study. The endosulfan-related effect on the immune system of the rat

did not appear to be secondary to other toxicity since the body weights of the animals were unaffected by the treatment and endosulfan is not known to affect the hormonal system. The effects were dose and time related. The results of the other assays of immune responsiveness although less reliable followed a similar trend. Immune responses were unaffected by endosulfan treatment at 5 ppm (0.25 mg/kg/day).

Conclusion

Results obtained in this study revealed marked suppression of the humoral and CMI responses in rats administered with sub-chronic doses of endosulfan. Both cellular and humoral immune responses were decreased in a dose-time dependent pattern. Suppression of immune responses by endosulfan is clearly an important aspect of its toxicology.

Hack & Leist, 1998 (IPCS 1998)

Material and Methods

Technical-grade endosulfan (purity 96%) was administered in sesame oil on 10 occasions by gavage to groups of 8 female Wistar rats at doses of 0.5, 1.5 or 4.5 mg/kgbw/day from 2 days before until seven days after infection by gavage with approximately 500 *Trichonella spiralis* larvae. As a positive control, prednisolone was administered by subcutaneous injection at a dose of 25 mg/kgbw/day two days before and three days after infection. Three rats from each group were killed seven days after infection so that the number of adult worms in the intestine could be counted; the remaining rats were killed 54-days after infection in order to count the number of larvae in the tongue.

Results

Thymus and spleen weights and the percentage lymphocytes in the white cell count were measured at both times. Body weights were measured weekly. There were no differences between endosulfan treated and untreated rats, whereas the prednisolone-treated group had a sevenfold higher tongue larval count and, at seven days, a 25% reduction in thymus weight, a 50% reduction in spleen weight, and lymphocyte count < 50% of the control value.

Vos et al., 1982 (IPCS 1998)

Endosulfan was included in the first part of a study to screen for immunotoxicity, but because no effect was observed it was not examined in greater detail.

Material and Methods

Groups of six male, weanling Wistar rats were given endosulfan in the diet at concentrations of 20, 100 or 250 ppm for three weeks. Body weights and food intake were recorded weekly. At autopsy, the weights of the liver , kidneys, spleen, thymus, pituitary, adrenals, thyroid, testis and mesenteric and popliteal lymph nodes were recorded, these organs were also examined histologically. Haematological examinations consisted of total and differential leukocyte counts. Serum IgM and IgG were determined by enzyme-linked immunosorbent assay.

Results

The only effects induced by endosulfan were considered to be expression of general toxicity, and there was no evidence for any specifically immunotoxic effects. The most sensitive parameter for the toxicity of endosulfan was a reduction in body weight gain, which was observed at 100 ppm.

B.6.8.1.6 Neurobehavioral studies

Summary

AgrEvo has included review documents of endosulfan prepared by the Australian Registration Authority (ANRA) for Agricultural and Veterinary Chemicals , which have been considered only as additional information because they are a summary of the published paper. These papers were conducted by the same group of investigators (Paul et al., 1993; 1994;1995) .Generally, at doses tested which resulted in signs of frank toxicity (reduce body weights, reduce food consumption, mortality, increased intensity of tremors, increased liver enzyme activity), some changes in behavioural were noted, including increased motor activity, and inhibition of conditioned and unconditioned escape and avoidance responses.

Other Neurobehavioral studies corresponding to Müllner (1989) and Bury, (1997) were summarised in IPCS.

B.6.8.1.6.1 Acute neurobehavioral toxicity study

Bury, (1997) (IPCS 1998)

The study was carried out in accordance with OEDC 401 guideline; “Acute Oral toxicity”

GLP: Yes

Technical-grade endosulfan (purity 98.6%) was administered to groups of 10 rats as a single dose of 25, 50 or 100 mg/kg bw/day to males and 3, 6 or 12 mg/kg bw/day to females. Deaths occurred t the highest doses, and there was a dose-related increase in the frequency of clinical signs, which were reversible and apparent only on the day of dosing. Theses was assumed to be due to the known affinity of endosulfan for the γ-aminonobutyric acid receptors in the brain. At 50 and 100 mg/kg bw/day in males and 6 and 12 mg/kg bw/day in females, various serious neuropharmacological effects were seen including coarse tremor and tonic-clonic convulsions. At 25 mg/kg bw/day in males and 3 mg/kg bw/day in females, the clinical signs seen were typical of general discomfort, such as stilted gait, squatting posture, and irregular respiration. No compounds-related effects on motor activity were observed at non-lethal doses. No effects were seen on the rearing frequency, fore-or hindlimb grip strength, or on landing foot-spread. No histopathological effects were found in the central or peripheral nervous system.

Müllner, (1989) (IPCS 1998)

No inhibition of rat brain Ache activity was observed in a preparation incubated with 10μmol/L-α-endosulfan for up to 75 min. A similar concentration of aldicarb produced 15% inhibition within 5 min and 80% inhibition within 75 min.

B.6.8.1.6.2 30-Day Dietary Study

Paul V, et al (1995) (AgrEvo: ANRA)
European Journal of Pharmacology-Environmental Toxicology and Pharmacology Section, 293, 355-360.

The objective of this study was to evaluate a sex-related differences in the neurobehavioral and hepatic effects following chronic endosulfan treatment in rats.

The study is considered as additional information because is only a review of the publisher paper.

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

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

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

B.6.8.1.6.3 90-Day Oral Study

Paul V et al., (1993) (AgrEvo:ANRA)

Indian Journal of Physiology and Pharmacology, 37, 204-208.

In this paper was studied the biochemical and behavioural changes produced by repeated oral administration of endosulfan in rats.

The study is considered only as an additional information because is only a review of the publisher paper.

Material and Methods

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

Animals were observed during the treatment period for mortality and any obvious behavioural changes such as tremors and convulsions. Food consumption and body weight were measured prior to commencement of treatment and then every 15 days until completion of treatment. Spontaneous motor activity and motor co-ordination were recorded every 15 days.

24 hours after the last treatment some animals were subjected to necropsy. The brain, heart, liver, spleen, kidneys and adrenals were dissected and weighed. Protein concentrations were estimated in the serum, brain, liver , heart and skeletal muscle. The activities of GOT and GPT were determined in the serum and liver of the remaining animals.

Results

No clinical signs of convulsions occurred due to treatment with endosulfan. A significant reduction ($P<O.05$) in food consumption (44% with respect to controls) and bodyweight gain (37%) occurred 16-30 days after the start of treatment, and continued throughout the treatment period. A significant increase ($P<0.05$) in motor activity was noted in animals treated with endosulfan, particularly at the 75th and 90th day of treatment (48% at this time). Motor co-ordination was not effected by treatment with endosulfan.

A significant ($P<0.05$) increase in liver weight (9%), liver GOT (64%) and GPT (44%) and increased serum GPT (39%) were found in animals treated with endosulfan. Animals treated with endosulfan showed significantly decreased myoclonus latency (1 5%), increased tonus (67%), mortality (9/12 animals) and intensity of myoclonus (33 %) (measured 1-10 minutes after injection) with respect to control animals.

Conclusion

Endosulfan-induced changes in the liver and central nervous system effects, but did not impair motor responses in male rats. Nevertheless, this study does not appear to use typical measures for neurobehavioral effects, and it is difficult to interpret the relevance of these results.

Paul V. *et al.,* (1994) (AgrEvo: ANRA)

European Journal of Pharmacology-Environmental Toxicology and Pharmacology Section, 270, 1-7.

The purpose oft his study was to evaluate the neurobehavioral toxicity of endosulfan.

The study is considered only as an additional information because is only a review of the publisher paper.

Material and Methods

Endosulfan (purity 95%) was administered by oral intubation to six groups of immature male rats (10-12 rats/group) at a single daily dose of 2 mg/kg/day for 90 days. A suspension of endosulfan was formulated in distilled water with an equivalent amount of tragacanth powder. A control group of 10- 1 2 rats received only tragacanth suspension. One of the groups was treated intraperitoneally with p-chlorophenylalanine (PCPA) methylester HCL (100 mg/kg/day) during the last 3 days of the 90 day treatment. PCPA is known to produce an 80% depletion of 5-HT in the brain.

Animals were observed during the treatment period for mortality and any obvious behavioural changes such as tremors and convulsions. Food consumption and body weight were measured.

Results

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

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

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

Endosulfan may impair learning and memory in rats as evidenced by suppression of escape (learning) and avoidance (memory) acquisition respectively. This was possibly facilitated by a serotonergic involvement (increased 5-HT levels) as PCPA reinstated significantly the escape response but not the avoidance response in endosulfan treated animals. In conclusion this suggested that there was an

endosulfan-induced increased serotonergic impairment of learning but that the contribution of this mechanism to memory disruption is negligible. Given the relatively large dose level employed, the effects seen in this study may reflect the high toxicity of endosulfan and the general physiological status of the animals rather than any learning impairment due to endosulfan administration.

Conclusion

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

B.6.8.2 Endosulfan-metabolites studies

Summary

Testing of endosulfan-diol (Hoe 51329) metabolite for acute oral toxicity, LD_{50} values for the male and female rats were higher than 5000 mg/kg b.w. (Ehling & Leist, 1991a). Studies of acute dermal toxicity with this metabolite determined that the LD_{50} values for male and female rats were higher than 2000 mg/kg b.w. (Ehling & Leist 1991c). Endosulfan-diol is irritant and the skin contact produce sensitisation in guinea pig (Hammerl, 1996a) and, however, the substance has not this effect when the Buehler Test has been applied (Hammerl 1996b). Whit respect to dermal and eye irritation, the test substance can not considered as irritant substance (Hammerl 1996c).

Testing of endosulfan-sulphate (Hoe 51327) metabolite for acute oral toxicity, LD_{50} values were 568 mg/kg b.w. for male rats, and 25-50 mg/kg b.w. for female rats (Ehling & Leist 1991b), whereas for acute dermal toxicity LD_{50} values were 2740 mg/kg b.w. for male rats and 280 mg/kg b.w. for female rats (Ehling & Leist 1991d).

It is impossible to obtain solvent data on endosulfan-lactone, endosulfan-hydroxyether, endosulfan-ether, and endosulfan-alcohol because the submitted studies have serious deficiencies, and they have been evaluated as unacceptable. More information is required.

Table 6.8.2-1: Acute toxicity of metabolites

Metabolite	Route/Species/Sex	Dose range (mg/kg bw)	Result	Reference
Hoe 51329	Oral Wistar rats (m/f)	5000	LD_{50} >5000 (m/f)	Ehling & Leist 1991a
Hoe 51327	Oral Wistar rats (m/f)	25, 31.5, 50, 63, 100, 200, 400, 800	LD_{50}= 568 mg/kg bw (m) LD_{50} = 25-50 mg/kg bw (f)	Ehling & Leist 1991b
Hoe 51329	Dermal Wistar rats (m/f)	2000	LD_{50} >2000 (m/f)	Ehling & Leist 1991c

Metabolite	Route/Species/Sex	Dose range (mg/kg bw)	Result	Reference
Hoe 51327	Dermal Wistar rats (m/f)	250, 315, 400, 1600, 2500, 4000	LD_{50}= 2740 mg/kg bw (m) LD_{50} = 280 mg/kg bw (f)	Ehling & Leist 1991d
Hoe 51329	Skin sensitisation Pirbright-White guinea pig (f)		Irritant. Sensitisation by skin contact.	Hammerl 1996a
Hoe 51329	Skin sensitisation Pirbright-White guinea pig (f)		No sensitising (Buehler Test)	Hammerl 1996b
Hoe 51329	Dermal irritation and eye irritation New Zealand White rabbits (f)		Dermal irritation: Not irritant. Eye irritation: Not subjet to labelling requeriments.	Hammerl 1996c

Subchronic toxicity data from two different Endosulfan metabolites were presented: the ones with Thiodan sulphate are done without GLP compliance, since the ones with Hoe 051329 fulfil the requirements of GLP. The results of these studies are summarised in Table 6.8.2-2.

Table 6.8.2-2: Summary of oral subchronic studies

Study	NOAEL (mg/kg bw/day)	Main adverse effect	LOAEL (mg/kg bw/day)	Reference and year
90-day, oral, dog. Hoe 051329	9.1 male 8.4 female	bile duct proliferated with fibrosis	89.4 male 82.9 female	Stammberger 1994.
90-day, oral, rat. Hoe 051329	7.8 male 8.0 female	haematotoxicity and liver toxicity.	40.2 male 40.7 female	Ebert and Hack, 1996a/b

Since the purity and the test method of the two Thiodan sulphate studies were not reported, these studies were not acceptable and due to the endosulfan sulphate was included in the residue definition for crop commodities a subchronic toxicity study of this endosulfan sulphate metabolite is required.

Three studies using endosulfan-diol, a endosulfan metabolite, were sponsored and presented by AgrEvo**.** They included *in vitro* (gene mutation and UDS) and *in vivo* (micronucleus) assays. These studies are summarised in Table 6.8.2-3.

All studies were performed according to specific test guidelines and were GLP compliant. They were reported over the period 1992 to 1993.
Negative results were obtained in all studies.

The available genotoxicity tests show that endosulfan-diol could be considered as non genotoxic.

Table 6.8.2-3: Summary of genotoxicity endosulfan-diol studies (AgrEvo)

Test	System	Dosage	Results	Comments	Reference

Test	System	Dosage	Results	Comments	Reference
Bacterial/ mammalian microsome plate incorporation assay	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, and TA100. *Escherichia coli* WP2 *uvrA*	First experiment: 4, 20, 100, 500, 2500 and 10000 µg/plate (±S9) Second experiment: 0.16, 0.8, 4, 20, 100 and 500 µg/plate (±S9)	Negative	Endosulfan-diol precipitated and was toxic (±S9) at 500 µg/plate.	Stammberger, 1992 (AgrEvo: IIA, 5.8.1.3/1) No published
In vitro UDS assay	Human cell line A 549	Experiment 1: 0.3, 1, 3, 10, 30, 100 and 300 µg/mL (±S9) Experiments 2: 0.03, 0.1, 0.3, 1, 3, 10 and 30 µg/mL (±S9)	Negative	An increasing cytotoxicity was observed from 100 µg/mL (-S9), and from 10 µg/mL (+S9). It should be justified the treatment time of 3 hours.	Stammberger,1993a (AgrEvo: IIA, 5.8.1.3/2) No published
In vivo micronucleus test	Bone marrow cells from male and female NMRI mice, strain NMRKf (SPF71).	Mice were treated by a single oral gavage at 500, 2500 and 5000 mg/kg.	Negative	No signs of toxicity were observed in mice. No toxicity for the bone marrow.	Stammberger,1993b (AgrEvo: IIA, 5.8.1.3/3) No published

B.6.8.2.1 Acute toxicity of metabolites

B.6.8.2.1 1 Oral toxicity

B.6.8.2.1.1.1 Rats

Weigand (1982a) (AgrEvo: IIA, 5.8.1/1/8)

Date of experimental work: Not available. (Study in German language).

The study was performed prior to GLP regulations.

The study is not acceptable. Purity grade, origin of animals, hausing, etc. are not included.

Material and methods

Test substance: Hoe 51329, technical substance, (Endosulfan-diol).

26 female Albino rats (4 per group, 10 per group in a 2º 15000 mg/l dose group) weighing 90-110 g were dosed with Endosulfan-diol administered as starch suspension (2 %), at dose level of 100, 320, 1000, 3200 and 15000 mg/kg bw.

Results

The highest administrable dose of 15.0 g/kg was tolerated without any reaction. No animal died during the follow-up period of 10 days after the treatment. Therefore, an exact determination of the LD_{50} was not possible.; the LD_{50} is with certainty above 15.0 g/kg body weight.

Conclusions

The determination of the acute oral toxicity of Hoe 51329 to female Albino rats resulted in an LD_{50} of > 15.0 g/kg body weight.

Ehling G & Leist KH (1991a) (AgrEvo: IIA, 5.8.1.2/1)

Date of experimental work: From 27 March 1991 to 10 April 1991.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: Hoe 51329, technical substance (Endosulfan-diol, 97.9% purity).

10 Wistar rats (5 males and 5 females; Hoechs AG, Kastengrund, SPF breeding colony)) of 7-8 weeks of age and weighing 172-192 g were dosed by gavage with 5000 mg/kg bw of Endosulfan-diol suspended in starch mucilage (2 %) During the acclimatisation period (5 days) the animals were maintained in fully air-conditioned rooms (22±3 ºC, 50±20 % relative humidity, 12 hr light-12h dark) in makrolon cages (5 animals per cage). Altromin 1324 rat diet (Altromin GmbH, Lage/lippe) and water *ad libitum*. The observation period following treatment lasted 15 days. A record was kept of the times at which signs of intoxication emerged and of the times of death. During this time, the animals were weighed weekly. At the end of the observation period, the animals were killed by CO_2 asphyxation, dissected and examined for macroscopically visible changes.

Results

No mortality occurred in the dose group tested. The clinical signs of intoxication were largely the same for males and females. Clinical signs such as decreased spontaneous activity, stilted gait and coat bristling were observed in nearly all animals during the day of administration only. There were no impairments of body weight gains. The animals killed at the end of the experiment were free of macroscopically visible changes. Based on the results of this study the median lethal dose (LD_{50}) for the male and female Wistar rat is greater than 5000 mg/kg body weight.

Conclusions

Testing of Hoe 051329, substance technical for acute oral toxicity in the male and female Wistar rat resulted in an LD_{50} for the male and the female rat of > 5000 mg/kg body weight.

Ehling G & Leist KH (1991b) (AgrEvo: IIA, 5.8.1.1/10)

Date of experimental work: From 7 May 1991 to 18 June 1991.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: Hoe 051327 substance, pure (Endosulfan-sulphate, 98.5% purity).

40 Wistar rats (Hoechs AG, Kastengrund, SPF breeding colony)) of 7-9 weeks of age and weighing 183-197 g were dosed by gavage with dosage levels of 25, 31.5, 50, 63, 100, 200, 400 and 800 mg/kg b.w. of Endosulfan-sulphate. The test groups are: 5 groups of 5 males and 3 groups of 5 females. The males received 63, 100, 200, 400 and 800 mg/kg b.w., the females 25, 31.5 and 50 mg/kg b.w. During the acclimatisation period (5 days) the animals were maintained in fully air-conditioned rooms (22±3 ºC, 50±20 % relative humidity, 12 hr light-12h dark) in makrolon cages (5 animals per cage). Altromin 1324 rat diet (Altromin GmbH, Lage/lippe) and water *ad libitum*. The observation period following treatment lasted 15 days. A record was kept of the times at which signs of intoxication emerged and of the times of death. During this time, the animals were weighed weekly. At the end of the observation period, the animals were killed by CO_2 asphyxation, dissected and examined for macroscopically visible changes. The LD_{50} values are calculated for males and females separately. LD_{50} and the limits of confidence are established by probit analysis.

Results

The results are summarised in Table 6.8.2.1.1.1-1. The deaths occurred on the day of treatment. Clinical signs of intoxication indicated no sex-specific differences. They began to emerge 10 - 60 minutes after administration and persisted in some cases up to day 9 of the study. The principal clinical signs included flanks drawn in, squatting posture, stilted gait, irregular respiration, decreased spontaneous activity and coat bristling, followed by a dose-related increase in the occurrence of tonoclonic convulsions. There were no impairments of body weight gains. Necropsy of the decedent animals revealed distinct injection of mesenteric vessels, an intestinal tract filled with a reddish-black mass (Haemoccult test positive), red discolouration of the lungs and lobular demarcation of the liver. The animals killed at the end of the experiment were free of macroscopically visible changes.

Table 6.8.2.1.1.1-1. Rate of mortality and LD_{50} values obtained

MALES		**FEMALES**	
DOSE (mg/kg bw)	**RATE**	**DOSE (mg/kg bw)**	**RATE**
63	0/5	25	2/5
100	0/5	31.5	0/5
200	0/5	50	5/5
400	1/5		
800	4/5		
LD_{50} (CONFIDENCE LIMITS) (mg/kg bw)		LD_{50} (CONFIDENCE LIMITS) (mg/kg bw)	
568 (305-1410)		>25 and <50	

Conclusions

Testing of Hoe 051327; substance, pure for acute oral toxicity in the male and female Wistar rat yielded LD_{50} values of 568 mg/kg b.w. for the male and > 25 to <50 mg/kg b.w. for the female rat.

Kramer & Weigand (1971) (AgrEvo: 5.8.1.1/1)

Date of experimental work: Not available.

The study was performed prior to GLP regulations.

The study is not acceptable. The purity grade of the test substance, the observation period of the survival animals (toxicity reactions, recovery period, etc.), the source of animals, housing, etc. are not included.

Material and methods

Test substance: Endosulfan-lactone.

80 SPF-Wistar K-rats (40 males and 40 females, 10 animals per group and dose), weighing 88-115 g in weight, were dosed by gavage with crescent doses of Endosulfan-lactone suspended in sesame oil.. In the follow-up period after the treatment (7 days), the rats received standard diet (Standard-Altromin R, Altromin GmbH, Lage/Lippe) and water *ad libitum*. The rats were deprived of food for 16 hr before the administration of the test substance. LD_{50} was determined by probit analysis, and the confidence limits were calculated according to Fieller method.

Results

The mortality data obtained is summarised in Table 6.8.2.1.1.1-2. Within 4 respective 4 ½ to 24 hours after treatment the male respective female animals died with violent convulsions in abdominal position. The macroscopic post-mortem examination of the animals that had died produced no conspicuous findings. The LD_{50} was found to be 105 (97-113) and 115 (99-135) mg/kg body weight for male and female SPF Wistar K-rats, respectively.

Table 6.8.2.1.1.1-2: Rate of mortality and LD_{50} (confidence limits) obtained in male and female SPF-Wistar K-rats.

MALES		FEMALES	
DOSE (mg/kg bw)	**RATE**	**DOSE (mg/kg bw)**	**RATE**
80	0/10	63	0/10
100	4/10	100	3/10
125	9/10	160	9/10
160	10/10	250	10/10
LD_{50} (confidence limits) (mg/kg bw)		LD_{50} (confidence limits) (mg/kg bw)	
105 (97.049-113.302)		115 (98.913-134.602)	

Conclusions

The determination of the acute oral toxicity of endosulfan-lactone to the male and female SPF Wistar K-rat resulted in LD_{50} values of 105 and 115 mg/kg body weight for male and female animals, respectively.

Hollander & Kramer (1975a) (AgrEvo: 5.8.1.1/2)

Date of experimental work: Not available.

The study was performed prior to GLP regulations.

The study is not acceptable. The purity grade of the test substance, the observation period of the survival animals (toxicity reactions, recovery period, etc.), the source of animals, housing, etc. are not included.

Material and methods

5 Test substance: Endosulfan-lactone.

0 male SPF-Wistar K-rats (10 animals per group and dose), weighing 92-120 g in weight, were dosed by stomach tubing with crescent doses of Endosulfan-lactone suspended in starch slurry (1 %). In the follow-up period after the treatment (7 days), the rats received standard diet (Standard-Altromin R, Altromin GmbH, Lage/Lippe) and water *ad libitum*. The rats were deprived of food for 16 hr before the administration of the test substance. LD_{50} was determined by probit analysis, and the confidence limits were calculated according to Fieller method.

Results

The mortality data obtained is summarised in Table 6.8.2.1.1.1-3. Within 4 respective 4 ½ to 24 hours after treatment the male respective female animals died with violent convulsions in abdominal position. The macroscopic post-mortem examination of the animals that had died produced no conspicuous findings. The LD_{50} was found to be 105 (97-113) and 115 (99-135) mg/kg body weight for male and female SPF Wistar K-rats, respectively. The LD_{50} was found to be 165 (128 - 216) mg/kg body weight.

Table 6.8.2.1.1.1-3: Rate of mortality and LD_{50} (confidence limits) obtained in male SPF-Wistar K-rats.

MALES	
DOSE (mg/kg bw)	**RATE**
80	0/10
125	5/10
200	7/10
320	7/10
500	10/10
LD_{50} (confidence limits) (mg/kg bw)	
165 (128.337-215.969)	

Conclusions

The determination of the acute oral toxicity of endosulfan-lactone in male SPF Wistar rats resulted in a LD_{50} of 165 mg/kg body weight.

Hollander & Kramer (1975c) (AgrEvo: IIA, 5.8.1.1/4)

Date of experimental work: Not available.

The study was performed prior to GLP regulations

The study is not acceptable.

Material and methods

Test substance: Endosulfan-sulphate.

The product was administered orally by stomach tubing at various dose levels to groups of 10 female mixed bred Albino-rats with a mean weight of 122 (82-150 g). A 0.4 % suspension in 2 % starch slurry was administered. The rats were deprived of food for 12 h prior to the administration of the test substance. After the treatment, the animals were kept in groups in metal cages and given food and water. The animals were observed for possible toxic effects and mortality, if any; the total follow-up period as 8 days. The LD_{50} was evaluated by probability graph.

Results

The results are summarised in Table 6.8.2.1.1.1-4.

Table 6.8.2.1.1.1-4: Rate of mortality and LD_{50} value obtained en female Albino-rats.

FEMALE	
DOSE (mg/kg bw)	**RATE**
25	0/10
40	0/10
63	3/10
100	7/10
160	10/10
LD_{50} (confidence limits) (mg/kg bw)	
76 (62-88)	

Conclusions

The determination of the acute oral toxicity of endosulfan-sulphate in female SPF Wistar rats resulted in a LD_{50} of 76 mg/kg body weight.

Hollander & Kramer (1975d) (AgrEvo: IIA, 5.8.1.1/5)

Date of experimental work: Not available.

The study was performed prior to GLP regulations

The study is not acceptable.

Material and methods

Test substance: Endosulfan-hydroxyether.

0 female BR 46-Wistar rats (10 animals per dose group), weighing 88-124 g, were dosed with Endosulfan-hydroxyeher (10 % starch suspension). The test suspension was administered once at various dose levels (630, 1000, 1600, 2500 and 4000 mg/kg bw) by stomach tubing. The follow-up period after the administration was 14 days. The rats were deprived of food for 12 hr prior to the administration of the test compound. The fellow-up period after the administration was 14 days. During this period, the rats received Standard-Altromin R (Altromin, Lage/lippe) and tap water. The LD 50 was evaluated by probability graph.

Results

The results are summarised in Table 6.8.2.1.1.1-5. The animals died within 1-8 days after the administration.

Table 6.8.2.1.1.1-5: Rate of mortality and LD_{50} value obtained en female BR 46-Wistar rats.

FEMALE	
DOSE (mg/kg bw)	**RATE**
630	0/10
1000	1/10
1600	5/10
2500	7/10
4000	10/10
LD_{50} (confidence limits) (mg/kg bw)	
1750 (1450-2100)	

Conclusions

The determination of the acute oral toxicity of 1-hydroxy endosulfan-ether in female SPF-Wistar rats resulted in a LD_{50} of 1750 mg/kg body weight.

Hollander & Kramer (1975e) (AgEevo: IIA, 5.8.1.1/6)

Date of experimental work: Not available.

The study was performed prior to GLP regulations

The study is not acceptable. Purity grade, origin of animals, housing, etc. are not included.

Material and methods

Test substance: Endosulfan-ether and Endosulfan-alcohol.

The substances were suspended in 2% starch slurry and administered once by stomach tubing to female mixed-bred Albino rats in a weight-range of 90-110 g. The animals remained unfed for 12 hr prior to the administration of the test compounds; tap water was always provided *ad libitum*. 1 %, 10 % or 25 % concentrations of the compounds depending on the administered dose were tested.

Results

The results are summarised in Table 6.8.2.1.1.1-6. With both test substances, the maximum dose of 15 g/kg b.w. that could be administered was tolerated by all animals without any reactions. It is notheworthy that no animal died during the 10-day follow-up period after dosing. An exact determination of the LD_{50} was therefore not possible; it is, however definitely above 15 g/kg body weight.

Table 6.8.2.1.1.1-6: Rate of mortality and LD_{50} value obtained in female Albino rats.

FEMALE			
ENDOSULFAN-ETHER		**ENDOSULFAN-ALCOHOL**	
DOSE (mg/kg bw)	**RATE**	**DOSE (mg/kg bw)**	**RATE**
100	0/4	100	0/4
320	0/4	320	0/4
1000	0/4	1000	0/4
3200	0/4	3200	0/4
15000	0/4	15000	0/4
15000	0/10	15000	0/10

Conclusions

The determination of the acute oral toxicity of endosulfan-ether and endosulfan-alcohol to female Albino rats resulted in an LD_{50} of > 15 g/kg body weight for both test substances.

Hollander & Kramer (1975f) (AgrEvo. IIA, 5.8.1.1/7)

Date of experimental work: Not available.

The study was performed prior to GLP regulations

The study is not acceptable. Purity grade, origin of animals, housing, acclimatisation period, etc. are not included.

Material and methods

Test substance: Endosulfan-lactone.

Endosulfan-lactone was administered once as a starch suspension by stomach tubing at various dose levels to female SPF-Wistar rats ranging in weight from 84-130 g. Each dosage was tested 16 hr prior to the administration of the test substance. The follow-up period after dosing was 7 days. During this period, the rats received Standard-Altromin R (Altromin GmbH, Lage/Lippe) and tap water. The LD_{50} was determined by probit analysis. The confidence limits were calculated by Fieller method.

Results

The results are summarised in Table 6.8.2.1.1.1-7. Within 5 to 24 hours following dosing the animals died in abdominal position displaying violent convulsions. The autopsy of the animals that had died produced no conspicuous gross findings. The LD_{50} was determined by probit analysis; the confidence limits were calculated by FIELLER method. The LD_{50} was calculated to be 290 (240 - 352) mg/kg body weight.

Table 6.8.2.1.1.1-7: Rate of mortality and LD_{50} values obtained in SPF-Wistar rats.

FEMALE	
DOSE (mg/kg bw)	**RATE**
100	0/10
160	0/10
250	5/10
400	7/10
630	10/10
LD_{50} (confidence limits) (mg/kg bw)	
290 (240.-351)	

Conclusions

The determination of the acute oral toxicity of endosulfan-lactone in female SPF Wistar rats resulted in a LD_{50} of 290 mg/kg body weight.

B.6.8.2.1.1.2 Dogs

Hollander & Kramer (1975b) (AgrEvo: IIA, 5.8.1.1/3)

Date of experimental work: Not available.

The study was performed prior to GLP regulations

The study is not acceptable. The purity grade of the test substance, origin of the animals, changes of body weight during the experience, etc., are not included. Without statistical analysis for determination of LD_{50}.

Material and methods

Test substance: Endosulfan-sulphate.

1 male beagle dogs (2 animals per group and dose), weighing 10.2-15.8 kg., were dosed by stomach tubing with crescent doses of Endosulfan-sulphate suspended in starch suspension (1 or 4 %). Prior to the commencement of the experiment, all animals were dewormed and vaccinated against distemper, hepatitis contagiosa anis and leptospirosis. The dogs were housed individually in kennels and received a cooked feed mash and water. During the first 8 hr after dosing, the dogs were kept under constant observation to record possible toxic symptoms. The total observation period was 8 days.

Results

The mortality data obtained is summarised in Table 6.8.2.1.1.2-1. The clinical findings in dose group 2.5 mg/kg were emesis and salivation, in the other 4 dose groups (5.0, 10.0, 20.0 and 40.0 mg/kg) emesis, convulsions and salivation were observed. The LD_{50} was found to be 15 mg/kg body weight.

Table 6.8.2.1.1.2-1: Rate of mortality and LD_{50} (confidence limits) obtained in male beagle dogs.

MALE	
DOSE (mg/kg bw)	**RATE**
2.5	0/2
5.0	0/2
10.0	1/2
20.0	1/2
40.0	2/2
LD_{50} (confidence limits) (mg/kg bw)	
15 (10.0-20.0)	

Conclusions

The determination of the acute oral toxicity of endosulfan-sulphate in male Beagle dogs resulted in a LD_{50} of 15 mg/kg body weight.

B.6.8.2.1.2 Dermal toxicity

B.6.8.2.1.2.1 Rat

Ehling G & Leist KH (1991c) (AgrEvo: IIA, 5.8.1.2/2)

Date of experimental work: From 7 May 1991 to 11 June 1991.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: Hoe 051329 substance, technical (Endosulfan-diol, 98.5% purity).

0 male and female Wistar rats (Hoechst AG, Kastengrund, SPF breeding colony) of 8-10 weeks of age and weighing 204-240 g were dosed by dermal application with Endosulfan-diol at 2000 mg/kg bw. Before dermal treatment, the hair was mechanically removed from the dorsal skin (30 cm^2 approx.) The treated skin area was covered with and aluminium foil (6x8 cm), which was held in place with an elastic plaster bandage fixed around the body. After a dermal exposure period of 24 hr, the bandage was removed and treated skin area washed with warm water in order to remove any unabsorbed remnants of the test substance During the acclimatisation period (5 days) the animals were maintained in fully air-conditioned rooms (22±3 ºC, 50±20 % relative humidity, 12 hr light-12h dark) in makrolon cages). Altromin 1324 rat diet (Altromin GmbH, Lage/lippe) and water *ad libitum*. The observation period following treatment lasted 15 days. A record was kept of the times at which signs of intoxication emerged and of the times of death. During this time, the animals were weighed weekly. At the end of the observation period, the animals were killed by CO_2 asphyxation, dissected and examined for macroscopically visible changes.

Results

No mortality occurred in the dose group tested. There were no clinical signs of intoxication. The following signs of skin irritation were observed: the treated skin showed erythema. In addition, the skin surface was dry, rough and covered with fine and coarse scales. There were no impairments of body weight gains. The animals killed at the end of the experiment were free of macroscopically visible changes.

Conclusions

Testing the acute dermal toxicity of Hoe 051329, substance technical in male and female Wistar rats resulted in a LD_{50} of > 2000 mg/kg body weight.

Ehling G & Leist KH (1991d)(AgrEvo: IIA, 5.8.1.1/11)

Date of experimental work: From 7 May 1991 to 11 June 1991.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: Hoe 051327; substance, pure;(Endosulfan-sulphate, 98.5% purity).

30 male and female Wistar rats (15 males an 15 females; Hoechst AG, Kastengrund, SPF breeding colony) of 9-15 weeks of age and weighing 225-256 g were dosed by dermal application with Endosulfan-sulphate at dosage level of 250, 315, 400, 1600, 2500 and 4000 mg/kg bw. 3 groups consisting of 5 males and 5 females. The males received 4000, 2500 and 1600 mg/kg body weight, the females received 400, 315 and 250 mg/kg body weight. Conditions of the experiment: See Ehling G & Leist KH (1991c).

Results

The course of mortality are summarised in Table 6.8.2.1.2.1-1. The death occurred between 1 and 4 days after treatment. In the highest dose group tested 3 males and 5 females, in the medium dose 3 males and 4 females animals and in the low dose 1 male and 1 female died. Clinical signs were nearly comparable in both sexes. They began to emerge 1 day after administration and persisted in some cases up to day 8 of the study. Clinical signs such as irregular respiration, exophtalmos, uncoordinated gait, squatting posture, decreased spontaneous activity, coat bristling, flanks drawn in, stilted gait and tonoclonic convulsions were observed in most animals. In addition, the following signs of skin irritation were observed: slight erythema, dry and rough skin, skin covered with fine and coarse scales. The body weight gains were generally impaired, but returned to normal until the end of the study.Necropsy of the decedent animals revealed: Intestinal tract full of a reddish black mass (haemoccult test positive), full of gas, mesenteric vessels distinctly injected; small intestine full of a reddish black mass (haemoccult test positive); stomach full of gas; lungs discoloured red. The animals killed at the end of the experiment were free of macroscopically visible changes.

Table 6.8.2.1.2.1-1: Course of mortality and LD_{50} values obtained.

DOSAGE (mg/kg bw)	DAY 2	DAY 3	DAY 4	DAY 5	TOTAL (15 d)
Males					
1600		1			1/5
2500		3			3/5
4000	1	2			3/5
Females					
250		1			1/5
315	4				4/5
400	2	2		1	5/5
MALES LD_{50} (confidence limits) (mg/kg bw)			FEMALES LD_{50} (confidence limits) (mg/kg bw)		
2740			280 (204-349)		

Conclusions

Testing the acute dermal toxicology of Hoe 051327, substance, pure in the male and female Wistar rat resulted in LD_{50} of 2740 and 280 mg/kg body weight for males and females, respectively.

B.6.8.2.1.3 Skin Sensitisation

Hammerl R (1996a) (AgrEvo: IIA, 5.8.1.4/1)

Date of experimental work: From 2 January 1996 to 2 February 1996.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: Hoe 051329; susbstance, technical, 99.6 % purity.

39 female Pirbright-White guinea pig (Hoechst AG, Kastengrund, SPF breeding colony), weighing 262-332 g, were used in this experiment. The following test groups were used for the study: 6 animals for determination of primary non-irritant concentration, 3 animals for determination of the tolerance of intradermal injections, 10 animals as control group and 20 animals as treatment group. During acclimatisation period (5 days), the animals were housing in fully air-conditioned rooms (20±3 ºC, 50± 20 % humidity, 12 hr light – 12 hr dark), in macrolon cages, and with diet (ssnif® Ms-H V2233) and water *ad libitum*. Induction was carried out both by dermal application and intradermal injection. Freund's Complete Adjuvant was added to the solution designated for intradermal injection in order to maximise the response of the immune system. Challenge treatment comprised dermal application only. The test substance is considered to be sensitised if 30 % or more of the treated animals show a positive reaction (erythema and/or oedema) and at the same time no irritant effects emerge in the control group.

Results

Based on the results of a pilot test prior to the main study, the intradermal induction of the treatment group was performed with 5.0 % (w/v) in isotonic saline using 20 animals. Dermal induction and challenge treatment were carried out with 25.0 % (w/v) in isotonic saline. There were no clinical signs of intoxication. The bw gains of the animals were not impaired. Under the conditions of the present study, 11 of 20 animals of the treatment group showed a positive skin response after the challenge treatment, with moderate erythema, very slight oedema and crusted skin.

The validity of the test system was confirmed in positive control tests using dinitrochlorbenzene (DNCB) for the Buehler test, and benzocain for the Mafnusson & Kligman test, at intervals of approx. 6 months. In these studies, the positive control substances gave the expected positive response.

Conclusions

Hoe 051329 is sensitising and thus subject to labelling requirements. The product is irritant and may cause sensitisation by skin contact.

Hammerl R (1996b) (AgrEvo: IIA, 5.8.1.4/2)

Date of experimental work: From 5 March 1996 to 4 April 1996.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: Hoe 051329; susbstance, technical, 99.6 % purity.

20 female Pirbright-White guinea pigs (Hoechst AG, Kastengrund, SPF breeding colony), weighing 337-415 g, received a total of 3 topical 6 hr applications of the test material at weekly intervals. A control group of 10 animals was treated with deionised water only. 2 weeks after the last induction treatment, all animals received a topical 6 hr challenge application of the test material. Based on irritation findings during pre-testing, 50.0 % for induction phase (no erythema, no oedema in pre-test) and 50.0 % for challenge phase (no erythema, no oedema in pre-test) were used. The validity of the test system was confirmed by the periodically conducted positive control test using dinitrochlorbenzene (DNCB) for the Buehler test.

Results

During the induction phase, the animals of the control and treatment groups showed no signs of irritation. After challenge treatment, no dermal reactions could be observed in the treatment and control groups. There were no clinical signs of intoxication, and body weight gains remained unaffected.

Conclusions

Hoe 051329 did not show sensitising potential under the conditions of the test according to Buehler.

B.6.8.2.1.4 Acute Dermal Irritation

Hammerl R (1996c) (AgrEvo: IIA, 5.8.1.4/3)

Date of experimental work: From 5 December 1995 to 8 December 1995.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: Hoe 051329; susbstance, technical, 99.6 % purity.

An amount of 500 mg of the test substance moisted with 0.25 ml distilled water was tested for primary dermal irritation in 3 female New Zealand White rabbits (Chemical Pharmaceutical Factory Dr. K. Thomae, 88400 Biberach) of 3-5 months of age and weighing 3.3-3.5 kg. During acclimatisation period (5 days), the animals were housing in fully air-conditioned rooms (20±3 ºC, 50±20 % humidity, 12 hr light – 12 hr dark), in separate cages, and with diet (ssnif® K-H V2333) and water *ad libitum*.

Results

No signs of irritation occurred during the whole observation period. No clinical signs of systemic toxicity were observed.

Conclusions

Based on the results obtained in this study, the substance Hoe 051329 is not irritant in female New Zealand White rabbits.

B.6.8.2.1.5 Eye Irritation (Rabbit)

Hammerl R (1996c) (AgrEvo: IIA, 5.8.1.4/4)

Date of experimental work: From 5 December 1995 to 8 December 1995.

The study was conformed to GLP regulations and the applicant include QA Statement.

The study is acceptable.

Material and methods

Test substance: Hoe 051329; substance, technical, 99.6 % purity.

About 24 hr before the start of the study, the eyes of all animals were examined under UV light for corneal lesions after instillation of 1 drop of a 0.1 % fluorescein-sodium solution. Only animals without ocular abnormalities were used for the study. An amount of 100 mg of test substance was applied as a single dose to the conjunctival sac of the left of 3 New Zealand albino rabbits (Chemical Pharmaceutical Factory Dr. K. Thomae, 88400 Biberach) of 3-5 months of age and weighing 3.3-3.5 kg. The untreated eyes served in each case as a control. The animals were housing in fully air-conditioned rooms (20±3 ºC, 50±20 % humidity, 12 hr light – 12 hr dark), in separate cages, and with diet (ssnif® K-H V2333) and water *ad libitum* At 24 hr after application and at all other designated examination times at which the treated eyes still showed discharge or at which a corneal examination with fluorescein-sodium solution took place, the treated eyes were washed out thoroughly with isotonic saline at 37 ºC. The eyes were examined 1, 24, 48 and 72 hr after application of the test substance. At 24 and 72 hr, the eyes were also examined for corneal lesions under UV light (0.01 % fluorescein-sodium solution). Lesions in cornea, iris or conjunctivae were graded. All other changes or toxic effects were recorded.

Results

1 hr up to 2 days after application, the conjunctivae of 2 animals showed an evident hyperaemia of the blood vessels up to a diffuse, deeper crimson reddening. Additionally a clear, colourless discharge was noted 1 hr up to 1 day after application. 3 days after application, all signs or irritation had reversed.

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

Redness of conjunctiva:	0.3
Chemosis of conjunctiva:	0.0
Opacity of cornea:	0.0
Iris:	0.0

No clinical signs of systemic toxicity were observed.

Conclusions

Based on the results obtained in this study, Hoe 051329 is not subject to labelling requirements, according to the criteria for CEE classification

B.6.8.2.2 Subchronic studies of metabolites

B.6.8.2..2.1 90-Days in Beagle dogs

Cervenka,H, *et al*., (1964) (AgrEvo: IIA, 5.8.1.5/1)

Date of report: 20 Aug 1964.

Test method: not reported.

GLP: No.

The study is not acceptable (purity not reported).

Material and methods

Groups of 3 male and 3 female Beagle dogs were given orally in gelatine capsules daily doses of 0, 0.075, 0.75 and 2.5/1.5 mg/kg bw/day of endosulfan-sulphate (purity not reported) for 90 days. Lactose was added to the test material to facilitate accurate weighing of the daily dosing. Determinations like heamatologic studies, clinical blood chemistry, urine analysis and liver function test were performed.

Results

No effects were seen at 0.075 and 0.75 mg/kg/day. Salivation, muscular tremors and tonic-clonic convulsions, as well as food and body weight gain reduction were seen in the dogs at 2.5 mg/kg up to day 18 when dosing was stopped. These signs became less but did not totally disappear, when this group was changed on day 20 to 1.5 mg/kg.

Conclusion

The No Adverse Effect Level (NOAEL) of Thiodan sulphate in this study for the dog is 0.75 mg/kg bw/day.

B.6.8.2.2.2 90-Days in rats

Wolf, C. & Calandra J.C., (1965) (AgrEvo: IIA, 5.8.1.5/2)
Date of report: 13 Oct 1965

Test method: not reported.

GLP: No.

The study is not acceptable (purity not reported)

Material and methods
Groups of 15 male and 15 female Sprague Dawley rats (a total of 180 rats) were given daily nominal dietary doses of 0, 3, 10, 30, 50 or 500 ppm endosulfan-sulphate (purity not reported) for 90 days.

Results
No effects were seen up to 10 ppm. Liver weights were increased in females at 30 ppm and above and in the males at 50 ppm and above.

Conclusion
Thus the No Adverse Effect Level (NOAEL) could not be evaluated.

Stammberger (199) (AgrEvo: IIA, 5.8.1.5/3)4
Date of report: 22, August 1994

Test method: The study was conducted in accordance with EEC Test Guideline No. 87/302/ EEC of 18. November 1987.

GLP: Yes.

The study is acceptable.

Material and methods
Groups of 5 male and 5 female Beagle dogs were given in the diet 0. 100, 1000 or 10.000 ppm of endosulfan-diol, purity 99.8%, corresponding to a mean daily intake of 0, 9.1, 89.4 or 910.6 mg/kg bw/day for males and 0, 8.4, 82.9, or 870.9 for females for three months. Bipromix was used as vehicle. Clinical observation and examination were: mortality, general health check, food consumption, body

weight, behaviour, neurological status, ophtalmological examination, hearing test, dental inspection and laboratory examination (heamatology, clinical chemistry, urinalysis) and postmortem examination.

Results

One male and two females in the high dose group had to be killed *in extremis* due to clinical signs of intoxication and poor condition. A dose-related reduction in body weight gain was observed at all doses. Serum GPT and ALAT activity was increased at all doses. Serum AP and γ-GT activity were increased in the middle and high dose. Histology showed bile duct proliferation at all doses, and thickened gallbladder walls and reduced iron in bone marrow of the middle and high dose animals.

Conclusion

The NOAEL in dogs in this study was concluded to be 8.7 mg/kg bw/day (9.1 mg/kg bw/day male and 8.4 mg/kg bw/day female).

Ebert, E. &Hack, R. (1996a/b((AgrEvo: IIA, 5.8.1.5/4/5)

Date of report: 04 April 1996

Test method: The study was conducted in compliance with EEC Directive 87/302/EEC.

GLP: Yes.

The study is acceptable.

Material and methods

Groups of 10 male and 10 female Wistar rats were given standard diet containing 0, 100, 500, 1000 or 10000 ppm of endosulfan-diol (purity 99.8%) for 90 days. Recovery groups of 10 males and 10 females were given 0. 1000 or 10000 ppm in the diet for 90 days followed by a 30-day period on standard diet. In addition, satellite groups of 5 male and 5 female rats per dose group were included for neurotoxicity screening.

Results

The animals at the high dose of 10000 ppm had to be killed *in extremis* at the end of the 2nd study week, due to a clearly impaired general health. At the high dose, many animals had straddling hindlimbs. Two males exhibited muscle twitching and clonic convulsions. One male died on day 13. All animals at 10000 ppm had a markedly reduced body weight, food and water (males only) intake. At doses of 1000 ppm or below, no substance related clinical signs were found. The body weights of the males at 1000 ppm showed a tendency to a decrease. At 1000 ppm a decrease in erythrocytes, haemoglobin and haematocrit values together with an increase in reticulocytes was recorded for both males and females. This effect was already recognisable in females at 500 ppm. The changes proved reversible after the recovery period with a tendency towards overcompensation. An increase in bilirubin levels was observed in males and a tendency towards this effect in females at 1000 ppm. These findings

were considered related to the haematological effects. In addition, increases in transaminase and γ-GT activities in males at 1000 ppm indicated the liver as target organ; the slight increases in protein, albumin and β1)-globulin in these animals may be interpreted similarly. Decreased triglycerides together with increased lipase activities, found at 500 ppm and above, indicated enhancement of catabolic lipid metabolism. All changes proved to be reversible after a 4-week recovery period. Liver and spleen weights were increased from 500 ppm onwards. A minimal increase (ca. 8%) in relative (not absolute) liver weight attaining statistical significance, was already recorded among males at 100 ppm. However, no toxicological relevance in the sense of an adverse effect can be assigned to this minimal change in absence of further toxicological correlates. In addition, heart weights were slightly decreased and kidney weights increased in males at 1000 ppm. All these changes proved to be reversible or showed clear remission (liver) at the end of the recovery period. At 1000 ppm, a marked degree of bile duct proliferation, undergoing atrophy during recovery, combined with cystic cholangiofibrosis and centrilobular fatty changes, was found. Focal hepatic necrosis was seen in one male. In addition, medullary and extra-medullary erythropoiesis was increased. At 500 ppm, discrete hepatic bile duct proliferation was also present in about half of the animals and also enhanced erythropoiesis in several animals.

Conclusion

Dietary treatment of rats with 10000 ppm endosulfan-diol caused neurological disturbances such as clonic convulsions and also death. At 500 and 1000 ppm, endosulfan-diol caused haematotoxicity and liver toxicity. Haematotoxicity consisted of haemolytic anaemia together with compensatory medullary and extra-medullary (liver, spleen) erythropoiesis and increased bilirubin levels. Liver toxicity was characterised by bile duct proliferation combined with distinct cholangiofibrosis and centrilobular fatty changes, increased organ weights and transaminase and glutamyl transferase activities in males at 1000 ppm. In addition enhancement of catabolic lipid metabolism was observed particularly in males from 500 ppm onwards. No signs of neurotoxicity were found at levels up to and including 1000 ppm.

The NOAEL of endosulfan-diol in this 90-day study in the rat is 100 ppm equivalent to 7.8 mg/kg bw/day in male rats and 8.0 mg/kg bw/day in female rats, on aggregate 7.9 mg/kg bw/day for male and female rats.

B.6.8.2.3 Genotoxicity Testing of Metabolites

B.6.8.2.3.1 Bacterial Gene mutation

Stammberger, I. , 1992 (AgrEvo: IIA, 5.8.1.3/1)

Dates of experimental work: The study was performed between 29 September and 29 October, 1992. Date of report: 25, November, 1992.

The objective of the study was to determine the mutagenic potential of endosulfan-diol in bacteria using the reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli*.

This study was performed according to OECD Guidelines (471 and 472, adopted May 26th, 1983), U.S. EPA Guidelines (HG- Gene Muta- *S. typhimurium* and HG- Gene Muta- *E. coli*, August, 1982) and EEC Directive 79/831 Annex V, 4.3.1.

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

Endosulfan-diol test substance was Code Hoe 051329 00 ZD99 0001 (Batch No. C 0232 2019), with purity 99.8%. The test article was reported to be stable in the solvent, DMSO, for 4 hours. The study was conducted using five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) and one strain of *Escherichia coli* (WP2 *uvrA*). S9 was derived from the liver of male Sprague-Dawley rats induced with Aroclor 1254. Appropriate positive controls (SA, 9-AA, 2-NF, MNNG and 2-AA) were included.

The plate incorporation assay was performed according to published methods (Ames *et al,* 1973; Ames, McCann and Yamasaki, 1975). Two independent experiments were carried out.

The first experiment was performed with all tester strains using three plates per concentration to get information on mutagenicity and toxicity for calculation of an appropriate concentrations range. Endosulfan-diol was tested at concentrations of 4, 20, 100, 500, 2500 and 10000 µg/plate with and without S9 metabolic activation and along with concurrent negative and positive controls. A reduced rate of spontaneously occuring colonies as well as visible thinning of the bacterial lawn were used as indicator for toxicity. Endosulfan-diol was toxic to the bacterial strains at 500 µg/plate. Visible precipitation of the test compound was observed at 500 µg/plate.

Based on the results obtained at the first experiment, endosulfan-diol was tested at concentrations of 0.16, 0.8, 4, 20, 100 and 500 µg/plate with and without S9 metabolic activation and along with concurrent negative and positive controls. A toxicity testing was also performed for TA100 strain; the surviving fraction was 0 (±S9) at 500 µg/plate, and 0.6 (-S9) and 0.8 (+S9) at 100 µg/plate. A test article is classified mutagenic if it produces at least a 2-fold increase in the mean number of revertants per plate of at least one of the tester strain over the mean solvent control value. A dose-related increase is also required. Test results must be reproducible.

Endosulfan-diol did not cause a significant increase in the number of revertant colonies with any of the tester strains either in the absence or presence of S9 mix. No dose dependent effect was obtained. Positive controls gave a satisfactory response.

Conclusion

Endosulfan-diol did not induce gene mutation in any of the bacterial tester strains, under the conditions of this study. Nevertheless, standard deviations should have been showed.

B.6.8.2..3.2 DNA effects – UDS assay in mammalian cells *in vitro*

Stammberger, I. , 1993a (AgrEvo: IIA, 5.8.1.3/2)

Dates of experimental work: The study was performed between 5 November and 22 December, 1992. Date of report: 27, January, 1993.

The objective of this study was to assess the potential of endosulfan-diol to induce unscheduled DNA synthesis (UDS) in the human cell line A 549.

The study was performed according to the OECD-guideline (Gen 85.4, Genetic Toxicology: DNA Damage and Repair / Unscheduled DNA Synthesis in Mammalian Cells *in vitro*, 1986) and the EPA-guideline (Unscheduled DNA Synthesis in Mammalian Cells in Culture, HG-DNA-Unsched., August 1982).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

Endosulfan-diol test substance was Code Hoe 051329 00 ZD99 0001 (Batch No. C 0232 2019), with purity 99.8%. The test article was reported to be stable in the solvent, DMSO, for 4 hours. The study was conducted using the permanent human cell line A 549 (American Type Culture Collection no CCL 185). S9 was derived from the liver of male Sprague Dawley rats induced with Aroclor 1254. Appropriate positive controls (NQO and BP) were included.

Preliminary experiments were performed to determine the solubility and microscopic cytotoxicity of the test compound. Endosulfan-diol appeared soluble in the culture medium up to 30 µg/mL. Indication of visible microscopic cell toxicity was observed at 100 µg/mL and higher dose levels. $4x10^5$ cells/35 mm culture dish were seeded and cultured. Six cultures were used for each experimental point. Two days before the start of the experiment the medium was replaced by an arginine-deficient medium which contains 10 mM hydroxyurea to reduce or inhibit semi-conservative DNA replication. The incubation of the test substance at various concentrations, with and without S9, was performed at 37^o C for 3 hours. Tritiated thymidine was added to the cell culture immediately after the test compound. Two experiments were carried out. In Experiment 1, cells were exposed to endosulfan-diol at 0.3, 1, 3, 10, 30, 100 and 300 µg/mL, with and without S9. An independent cytotoxicity test was performed under the same experimental conditions as the UDS test. In this test, cytotoxic effects were determined by photometric measure of A 549 cell cultures breeded in microwell plates and stained with crystal violet after treatment with the test substance. An increasing cytotoxicity was observed from 100 to 300 µg/mL

in the absence, and from 10 to 300 µg/mL in the presence of S9. In Experiment 2, cells were exposed to endosulfan at 0.03, 0.1, 0.3, 1, 3, 10, and 30 µg/mL (± S9). Concurrent negative (solvent) and positive controls were included in each experiment. At the end of the incubation period DNA was extracted from the cells. The DNA concentration was determined colorimetrically using the diphenylamine reaction of deoxyribonucleic acid (Burton, 1956). The incorporation of radiolabel into DNA was determined by liquid scintillation counting. Results were given as dpm/µg DNA and data were statistically evaluated using Student's t-test.

No relevant reproducible increase in the rate of UDS was observed at any concentration of endosulfan-diol. Positive controls gave a satisfactory response.

Conclusion

Endosulfan-diol was not genotoxic under the conditions of this study. The study is considered acceptable. Nevertheless, it should be advisable to justify the treatment time of 3 hours and to report the cell density obtained at time of treatment.

B.6.8.2.3.3 *In vivo* mouse micronucleus test

Stammberger, I. , 1993b (AgrEvo: IIA, 5.8.1.3/3)

Dates of experimental work: The study was performed between 2 November, 1992 and 22 April, 1993.
Date of report: 12, May, 1993.

The objective of this study was to determine the potential of endosulfan-diol to induce micronuclei in the bone marrow polychromatic erytrocytes of NMRI mice.

The study was performed according to the EPA-guideline, *In vivo* mammalian bone marrow cytogenetic tests: micronucleus assay. HG-Chromo-Micronuc, August 1982, and took into consideration the proposals and recommendations given in the EPA Gene-Tox Program, 1983.

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

Endosulfan-diol test substance was Code Hoe 051329 00 ZD99 0001 (Batch No. C 0232 2019), with purity 99.8%. It was suspended in starch mucilage. The test article was reported to be stable in the vehicle for 4 hours. The test compound dilutions were freshy prepared at the day of administration. The study was conducted using male and female NMRI mice, strain NMRKf (SPF71).

A preliminary study was conducted to determine the highest administrable non lethal dose level. An oral administration of 5000 mg/kg did not lead to lethality in male and female mice. No signs of

toxicity were observed. It is considered the maximum applicable dose and was selected as the high dose level for the main study. Endosulfan-diol was administered by single oral gavage to mice at 0 (starch mucilage control), 500, 2500 and 5000 mg/kg in aliquots of 10 mL/kg. Dose groups consisted of 5 males and 5 females for each sacrifice time (24, 48 and 72 hours post treatment). Animals treated with Endoxan^{R} and sacrificed at 24 hours served as positive controls. Bone marrow cells were collected and stained. 1000 immature polychromatic erythrocytes (PCE) were counted from each animal and examined microscopically for micronuclei. As a control measure 1000 mature normochromatic erythrocytes (NCE) were also counted and examined for micronuclei. In addition, the ratio of PCE:NCE was determined. The results of the treatment group in the micronucleus test at each dose and killing time were compared with corresponding control values according to a Wilcoxon test (one-sided). The ratio of PCE:NCE was also statistically evaluated by the method of Wilcoxon (two-sided). All statistical results were based on a 95% level of significance. Actual data were also compared with historical controls.

All animals survived after application endosulfan-diol at 500, 2500 and 5000 mg/kg. No signs of toxicity were observed. The dissection of the animals revealed no test substance related macroscopic findings. The incidence of both micronucleated PCE and micronucleated NCE in animals treated with endosulfan-diol was within the normal range of the negative control groups. The ratio of PCE:NCE remained essentially unaffected by the test compound. A statistically significant increase in the 72-hour group at 5000 mg/kg was of no biological relevance. The positive control gave a satisfactory response.

Conclusion

Endosulfan-diol was considered negative in the mouse bone marrow micronucleus test, under the conditions of this study.

B.6.9 Medical data and information (IIA, 5.9)

B.6.9.1 Medical surveillance on manufacturing plant personnel

The active ingredient endosulfan has been produced by Hoechst AG since 1957. Company employees handling endosulfan are subjected to routine medical examinations by the Department of Occupational Medicine.

These examinations comprise:

- Anamnesis: Questions on illnesses, medicaments taken, feelings of ill health
- Physical examination: Heart, lungs, liver, spleen, oral cavity, throat, skin, current status of lymph nodes, thyroid, blood pressure, pulse, weight.
- Neurological examination: Reflexes, pupils, behaviour

- Urinalysis: Bilirubine, ketone bodies, glucose, proteins, leukocytes, erythrocytes
- Clinical Laboratory: Differential blood count, transaminases, (-GT)

No abnormal changes were observed either at the routine examinations or at any check-up following accidental skin contamination.

As a result of a disturbance in the production process in 1971 (manual shovelling of material into the mixing apparatus due to untypical packaging of one endosulfan batch, without dust filter masks), two out of four workers involved showed signs of intoxication in the form of spasms.

B.6.9.2 Direct observation, e.g. clinical cases and poisoning incidents

Several cases have been reported over the last ten years where larger quantities of endosulfan formulations were ingested with the intent to commit suicide (quantities given have been converted to give endosulfan active ingredient).

Lo, *et al* (1995) and Garnier, *et al* (J. Toxicol clin Toxicol 1995;33(4):375 report some cases of renal failure due to acute tubular necrosis following a suicidal attempt.

The lowest dose with a lethal effect was 41 mg/kg body weight (Geissbuehler *et al*., 1989, Doc. No.: A57045). Deaths have also been reported after ingestion of 295 and 467 mg/kg body weight (Geisbuehler *et al.,* 1989, Doc. No.: A57045; Bernardelli and Gennari, 1987, Doc. No.: A43387).

In two cases where it was possible to save the patients' lives despite oral ingestion of extremely high doses (100 and 1000 mg/kg body weight), the patients were subjected to intensive medical treatment within 1 h after intoxication (Shemesh *et al*., 1988; Sauer *et al.,* 1989,). Clinical signs in these patients were largely the same as those observed in acute studies on rats and dogs, and dominated by generalised recurrent tonoclonic spasms. Nine cases of convulsions in workers following Thiodan handling and feel were refereed by Ely *et al.* (1967). Benzodiazepines had no influence on the attacks, only barbiturates were effective. In the case of the suicide attempt with the active ingredient dose of 100 mg/kg body weight a restitutio *ad integrum* was achieved (Shemesh *et al*., 1988,). In the case of the attempt with 1000 mg/kg, neurological symptoms requiring anti-epileptic therapy were still present after one year (Sauer *et al*., 1989).

A report from Bulgaria describes the clinical symptoms, and morphological changes in circumstances, 5 cases associated with endosulfan poisoning,- (Terziev *et al*., 1974). These cases comprised 2 suicides and 3 accidental poisoning. Death generally followed a few hours after ingestion. The clinical symptoms included vomiting, agitation, convulsions, cyanosis, dyspnoea, foaming- at the mouth, and noisy breathing.

Another report lists the findings on 2 cases (apparently suicides) of men who died after ingesting endosulfan (Demeter & Hendrickx, 1978). Again death was noted to occur within a few hours of ingestion, and significant post-mortem findings included congested and oedematous lungs and cyanosis. Tissue analysis for residues indicated the possible synergistic effect of endosulfan and alcohol in one patient (Demeter & Hendrickx, 1977), and endosulfan, alcohol, and dimethoate, an organophosphorous insecticide, in the second.

Three cases of poisoning,, in workers employed in a chemical factory have been reported (Israel¡ et al., 1969; Tiberin et al., 1970). Poisoning, occurred when the men filled bags with insecticide without wearing protective clothing- and masks. Symptoms developed after 3 weeks, 1 month, and 18 months, respectively, following daily exposure, and consisted of headaches, restlessness, irritability, vertigo, stupor, disorientation, and epileptiform convulsive seizures. Electroencephalogram changes were noted.

Endosulfan has been shown to persist on the hands of pest control operators for up to 3 1 days after exposure. No clinical symptoms were observed (Kazen, *et al*., 1974).

B.6.9.3 Observations on exposure of the general population and epidemiological studies, if appropriate

California has a thorough (and legally required) reporting system on occupational illnesses/injuries due to pesticide exposures. An evaluation of 40 years of pesticide incidents (1949-1988) is available from the Californian FDA (Maddy, Edmiston and Richmond, 1990). In California are more than 800 active ingredients registered as pesticides. Annual registered sales are between 500 and 700 million lbs. These pesticides are used by 650.000 active farmers, 8.000 professional pesticide applicators and 300.000 maintenance workers/applicators.

From 1951-1987, 48 deaths were recorded due to the (mis)use of pesticides. Of these deaths none was due to the use of endosulfan.

The average yearly incidence rate per million lbs. of pesticide is between 4 and 2. Almost half of the reported cases were occupational incidents. The amount of endosulfan sold over the period 1976-1986 was 4.225 million lbs. The number of incidents related to endosulfan is known for the 12 year period of 1976-1987 and amounts to 24 cases. This figure refers to all definite, probable and possible exposure cases and does not include exposures involving combination with other pesticides. Half of these incidents only involved skin exposure without any lost working time. The other half involved ‘systemic’ exposure and the highest recorded lost working time for a single case amounted to 7 working days. In conclusion, the exposure incidence rate of endosulfan in California lies below the average pesticide exposure incidence rate. Most exposure cases did not cause lost working time (Volger, 1988,)

Sancewicz-Pach, K., Groszek, B., Pach, D., Klys, M.
Acute pesticides poisonings in pregnant women.

Przegl Lek 1997;54(10):741-744

A 21-year-old woman, 5 months pregnant ingested an unknown amount of endosulfan to provoke abortion. Gynecological examination and abdominal ultrasonography revealed longitudinal pelvic presentation of fetus. Neither fetal movement nor heart tones were audible as early as four hours after the clinical symptoms occurred. Such low concentration of endosulfan in the blood of the mother as 0.47 microgram/g of the poison caused relatively quick fetus death. The highest levels of endosulfan were found in the liver and in the fetus kidneys.

Grimmett, W. G., Dzendolet, I., Whyte, I.

Intravenous thiodan (30% endosulfan in xylene).

J Toxicol Clin Toxicol 1996;34(4):447-452

A 28-year-old woman with a past history of epilepsy presented with refractory grand mal seizures after injecting 1 mL of Thiodan intravenously. She developed liver dysfunction, proximal myopathy secondary to rhabdomyolysis and renal failure.

Sood AK, Yadav SP, Sood S

Endosulfan poisoning presenting as status epilepticus.

Indian J Med Sci 1994 Mar;48(3):68-69

A case of generalised seizures following ingestion of 20 cc of endosulfan (Endocel), an organochloride insecticide is presented. The patient, a young male of 25 years made a complete recovery. The mode of action of endosulfan is due to involvement of cholinergic neuronal system and the management is on the line of status epilepticus.

Blanco-Coronado JL, Repetto M, Ginestal RJ, Vicente JR, Yelamos F, Lardelli A

Acute intoxication by endosulfan.

J Toxicol Clin Toxicol 1992;30(4):575-583

The authors report six patients with acute endosulfan intoxication. The symptoms of nausea, vomiting, headache, and dizziness began 2.7 +/- 0.5 h after ingestion; in four cases the patients had been hospitalised but were asymptomatic. All had severe metabolic acidosis with high anion gap and hyperglycemia; five of six had decreased blood platelets. Three patients had pulmonary aspiration, and five required mechanical ventilation. The one fatality followed acute renal failure, disseminated intravascular coagulation, thrombi in the pulmonary arteries and aorta, and cardiogenic shock. In this patient the blood endosulfan was 2.85 mg/L versus a mean of 0.48 mg/L in the survivors.

Aleksandrowicz, D. R.

Endosulfan poisoning and chronic brain syndrome.

Arch Toxicol 1979 Oct;43(1):65-68

The author describes a case of acute poisoning by endosulfan (a chlorinated hydrocarbon insecticide) in an industrial worker, with residual psychiatric syndrome. The acute phase was manifested by repeated convulsions and impaired consciousness. After recovery the patient became disoriented and agitated. The residual phase, 2 years after initial hospitalisation, was manifested by cognitive and emotional deterioration, severe impairment of memory and inability to perform any but the simplest tasks. Psychological tests revealed gross impairment of visual-motor co-ordination. The differential diagnosis of chronic brain syndrome requires accurate history and milder cases of endosulfan poisoning may easily be overlooked or misdiagnosed.

B.6.9.4 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Clinical signs of intoxication in humans were largely the same as those observed in acute studies in rats and dogs, and dominated by generalised recurrent tonoclonic spasms. Benzodiazepines had no influence on the attacks, only barbiturates were effective. (Shemesh, *et al*., 1988; Sauer, *et al*., 1989; see 6.9.2).

Investigations in female rats showed that phenobarbital (Luminal®) had a good anticonvulsive effect even after administration of otherwise lethal doses of endosulfan so appreciably reduced the mortality rates in comparison with the non-antidote control group, while diazepam had hardly if any effect (Ebert and Weigand, 1984).

B.6.9.5 Proposed treatment: first aid measures, antidotes, medical treatment

B.6.9.5.1 First aid measures

If poisoning symptoms occur, particularly if it is known that there has been gross overexposure, medical attention should be sought immediately.

Skin contact: Effective skin decontamination should take place after any skin exposure:

Remove contaminated clothing.
Wash exposed skin thoroughly with soap and water.

Eye contact: Flush eyes well with water for at least 10 minutes. If irritation persists, obtain medical attention.

Ingestion: Induce vomiting in patient is still conscious. Obtain medical attention.

Inhalation: Place the patient in an area of fresh air. Keep patient warm. and obtain medical attention immediately.

B.6.9.5.2 Antidotes

No antidote exists against endosulfan intoxication.

B.6.9.5.3 Advice to physicians

Make sure the patient is decontaminated thoroughly.

In case of over-exposure (also by dermal route) do not give milk, fats and oils by mouth, as this will promote intestinal absorption.

Following ingestion, specific treatment consists of induction of vomiting or gastric lavage, whereby aspiration into the lungs should be prevented. This should be followed by intragastric administration of 3 - 4 tablespoons of charcoal.

In case of ingestion of an emulsifiable concentrate or other solution, the possibility of chemical pneumonia due to aspiration of the solvent should not be overlooked.

If the patient shows signs of convulsions, anticonvulsive treatment may be given. Intravenous application of barbiturates and calcium gluconate is advised for this purpose. Benzodiazepines have been reported to be of no influence (Shemesh, *et al*., 1988; Ebert and Weigand, 1984.

Contra-indicated are oily purgatives, epinephrine, other adrenergic drugs and central stimulants of all types.

B.6.9.6 Expected effects of poisoning

The lowest human lethal dose was 41 mg/kg body weight (Geissbuehler *et al*., 1989). However full recovery with and without treatment has been reported after intake of higher doses (see above).

Clinical signs of intoxication consist of neurological effects and are characterised by generalised recurrent tonoclonic spasms, further by headaches, restlessness and irritability, vertigo, stupor, disorientation and epileptiform convulsive seizures. Endosulfan exerts only acute effects and no lasting effects on survivors of endosulfan intoxication are anticipated.

Supplementary information

Sancewicz-Pach K, Groszek B, Pach D, Klys M

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A 21-year-old woman, 5 months pregnant ingested an unknown amount of endosulfan to provoke abortion. Gynecological examination and abdominal ultrasonography revealed longitudinal pelvic presentation of fetus. Neither fetal movement nor heart tones were audible as early as four hours after the clinical symptoms occurred. Such low concentration of endosulfan in the blood of the mother as 0.47 microgram/g of the poison caused relatively quick fetus death. The highest levels of endosulfan were found in the liver and in the fetus kidneys.

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Taking into account the above studies additional information will be required so as to examine the interatiction between endosulfan and dieldrin in the activation of estrogen receptors (Wade *et al*, 1997).

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

B.6.10.1 Summary of mammalian toxicology

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

In mice endosulfan and its sulphate and diol metabolites were the major faecal excretion products, with the diol metabolite excreted in the urine, while in rats, biliary excretion was extensive (up to 50%), and there was a little enterohepatic circulation form the bile. There does not appear to be appreciable bioaccumulation of endosulfan residues in body tissues, with only trace amounts of endosulfan residues found in most tissues, including the fat, of most species. In Wistar rats, kidney and lover residues were highest, although the half life for residues in these organs was only 7 days and 3 days, respectively, and kidneys residues were also higher than other tissues in goats. No residues of endosulfan or its metabolites in cow or sheep milk were detected.

The metabolites of endosulfan include endosulfan sulphate, diol, hydroxyl-ether, ether, and lactone but of its metabolites are polar substances which have not yet been identified.

Dermal absorption studies *in vivo* (rats and monkeys) and *in vitro* (human:rats) were performed They suggest that initial absorption is dose related, movement through skin is low (occurring over 168 h in the rat in vivo study), endosulfan continues to be absorbed from skin reservoirs after skin washing and penetration as per cent rate is lower in human skin than rat skin. Dermal absorption was reported to be as high as 25% in rats, and about 20% in Rhesus monkeys.

Endosulfan has been tested for acute toxicity, primary irritation and sensitisation potential. Three notifier have submitted studies. Besides, AgrEvo has included review document of endosulfan prepared by the Australian National Registration Authority (ANRA) for Agricultural and Veterinary Chemicals, which includes studies previously presented and studies which have not been presented by any applicant. However, additional information to cover acute toxicity has been found from IPCS (1998). Nevertheless, these studies are only a little summary of the original papers, thus they have been considered only as additional information.

Purity, when reported, range between 96 and 97.3% among all the studies. The followed procedures were in accordance or without significant deviation from USEPA and OECD Guidelines. Not all the studies were performed to GLP.

The LD_{50} of endosulfan varies widely depending on the route of administration, species, vehicle, and sex of the animal. The male rats are clearly more sensitive than male rats, and, on the basis of a single study this sex differences appears apply to mice also (Bremmer & Leist, 1998). The lowest oral LD_{50} value is 9.6 mg/kgbw in female Sprague Dawley rats (Reno, 1975), however, this study is considered as not acceptable due the paucity of information provided.

The acute oral median lethal dose LD_{50} of Endosulfan Technical in rats was calculated to have a range between 48 and 160 mg/kg for male and 10 and 22.7 mg/kg for female rats. These results would require an EEC classification of “T+” (very toxic) for the technical active ingredient, if based on the more sensitive sex alone.

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

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).

Skin and eye irritation studies submitted were considered not acceptable because purity of the technical product was not reported and exposition period after instillation into the eyes was very short. On the basis of the only percutaneous study considered as acceptable (Diehl & Leist,, 1988) plus the

additional information (ANRA and IPCS documents reviews) about skin and eye irritation endosulfan could be considered as not irritating to skin and eyes. However, original studies should be provided.

Based on the skin sensitisation studies (Buehler test), there is no evidence that Endosulfan is a contact allergen and it is not classified based on EU criteria. Besides, a summary about skin sensitisation found in IPCS document showed that endosulfan was not sensitising for guinea-pig skin (Arcelin, 1996). However, original studies should be provided.

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 LD50 value in rats, it also should be classified as "Harmful" and be associated with the risk phrase "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.

The results obtained in the studies considered acceptable are summarised in Table 6.10.1-1

Table 6.10.1-1: Summary of acute acceptable 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 1988-b 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 sensitisation				
Guinea pig, SPF Pirbright-White f	-	Polyethylene glycol 40%	No Sensitizer	Jung 1983 AgrEvo IIA, 5.2.6/1

Several short-term toxicity studies were provided: a subacute oral toxicity study in rats, suchronic oral studies on rats and mice and, finally, dermal and inhalation studies on rats. The results of the studies considered as acceptable are summarised in table 6.10.1-2.

Table 6.10.1-2: Summary of acceptable short-term toxicity studies.

Study	NOAEL (mg/kg bw/day)	Main adverse effect	LOAEL (mg/kg bw/day)	Reference and year
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: 0, 10, 30, 60 and 360 mg/kg feed.(equal to 0, 0.64, 1.9, 3.8 and 23 mg/kg/day for males and 0.75, 2.3, 4.6 and 27 mg7kg/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 Concentration 0, 2, 6, 18, and 54 mg/kg feed. (equal to 0, 0.24, 0.74, 2.13 or 7.3 mg/kg/day for males and 0, 0.27, 0.80, 2.39,0r 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 levels 0, 18 ppm				Donaubauer *et al* 1985 AgrEvo IIA, 5.3.2.5/1
Other routes				
28-day dermal, rat 0, 1, 3, 9, 27 and 81 mg/kg bw/day				Ebert *et al* 1985 AgrEvo IIA, 5.3.3.1/1
28-day dermal, rat (males 0, 18.75, 37.50, 62.50 mg/kg bw/day, females 0, 9.83, 19.66, 32.00 mg/kg).		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 0.0005, 0.0010, 0.0020 mg /l		No symptoms up the highest dose tested were observed.		Hollander *et al* 1984 AgrEvo IIA, 5.3.3.2/1

The subchronic oral toxicity study in rat revealed a NOAEL of 3.85 mg/kg bw/day (m), and a NOAEL of 2.3 mg/kg bw/day (m/f) in mice A 90-days feeding study in dogs is required.

The endosulfan genotoxicity data base has been prepared using the documentation submitted by AgrEvo, Excel and Calliope in support of the application. Numerous genotoxicity tests have been conducted with endosulfan. However, evaluation of the mutagenicity is confined to tests using technical endosulfan of clearly defined specifications. Results of these tests together with the information, presented by AgrEvo, about the genotoxicity of endosulfan-diol, a endosulfan metabolite, are summarised in Table 6.10.1-3.

The conclusions about the mutagenicity of endosulfan, based in data from studies carried out with technical material of clearly defined specifications, 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 *Saccharomyces cerevisiae* mitotic gene conversion assay, cannot be considered conclusive because of its conduct.

4. Endosulfan appears to be non-clastogenic in mammalian somatic cells *in vivo.* Nevertheless, in the only study, considered acceptable in evaluating the mutagenicity of endosulfan, a micronucleus test, a dose greater than 10 mg/kg should have been tested. On the other hand, thiodan 35 induced chromosomal aberrations in hamster; although any mutagenic activity may have resulted from non active constituents included in the formulation, it could be advisable to performed one study on chromosomal aberration induction with technical endosulfan.

5. The information given by the two presented chromosome aberration studies precludes any conclusion on the endosulfan clastogenicity for rodent germ cells, because in both studies the purity of the test substance was not reported. On the other hand, it is unlikely that a single isolated increase in dominant lethal mutations at the high dose is related with endosulfan administration; the lack of detail in the published study makes the significance of the isolated finding questionable.

6. Endosulfan induced sperm abnormalities in rodents. Nevertheless, it is unclear whether this effect is biologically significant.

The overall weight of evidence from the *in vitro* and *in vivo* studies is that endosulfan does not induce gene mutation. Nevertheless, although it appears to be non-clastogenic, more studies are required in order to give a definitive conclusion.

Table 6.10.1-3: Genotoxicity studies

Type of study	Species	Result with most sensitive species
In vitro studies	Bacteria	Negative for gene mutation in *Salmonella typhimurium* & *Escherichia coli.*
		Negative for rec-assay with *Bacillus subtilis.*
	Yeast	Inconclusive negative for gene mutation in *Schizosaccharomyces pombe.*
		and for mitotic gene conversion in *Saccharomyces cerevisiae.*
	Mammalian	Negative for gene mutation in mouse lymphoma cells.
	cells	Inconclusive negative for CA in human lymphocytes.
		Negative for UDS in both rat hepatocytes and a human cell line.
In vivo studies with somatic cells	Rodent	Inconclusive negative for MN in mouse.
In vivo studies with germ cells	Rodent	Inconclusive positive for mouse dominant lethal test.
		Positive for mouse sperm abnormalities test.

The Long-term effect of endosulfan on rats, mice and dogs were evaluated from eight studies provided by different applicants and using the additional information found in IPCS document and Australian monograph (ANRA).

Four chronic toxicity studies, were performed on rats (Keller, 1959c), mice (Arai, 1981) and dogs (Keller, 1959b and Brunk 1989; 1990).

Chronic toxicity study on rats was carried out prior to GLP regulations and is not considered acceptable because the purity of the test substance was not reported The second study performed on mice is only a review of the original paper, thus only can be considered as additional information .

Finally, two 1-year feeding toxicity studies on dogs were presented by AgrEvo. The first study carried out on Mongrel dogs (Keller, 1959b), was performed prior to GLP regulations and is not considered acceptable for many reasons: the purity of the test substance was not reported, the higher dose level used did not induced any toxic effect and the number of dogs used by group does not permit obtaining significant results . Only, the other study carried out on Beagle dogs was conducted according to OCDE guidelines and GLPs compliance.

The possible carcinogenic effect of Endosulfan was studied in rats and mice by Thomas *et al*., (1978). The study correspond to a published work. in US National Cancer Institute. and it did not claim any adherence to a specific test guideline or GLPs compliance. No treatment-related neoplastic lesions were seen in female rats; owing to the high mortality in rat males, no valid conclusion could be drawn about carcinogenic effects in male rats A NOAEL for rats was not identified, as treatment-related changes occurred in the kidneys and the testis at all doses.

A high early mortality in male mice was observed but due the high mortality in control groups this results were doubtful. As consequence, it could not be established a NOAEL for male mice. The NOAEL for female mice was 0.58 mg/kg/day.

The combined chronic /carcinogenic studies were carried out on Charles River rats (Ruckman et al., 1989) and on NMRI mice (Donaubauer 1989a, 1989b).

In the first case, the study was performed according to OECD: "Short-term and Long-Term toxicology group guideline" and following the GLP regulations Progressive glomerulonephrosis and aneurysms among in male rats aneurysms were detected. and, both signs were studied with more detail by histophatology techniques by Gopinath & Cannon, (1990). A second addendum was provided by Leist et al., (1989a): 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.

In the second combined study was evaluated the chronic oral toxicity and carcinogenic potential of endosulfan in NMRI-mice during two years . The study was conducted according to OECD 451 guideline in compliance with EPA guideline and following the GLP regulations. In support of this study, the residues of α-endosulfan, β-endosulfan, endosulfan-hydroxiether, endosulfan-sulphate, endosulfan-lactone and endosulfan-diol, were determined in the liver and kidneys (Leist. 1989b).

Both combined chronic and carcinogenic studies were summarised by Hack and published in Fd. Chem. Toxic. Vol.33, nº 11, pp: 941-950 (1995)

On the overall of these studies, no carcinogenic effect was observed in rats and mice at any Endosulfan dose tested.

Table: 6.10.1-4: Summary of Long-Term and Carcinogenicity acceptable studies

Study	NOAEL		LOAEL		Main Adverse Effect	Reference/year
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d		
Chronic toxicity study						
1-year toxicity study in Beagle dogs. Oral. 1 year. Dose levels: 0, 3, 10,30 ppm.(equivalent to 0. 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)

	NOAEL		LOAEL			
Carcinogenic studies						
Osborne-Mendel rats Oral. (78 weeks) and average dose levels: 0,220, 410 or 950 ppm for males and 220 and 400 for females males/females;	Not identified				No tumours were found in females; and no valid conclusion can be drawn about carcinogenicty in males	Thomas, LW *et al* (1978) (AgrEvo: IIA, 5.5.1/2) (AgrEvo: ANRA) (Calliope: IIA, 5.5/01)
:B6C3F1mice (78 weeks Oral.)Average dose levels: 3.5 and 6.9 ppm for males and 2 and 3.9ppm for females	3.9 (f)	0.58 (f)			Owing the high early mortality rates, no conclusion can be drawn about carcinogenicty in males No carcinogenic effects in females.	Thomas, LW *et al* (1978) (AgrEvo: IIA, 5.5.1/2) (AgrEvo: ANRA) (Calliope: IIA, 5.5/01)
Combined chronic/carcinogenic studies						
Charles River rats Oral.104 weeks.. Dose levels: 0,3,7.5, 15 and 75 ppm (equivalent to 0, 0.1, 0.3, 0.6 and 2.9 for males and 0, 0.1, 0.4, 0.7 and 3.8 mg/kg/day for females)	15(m/f)	M 0.6 F: 0.7	75(m/f	M 2.9 F 3.8	LOAEL based on low body gain weigh (m/f), low food consumption in females and kidney alterations in both sexes No evidence of increased carcinogenicity findings at any dose tested.	Ruckman SA *et al*., (1989) (AgrEvo: IIA, 5.5.1/4) (AgrEvo: ANRA) Hack et al., (1995) (Published) (AgrEvo:IIA, 5.5.1/6)
NMRI mice. Oral, 24 months. Dose levels:0, 2, 6, 18 ppm (equivalent to 0.28, 0.84 and 2.51 for males and 0.32, 0.97,and .2.86 mg/kg/day for females)	6	0.84 (m) 0.97 (f)	18	2.51 m 2.86 f	LOAEL base on decreased body weight in males at 24 months and decreased weight in males at 24 months and decreased weights of the liver, ovaries and lung in males and females at 12 and/or 18 months. No carcinogenic properties in mice	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)

m =male
f = female

Eight studies have been conducted to evaluate endosulfan toxicity on reproductive system. They include three multigeneration studies on rats and five developmental studies, four on rats and only on rabbits- All these studies are sponsored mainly by AgrEvo company.(table 6.10.1-5).

Multigeneration toxicity

To establish, the maximum tolerated dosage of endosulfan for use in a multigenerational study in rats was performed a preliminary study by Edwards *et al*., (1982). This study does not claim adherence to specific guidelines and GLP compliance.. Under the conditions of this study, it was concluded that 75 ppm (equivalent to 8.26 mg (kg/day and 8.36 mg/kg/day in males and females respectively), would be suitable fur use as the highest dose level in the subsequent multigeneration studies.

Kennedy *et a*l., (1965) study was conducted prior to the requirement of GLP and did not claim adherence to a specific guideline besides, the purity of the endosulfan was not reported , thus this study is considered as not acceptable. In addition, the dosages employed are referred to mg/kg/diet , thus it has not been possible to relate diet concentration of endosulfan to mass of endosulfan/kg bw animal/day.

In the study carried out by Edwards et al (1984) and Offer (1985) was evaluate endosulfan effects on the reproductive performance and developmental of F0, F1B and F2B generation rats.

Both studies were conducted to GLP compliance. Endosulfan did not affect reproductive performance or the growth or developmental of the offspring of rat over the course of a two generation study. The NOAEL for maternotoxicity was 1 mg/kg bw/day and for reproduction toxicity was 6 mg/kg bw/day. Developmental NOAEL could not be stabilised.

Developmental toxicity studies:

Five studies on developmental toxicity were performed, four of them on rats and one on rabbits:

1.-The first teratology study submitted was performed prior to GLP regulations and no guideline method was available at the time of the study. The study was published in Acta Pharmacol. Toxicol. vol 42: 150-152.by Gupta *et al*., (1978). The level reporting in this published paper is not adequate for the purposes of defining an NOAEL for developmental toxicity Besides, the paper can not be considered acceptable because the purity of the test substance as the stability of the test substance and strain and age of the animals are not provided.

2.-An other study to determine the potential teratogenic of thiodan upon gravid albino rats was performed prior to GLP regulations and without any guideline specification (Haley, 1972). On the other hand, the dosages used in this study were not sufficiently high to induce any toxicity.

3.-.The only study performed according to OECD guideline referent to Teratogenicity studies and following the GLPs ,was carried out by Albrech and Baeder (1993). The NOAEL for maternotoxicty and for developmental toxicity was 2 mg/kgbw/day.

4.- A last report provide by AgrEvo company to evaluate the embriofetotoxicity in rats was designed by McKenzie et al (1980).The study was performed prior to GLP regulation and no guideline method was

available at the time of the study**.** This study is considered as acceptable with some reservation, mainly because the replacement of animals during the study made difficult to interpret the data .

5.- Finally, one year later, the same author studied the embrio-fetal and teratogenic method nor GLP compliance. Besides, the interpretation of data is not clear .because some animals were also replacement during the study .

On the overall of these studies, non critical effect was identified to reproduction after administration of endosulfan and the fetotoxicity effects appear at maternal toxic doses.

Table 6.10.1-5: Summary of acceptable reproduction toxicity studies

Study	NOAEL		LOAEL		Main Adverse Effect	Reference/year
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d		
Preliminary study to determine doses used in two generation study in rats .Dosages: 0, 50, 75, 100 ppm	Maternal.50	M 6.25 F 5.92	Maternal: 75	M 8.26 F 8.36	Maternal: decreased of food consumption and body weights. Litter weights of dams were significantly decreased	Edward et al (1982) AgrEvo: IIA, 5.6.1/2
Two generation reproduction toxicity in rats. Dose levels: 0, 3, 15, 75 ppm (0.2,1, 4.99 mg/kg bw/day for males and 0.24, 1.23, 6.18 mg/kg bw/day for females)	Maternal 15 Reprod 75:	Maternal 1 Reprod 6	Maternal:75	Maternal:1	Maternal: Increased relative liver and Kidney weights-	Edwards et al., (1984) AgrEvo:IIA, 5.6.1/1 Offer., (1985) AgrEvo, IIA: 5.61/4
Developmental toxicity in rats. Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal:2 Develop::2		Maternal:6 Develop:6	Maternal:. On the basis of the deaths, clinical signs and decreased body weight Develop: increase incidence of fragmented thoracic vertebral centra No teratogenic effects	Albrech & Baeder, 1993 AgrEvo: IIA, 5.6.2.1/4
Developmental toxicity in rats Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal.. 0.66 Develop:2		Maternal:2 Develop:6	Maternal: decreased body weight gain and clinical signs. Develop: delayed development and a low incidence of isolated skeletal variation No teratogenic effects	McKenzie (1980) AgrEvo: IIA, 5.6.2.1/3)
Developmental toxicity in rabbits. Dose levels: 0, 0.3, 0.7, 1.8 mg/kgbw/day		Maternal 0.7 Develop: 1.8		Maternal:1.8	Maternal: based on Clinical signs (noisy, rapid breathing, hyperactivity and convulsions) No teratogenic effects	McKenzie et al., 1981 AgrEvo: IIA, 5.6.2.2/1

Two studies were reported by AgrEvo and Excel companies to evaluate delayed neurotoxicity of endosulfan (Robert & Phillips, 1983 and Gupta, 1976) , nevertheless the second study was considered as not acceptable because any reference about the purity of the test substance was provided. (table 6.10.1-6).

Robert & Phillips,(1983) designated a study to determine LD_{50} and delayed neurotoxicity of endosulfan in hens 200. birds were used and allocated in three different treatment: LD_{50} determination, protection assessment and neurotoxicity assessment. To determine LD_{50} was developed a preliminary range finding study on 5 groups of 2 birds doses with different concentrations to endosulfan. On the basis of this results, 30 birds were allocated to 6 treatment groups of 5 birds each,, at doses to 0, 40, 60, 90,135 and 110 mg/kg of endosulfan.

A small study was carried out to determine the protective effects of phenobarbitone, diazepam, atropine and 2-PAM when administered prior to dosing with endosulfan.

For neurotoxicity determination were used six groups of 10 birds each (including positive and negative control),treated with 96 mg/kg endosulfan (LD_{50} calculated).Negative control birds were dose only with corn oil and positive control with 500mg/kg TOCP in corn oil Under the conditions of this study, endosulfan did not produce any clinical signs of neurotoxicity at the LD_{50} calculated .

Table 6.10.1-6: Neurotoxicity studies

Study type/species/ dose levels	Comments	Reference and years
Acute Delayed Neurotoxicity in hens. Dose levels 0,40,60,90,110, 135mg/kg	Any clinical signs of neurotoxicity at the LD_{50} calculated (LD_{50} value of the 96 mg/Kg	Roberts & Phillipps (1983) AgrEvo: IIA, 5.7/1
Neurotoxicity in Rats and mice	Endosulfan produce toxic effects due to CNS stimulation and the death may be due to direct depressant effect on some vital organ oft he body.	Gupta P(1976) Excel: IIA, 5.7/02)

There are several supplemental studies about,enzyme induction (endosulfan not induce hepatic microsomal enzyme activities on mice and rats), tumour promotion (No inhibition to enhance the incidence of GGT-positive hepatocyte in NDEA initiated was found in male rats treated with endosulfan.),endocrine system (endosulfan alone and in combination, may bind to estrogen receptors and may perturb the endocrine system), sperm effect (endosulfan does not produced significant changes), inmunotoxicity (endosulfan does not have any adverse effect on the immune function of laboratory animals) and neurobehaviour (at highest dose levels alterations in neurobehaviour were observed with signs of frank toxicity), which them the almost were provided by the applicants and . additional information to cover these items has been found from IPCS (1998). Nevertheless, this information is only a little summary of the original papers, thus they have been considered only as additional information within of summary of each item. Table 6.10.1-7.

Table 6.10.1-7 Summary of supplemental studies

Study	Dose levels	Main Effects	Reference
Enzyme induction			
3-days. Oral gavage in male mice.	5 mg/kg/day	Cytochrome P-450 group of enzymes is not significantly activated.	Robacker *et al*., (1981) (AgrEvo: IIA, 5.1.3.2/2):
Promotion study			
In vitro metabolic co-operation (V79 cells) and scrape loading/dye transfer (WB cells) assays *Invivo* EAF incidence assay, Oral gavage10-weeks, rats(m),	Doses:1 and 5 mg /Kg/ bw/day	*In vitro*: ENDOαβ, ENDOα , ENDOβ, technical Endosulfan and Endosulfan-sulphate metabolite were potent inhibitors of intracellular communication in both assays *in vitro*. In addition Endosulfan-ether inhibited transfer in WB cells. *In vivo*: Technical endosulfan produced congestion of the peritoneum and inner organs, and increased liver weights	Flodström *et al*, (1988) (AgrEvo:
Endocrine system			
In vitro and In vivo studies		Endosulfan does not meet the criteria of a endocrine disrupter	Bremmer & Leist (1998) AgrEvo review
Effects on sperm			
Oral short-term/chronic study in male rats	2.5, 5. 7.5.10 mg/kg	Possible deleterious effects on male reproductive organs (testis) and biosynthesis and secretion of testosterone	Singh & Padney(1989) (Excel, IIA, 5.5/01
Oral subchronic study in male Wistar rats	0, 7.5, 10 mg/kg/day	Testicular testosterone levels remained significantly decreased.	Singh & Padney(1990) (Excel, IIA, 5.5/03
Immunotoxicity studies			
Oral, six week study in male Wistar rats	0,10,30,50 ppm	Humoral and cellular immunity was depressed at doses of 30 and 50 ppm	Banerjee & Hussain (1987) (AgrEvo: IIA, 5.8.2.1/3)
Oral study in albino rats for up to 22 weeks	0,5,10,20 ppm	Marked suppression of the humoral and CMI responses in rats. Cellular and humoral immune responses were decreased in a dose-time dependent pattern.	Banerjee & Hussain (1986) (AgrEvo: IIA, 5.8.2.1/2)
Oral Wistar rats study	0.5, 1.5, 4.5 mg/kgbw/day		Hack & Leist (1988) (IPCS 1998)
Oral study in Wistar rats (3-weeks)	20, 100, 250 ppm	At 100 ppm: reduction in body weight gain.	Vos et al, (1982) (IPCS 1998)

Study	Dose levels	Main Effects	Reference
Neurobehavioral studies			
Oral acute study in rats	25, 50, 100 mg/kg/day (males) 3,6,12 mg/kg/day (females	LOAEL: 50 and 6 mg/kg/bw/day male and female respectively, based on serious neuropharmalcological effects.	Bury (1997) (IPCS 1998)
Rats	10mmol/L	No inhibition of rat brain AChE activity was observed for up to 75 min treatment.	Müller (1989) (IPCS 1998)
30-daysdietary study in Wistar rats	0, 3 and 6 mg/kg/day	A significant dose–related increase in motor activity in both sexes at low and high dose.	Paul , V et al., (1995) (AgrEvo:ANRA)
90-Days oral study in male rats	2 mg/kg/day	Changes in central nervous system, but not impair motor responses	Paul , V et al., (1993) (AgrEvo:ANRA)
90-Days oral study in male rats	2 mg/kg/day		Paul , V et al., (1994) (AgrEvo:ANRA)

Testing of endosulfan-diol (Hoe 51329) metabolite for acute oral toxicity, LD_{50} values for the male and female rats were higher than 5000 mg/kg b.w. (Ehling & Leist, 1991a). Studies of acute dermal toxicity with this metabolite determined that the LD_{50} values for male and female rats were higher than 2000 mg/kg b.w. (Ehling & Leist 1991c). Endosulfan-diol is irritant and the skin contact produce sensitisation in guinea pig (Hammerl, 1996a) and, however, the substance has not this effect when the Buehler Test has been applied (Hammerl 1996b). Whit respect to dermal and eye irritation, the test substance can not considered as irritant substance (Hammerl 1996c).

Testing of endosulfan-sulphate (Hoe 51327) metabolite for acute oral toxicity, LD_{50} values were 568 mg/kg b.w. for male rats, and 25-50 mg/kg b.w. for female rats (Ehling & Leist 1991b), whereas for acute dermal toxicity LD_{50} values were 2740 mg/kg b.w. for male rats and 280 mg/kg b.w. for female rats (Ehling & Leist 1991d).

It is impossible to obtain solvent data on endosulfan-lactone, endosulfan-hydroxyether, endosulfan-ether, and endosulfan-alcohol because the submitted studies have serious deficiencies, and they have been evaluated as unacceptable. More information is required.

Table 6.10.1-8: Acute toxicity of metabolites

Metabolite	Route/Species/Sex	Dose range (mg/kg bw)	Result	Reference
Hoe 51329	Oral Wistar rats (m/f)	5000	LD_{50} >5000 (m/f)	Ehling & Leist 1991a
Hoe 51327	Oral Wistar rats (m/f)	25, 31.5, 50, 63, 100, 200, 400, 800	LD_{50}= 568 mg/kg bw (m) LD_{50} = 25-50 mg/kg bw (f)	Ehling & Leist 1991b
Hoe 51329	Dermal Wistar rats (m/f)	2000	LD_{50} >2000 (m/f)	Ehling & Leist 1991c
Hoe 51327	Dermal Wistar rats (m/f)	250, 315, 400, 1600, 2500, 4000	LD_{50}= 2740 mg/kg bw (m) LD_{50} = 280 mg/kg bw (f)	Ehling & Leist 1991d
Hoe 51329	Skin sensitisation Pirbright-White guinea pig (f)		Irritant. Sensitisation by skin contact.	Hammerl 1996a
Hoe 51329	Skin sensitisation Pirbright-White guinea pig (f)		No sensitising (Buehler Test)	Hammerl 1996b
Hoe 51329	Dermal irritation and eye irritation New Zealand White rabbits (f)		Dermal irritation: Not irritant. Eye irritation: Not subject to labelling requirements.	Hammerl 1996c

Subchronic toxicity data from two different Endosulfan metabolites were presented: the ones with Thiodan sulphate are done without GLP compliance, since the ones with Hoe 051329 fulfil the requirements of GLP. The results of these studies are summarised in Table 6.10.1-9.

Table 6.10.1-9: Summary of oral subchronic studies

Study	NOAEL (mg/kg bw/day)	Main adverse effect	LOAEL (mg/kg bw/day)	Reference and year
90-day, oral, dog. Hoe 051329	9.1 male 8.4 female	bile duct proliferated with fibrosis	89.4 male 82.9 female	Stammberger 1994.
90-day, oral, rat. Hoe 051329	7.8 male 8.0 female	haematotoxicity and liver toxicity.	40.2 male 40.7 female	Ebert and Hack, 1996a/b

Since the purity and the test method of the two Thiodan sulphate studies were not reported, these studies were not acceptable and due to the endosulfan sulphate was included in the residue definition for crop commodities a subchronic toxicity study of this endosulfan sulphate metabolite is required.

Three studies using endosulfan-diol, a endosulfan metabolite, were sponsored and presented by AgrEvo**.** They included *in vitro* (gene mutation and UDS) and *in vivo* (micronucleus) assays. These studies are summarised in Table 6.10.1-10.

All studies were performed according to specific test guidelines and were GLP compliant. They were reported over the period 1992 to 1993.

Negative results were obtained in all studies.

The available genotoxicity tests show that endosulfan-diol could be considered as non genotoxic.

Table 6.10.1-10: Genotoxicity tests of metabolites (endosulfan-diol)

In vitro studies	Bacteria	Negative for gene mutation in *Salmonella typhimurium* & *Escherichia coli.*
	Mammalian cells	Negative for UDS in a human cell line.
In vivo studies with somatic cells	Rodent	Negative for MN in mouse.

In summary, of case report of human poisoning incidents, the lowest reported dose that caused death was 35 mg/kgbw. Higher doses caused death within 1 h. The clinical signs in these patients were dominated by tonic-clonic convulsion, consistent with the observations in experimental animal.

B.6.10.1.2 Overall Evaluation of Mammalian Toxicology

Study	NOAEL		LOAEL		Main Adverse Effect
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d	
Short-term toxicity studies					
28-days oral, rats. Dose levels:360 and 720 ppm (equal to 34 and 67.8 mg/kg/day)					
28-day dermal, rat 0, 1, 3, 9, 27 and 81 mg/kg bw/day	Not identified.		Not identified.		
28-day dermal, rat (males 0, 18.75, 37.50, 62.50 mg/kg bw/day, females 0, 9.83, 19.66, 32.00 mg/kg).	Not identified.		Not identified.		
42 day, diet, mouse NMRKf. Dose levels 0, 18 ppm	Not identified.		Not identified.		
29- days, nose-only inhalation, rat 0.0005, 0.0010, 0.0020 mg /l	Not identified.		Not identified.		
90-day, diet, rat. Concentrations: 0, 10, 30, 60 and 360 mg/kg feed. d (equivalent to 0, 0.64,1.9, 3.8 and 23 mg/kgbw/day for males and 0, 0.75, 2.3, 4.6 and 27 mg/kgbw/day for females	60	3.85 (m/f)	360	23.41 (m/f)	Haematological changes
90-day, diet, mouse CD-1 Concentration 0, 2, 6, 18, and 54 mg/kg feed (equal to 0, 0.24., 0.74, 2.13 or 7.3 mg/kg/day for males and 0, 0.27, 0.80, 2.39 or 7.5 mg/kg/day for females).	18	2.3 m/f	54	7.4 m/f	LOAEL: based on lethality and neurological signs

	NOAEL		LOAEL		
Genotoxicity studies					
In vitro studies in bacteria					Negative for gene mutation in *Salmonella typhimurium* & *Escherichia coli*. Negative for rec-assay with *Bacillus subtilis.*
In vitro studies in Yeast					Inconclusive negative for gene mutation in *Schizosaccharomyces pombe.* and for mitotic gene conversion in *Saccharomyces cerevisiae.*
In vitro studies in Mammalian cells					Negative for gene mutation in mouse lymphoma cells. Inconclusive negative for CA in human lymphocytes. Negative for UDS in both rat hepatocytes and a human cell line.
In vivo studies with somatic cells in rodents					Inconclusive positive for MN mouse
In vivo studies with germ cells in rodents					Inconclusive positive for mouse dominant lethal test. Positive for mouse sperm abnormalities test
Long-term and carcinogenic studies					
1-year oral toxicity study in Beagle dogs. Oral. 1 year. Dose levels: 0, 3, 10,30 ppm.(equivalent to 0. 0.23, 0.77 and 2.3 mg/kgbw/day).	10	0.65 m 0.57 f	30	2.3	LOAEL based on clinical signs (violent contractions of the abdominal muscles) and reductions in body weight gain

	NOAEL		LOAEL		
Carcinogenic study: Osborne-Mendel rats Oral. (78 weeks) and average dose levels: 0,220, 410 or 950 ppm for males and 220 and 400 for females males/females;	Not identified				No tumours were found in females; and no valid conclusion can be drawn about carcinogenicty in males
Carcinogenic study: in B6C3F1mice (78 weeks Oral.)Average dose levels: 3.5 and 6.9 ppm for males and 2 and 3.9ppm for females	3.9 (f)	0.58 (f)			Owing the high early mortality rates, no conclusion can be drawn about carcinogenicity in males No carcinogenic effects in females.
Combined toxicity/carcinogenic study. in Charles River rats Oral.104 weeks.. Dose levels: 0,3,7.5, 15 and 75 ppm (equivalent to 0, 0.1, 0.3, 0.6 and 2.9 for males and 0, 0.1, 0.4, 0.7 and 3.8 mg/kg/day for females)	15(m/f)	M 0.6 F: 0.7	75(m/f	M 2.9 F 3.8	LOAEL based on low body gain weigh (m/f), low food consumption in females and kidney alterations in both sexes No evidence of increased carcinogenicity findings at any dose tested.
Combined toxicity/carcinogenic study, in NMRI mice. Oral, 24 months. Dose levels:0, 2, 6, 18 ppm (equivalent to 0.28, 0.84 and 2.51 for males and 0.32, 0.97,and .2.86 mg/kg/day for females)	6	0.84 (m) 0.97 (f)	18	2.51 m 2.86 f	LOAEL base on decreased body weight in males at 24 months and decreased weight in males at 24 months and decreased weights of the liver, ovaries and lung in males and females at 12 and/or 18 months. No carcinogenic properties in mice
Reproduction and developmental studies					
Preliminary study to determine doses used in two generation study in rats .Dosages: 0, 50, 75, 100 ppm	Maternal.50	M 6.25 F 5.92	Maternal: 75	M 8.26 F 8.36	Maternal: decreased of food consumption and body weights. Litter weights of dams were significantly decreased
Two generation reproduction toxicity in rats. Dose levels: 0, 3, 15, 75 ppm (0.2,1, 4.99 mg/kg bw/day for males and 0.24, 1.23, 6.18 mg/kg bw/day for females)	Maternal 15 Reprod 75:	Maternal 1 Reprod 6	Maternal:75	Maternal:1	Maternal: Increased relative liver and Kidney weights-
Developmental toxicity in rats. Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal:2 Develop::2		Maternal:6 Develop:6	Maternal:. On the basis of the deaths, clinical signs and decreased body weight Develop: increase incidence of fragmented thoracic vertebral centra No teratogenic effects

	NOAEL		LOAEL		
Developmental toxicity in rats Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal..0.66 Develop:2		Maternal:2 Develop:6	Maternal: decreased body weight gain and clinical signs. Develop: delayed development and a low incidence of isolated skeletal variation No teratogenic effects
Developmental toxicity in rabbits. Dose levels: 0, 0.3, 0.7, 1.8 mg/kgbw/day		Maternal 0.7 Develop: 1.8		Maternal:1.8	Maternal: based on Clinical signs (noisy, rapid breathing, hyperactivity and convulsions) No teratogenic effects
Neurotoxicity studies					
Acute Delayed Neurotoxicity in hens. Dose levels 0,40,60,90,110, 135mg/kg					Any clinical signs of neurotoxicity at the LD_{50} calculated . the 96 mg/Kg

B.6.10.2.1 Acceptable Daily Intake (ADI)

The calculation of an ADI is based on the more sensitive of the following studies, chronic, carcinogenic and reproduction toxicity in dogs, rats and mice.

Dogs: 1-years.chronic study

NOAEL= 10 ppm (equivalent to 0.57 mg/kgbw/day in females)

104-weeks dietary study in rats

The NOAEL was 15 ppm (equivalent to 0.6 mg/kgbw/day in males and 0.7 mg/kgbw/day in females)

78 weeks dietary study in mice:

The NOAEL= 3.9 ppm (equivalent to 0.58 mg/kgbw/day in females)

Developmental toxicity in rats:

NOAEL for maternotoxicity= 0.6 mg/kgbw/day

ADI was established in 0.006 mg/kg/day based on the lowest NOAEL obtained in the most sensitive specie, rat , and using a safety factor of 100. (104-weeks dietary study in rats)

B.6.10.2.2 Acceptable Operator Exposure Level (AOEL)

Systemic AOEL was 0.006 mg/kgbw/day based on the lower NOAEL obtained in subchronic, chronic and reproduction studies on the most sensitive specie and using a safety factor of 100. (104-weeks dietary study in rats).

(Oral absorption > 90%, assessment factor =1)

B.6.10.2.3 Parametric Value for Drinking Water

On basis that exposure through drinking water should not account for more than 10% of the ADI and that the average consumption is 2 litres of water/day for a 60 kg person, we propose a **Parametric Value for Drinking Water =0.018 mg/l**

B.6.11a Acute toxicity including irritancy and skin sensitisation of preparation (IIIA, 7.1)

Applicant: Hoechst Schering AgrEvo GmbH - Makhteshim Agan International Co-ordination Centre (Task force)

Manufacture: Hoechst Schering AgrEvo GmbH

Tradenames: Thiodan, Cyclodan, Thionex, Endofan, Thyonex, FAN 35

Thiodan has been thoroughly tested for acute toxicity(the inhalation study was performed with Endosulfan emulsifiable concentrate (500 g/l)), primary irritation and sensitization potential. Results obtained in these studies are summarised in Table 6.11-1. All studies were performed according procedures of the OECD and EPA and in compliance with GLP.

The acute oral median lethal dose (LD_{50}) of Thiodan in rats was calculated to be 67 mg/kg for male and 17 mg/kg for female. According to the EU Criteria, Thiodan should be classified with the symbol T+ (very toxic) and the risk expression R28 in rats.

The acute oral median lethal dose (LD_{50}) of Thiodan in mice was calculated to be 39 mg/kg for both male and female. According to the EU Criteria, Thiodan should be classified with the symbol T (toxic) and the risk expression R25 in mice.

The acute oral median lethal dose (LD_{50}) of Thiodan in rabbit was determined to be 75 mg/kg for male. In the female rabbit, the oral LD_{50} was determined to be 34 mg/kg. In the sexes combined the oral LD_{50} was determined to be 50 mg/kg. According to the EU Criteria, Thiodan should be classified with the symbol T (toxic) and the risk expression R25 in rabbit.

The acute dermal median lethal dose (LD_{50}) of Thiodan for male rat was determined to be 412 mg/kg. For the female rat, the LD_{50} was approximately 266 mg/kg. According to the EU Criteria, Thiodan should be classified with the symbol T (toxic) and the risk expression R24.

The acute dermal median lethal dose (LD_{50}) of Thiodan for rabbit was greater than 400 mg/kg. According to the EU Criteria, Thiodan should be classified with the symbol Xn (harmful) and the risk expression R21.

The inhalation study was performed with Endosulfan-emulsifiable concentrate (500 g/l). (Hoe 002671 OI EC43 A103). The acute inhalation median lethal concentration (LC_{50}) of Endosulfan-emulsifiable concentrate (500 g/l) was determined to be 0.263 mg/l for male rats and 0.0594 for female rats. According to the EU Criteria, Endosulfan-emulsifiable concentrate (500 g/l) should be classified with the symbol T+ (very toxic) and the risk expression R26.

Material test (Thiodan) was considered to be irritant to rabbit skin. According to the EU Criteria, Thiodan should be classified as skin irritant (Xi) and the risk expression R38.

The acute eye irritation/corrosion test with Thiodan were irritant to rabbit eye. According to the EU Criteria, Thiodan should be classified as eye irritant and the risk expression R41.

A skin sensitization study in guinea pig using the Buehler method demonstrated that Thiodan is not considered to be a skin sensitizer.According to the EU Criteria, Thiodan should not be classified as skin sensitising.

In conclusion, Thiodan might be considered very toxic by oral route in rats, and toxic for mice and rabbit. By dermal route, material test is considered toxic for rat and harmful for rabbit. Endosulfan emulsifiable concentrate (500 g/l) is very toxic by inhalation. Thiodan is irritant to skin, irritant to eye and not a skin sensitizer.

Table 6.11a-1

Species/strain	Sex	Route/Method	Result	Reference
Rat/Wistar	Both	Oral	LD_{50}(male)=67 mg/kg LD_{50}(female)=17 mg/kg	Ebert. 1989
Mice/NMRI	Both	Oral	LD_{50}=39 mg/kg	Ebert 1989
Rabbit/NZ	Both	Oral	LD_{50}(male)=75 mg/kg LD_{50}(female)=34 mg/kg	Ebert 1989
Rat/Wistar	Both	Dermal	LD_{50}(male)=412 mg/kg LD_{50}(female)=266mg/kg	Ebert.1989
Rabbit/NZ	Both	Dermal	LD_{50}>400mg/kg	Ebert.1989
Rat/Wistar	Both	*Inhalation	LC_{50}(male)=0.263 mg/l LC_{50}(female)=0.0594 mg/l	Hollander 1984
Rabbit/NZW	Both	Dermal	Skin Irritant	Ebert.1989
Rabbit/NZW	Female	Eye	Eye Irritant	Ebert.1989
Albino Guinea pig/Himalaya	Both	Sensitization (Buehler)	Not Sensitizing	Ullmann.1986

* Material test: Endosulfan emulsifiable concentrate (500 g/l). code: Hoe 002671 OI EC 43 A103

B.6.11.1a Oral studies

B.6.11.1.1a Oral study in rats

Ebert,E. 1989a (AgrEvo: IIIA, 7.1.1)

Dates of experimental work: August-October 1989.

The study was performed according to EPA Guideline 81-1, OECD Guideline nº 401 and Agricultural chemicals, Laws and Regulations Japan, MAFF (p.19-20).

GLP: yes

The study is acceptable.

Materials and methods

A group of 30 (15 males and 15 females) Wistar WISKf (SPF71) rats, source Hoechst AG, Kastengrund, weighing between 190-221 the males and 157-190 the females were used for the determination of acute oral toxicity of material test Hoe 002671 00 EC33 B317 (Thiodan). After an acclimatization period of five days, the animals were divided into 3 groups, each consisting of 5 males and 5 females. The males received 40, 63 and 100 mg/kg bodyweight and the females 10, 16 and 25 mg/kg bodyweight. The test material was prepared at the appropriate concentration in desionised water, to permit administration at a constant volume of 10 ml/kg. The prepared test substance was administered by gavage to fasted animals at the stated dose levels. The animals were housed individually in Makrolon cages under standardised conditions (temperature: 22 ± 3ºC; relative humidity: 50 ± 20%; lighting time: 12 hours daily). The animals had free access to drinking water and food (Altromin 1324 rat diet), except 16 hours before dosing to 3-4 hours after treatment. After dosing, the animals were kept under observation for 15 days. During the observation period, the animals were weighed weekly, except the males of the 63 mg/kg dose group.. At the end of the observation period, the surviving animals were killed by carbon dioxide asphyxiation, dissected and also examined for macroscopically visible changes. Lethally intoxicated animals were also dissected and examined macroscopically.

Findings

No deaths occurred at the low dose level among either males or females. 2 out of 5 animals died in each of the middle dose level groups, and all of the animals in the high dose level groups, as noted in Table 6.11.1a-1.

Table 6.11.1a-1: Motalities

Sex	Dose Level (mg/kg)	Nº of animals	Mortalities (%)
Male	40	5	0 (0)
	63	5	2 (40)
	100	5	5 (100)
Female	10	5	0 (0)
	16	5	2 (40)
	25	5	5 (100)

Clinical signs: Reduced spontaneous activity, irregular breathing, narrowed palpebral fissures, straddling of hindlimbs, forward crawling, trembling, twitching, tonic spasms, tonoclonic spasms, blood-crusted eye margins and snout, increased salivation, and increased vocalization. The symptoms already emerged shortly after administration of the test substance. One day after treatment, most of these signs subsided and afterwards only flanks drawn in, squatting posture and decreased spontaneous activity in individual cases were observed up to 6 days after treatment. These clinical signs indicate the CNS as the primary target organ of the test substance.

There were no disturbances of body weight gains.

Gross pathology: Macroscopic examination of the males and females found dead during the study revealed the following abnormalities: light or dark discolouration of the kidneys, dark discolouration of the liver, small intestine filled with reddish black mass or reddish mucus and filled with test compound, large intestine filled with a reddish black mass or a reddish mucus, stomach filled with either test compound, clear fluid or gas, and lungs congested with blood. The animals killed at the end of the observation period showed no macroscopically visible changes.

Conclusions

The acute oral median lethal dose of Thiodan was 67 mg/kg for male rat and 17 mg/kg for female rat. Accordintg to the EU Criteria, Thiodan should be classified with the symbol T+ (very toxic) and the risk expression R28.

B.6.11.1.2a Oral study in mouse

Ebert, E. 1989b (AgrEvo: IIIA, 7.1.1)

Dates of experimental work: September-October 1989.

The study was performed according to EPA Guideline 81-1, OECD Guideline nº 401 and Agricultural chemicals, Laws and Regulations Japan, MAFF (p.19-20).

GLP: yes

The study is acceptable.

Materials and methods

A group of 30 (15 males and 15 females) NMRI mouse, source HOECHST AG, Kastengrund, SPF breeding colony weighing between 19-24 g the males and 18-22 g the females were used for the determination of acute oral toxicity of material test Hoe 002671 00 EC33 B317 (Thiodan). After an acclimatization period of five days, the animals were divided in 3 groups, each consisting of 5 males and 5 females. The dose levels tested were 25, 37.5 and 50 mg/kg bodyweight. The test material was prepared at the appropriate concentration in desionised water, to permit administration at a constant volume of 10 ml/kg. The prepared test substance was administered by gavage to fasted animals at the stated dose levels. The animals were housed by sex in groups of 5 in Makrolon cages under standardised conditions (temperature: 22 ± 3ºC; relative humidity: 50 ± 20%; lighting time: 12 hours daily). The animals had free access to drinking water and food (Altromin 1324 rat diet)), except 16 hours before dosing to 3-4 hours after treatment. After dosing, the animals were kept under observation for 14 days. During the observation period, the animals were weighed weekly. At the end of the observation period, the surviving animals were killed by carbon dioxide asphyxiation, dissected and

also examined for macroscopically visible changes. Lethally intoxicated animals were also dissected and examined macroscopically.

Findings

In the low, middle, and high dose level groups 1, 3 and 9 out of 10 animals died on the first day of the study. The mortality rates showed in Table 6.11.1.2a-1.

Table 6.11.1.2a-1: Mortalities

Sex	Dose Level (mg/kg)	Nº of animals	Mortalities (%)
Male	25.0	5	0 (0)
	37.5	5	2 (40)
	50.0	5	5 (100)
Female	25.0	5	1 (20)
	37.5	5	1 (20)
	50.0	5	4 (80)

Clinical signs: Reduced spontaneous activity, irregular breathing, narrowed palpebral fissures, exophtalmos, straddling of the hind limbs and tonoclonic spasms during the first 6 hours after treatment. The clinical signs already emerged shortly after administration of the test substance. One day after treatment, most of these signs subsided and afterwards only swollen abdomen, particularly in the males, and squatting position in individual cases were detected in the surviving animals. There were no disturbances of bodyweight gains.

Gross pathology: Macroscopic examination of the males and females found dead during the study revealed dark discolouration of the liver. The animals killed at the end of the observation period showed no macroscopically visible changes.

Conclusions

The acute oral median lethal dose (LD_{50}) of Thiodan was calculated to be 39 mg/kg for the male and female mouse. According to the EU Criteria, Thiodan should be classified with the symbol T (toxic) and the risk expression R25.

B.6.11.1.3a Oral study in rabbit

Ebert, E. 1990a (AgrEvo: IIIA, 7.1.1)

Dates of experimental work: January-February 1990.

The study was performed according to internal method in compliance with the OECD and EPA Guidelines.

GLP: yes

The study is acceptable.

Materials and methods

A group of 30 (15 males and 15 females) New Zealand albino rabits, source HOECHST AG, Kastengrund, weighing between 2370-3195 g the males and 2050-3360 g the females were used for the determination of acute oral toxicity of material test Hoe 002671 00 EC33 B317 (Thiodan). After an acclimatization period of five days, the animals were divided into 3 groups, each consisting of 5 males and 5 females. The dose levels tested were 25, 50 and 80 mg/kg bodyweight. The test material was emulsified in desionised water to permit the administration at a constant volume of 5 ml/kg and was administered by gavage to fasted animals at the stated dose levels. The animals were housed individually in a room under standardised conditions (temperature 20 ± 3 ºC, relative humidity: 50 ± 20%; lighting time: 12 hours daily). The animals had free access to drinking water and food (Altromin 2123 maintenance diet-rabbit) except 1 hour before and 1 hour after treatment. After dosing, the animals were kept under observation for 15 days. During the 15-day observation period the animals were weighed weekly. At the end of the observation period the surviving animals were killed by injection, dissected and also examined for macroscopically visible changes. Lethaly intoxicated animals were also dissected and examined macroscopically.

Findings

Two females at 25 mg/kg, four animals (3 females and one male) at 50 mg/kg and eight rabbits (5 females and 3 males) at 80 mg/kg died as noted in Table 6.11.1.3-1.

Table 6.11.1.3a-1: Mortalities

Sex	Dose Level (mg/kg)	Nº of animals	Mortalities (%)
Male	25	5	0 (0
	50	5	1(20)
	80	5	3 (60)
Female	25	5	2 (40)
	50	5	3 (60)
	80	5	5 (100)

Clinical signs: Periodic generalized convulsions, biting convulsions and salivation. They began to emerge shortly after application and persisted in some cases up to day 6 of treatment. In addition, increased respiratory rate, hyperactivity and reddish nasal discharge were observed. After few days paresis of hind limbs occurred in a few animals from the middle dose group; in the case of two animals this had not completely receded by the end of the observation period. One male from the 50 mg group had to be sacrificed on day 10 of the study *in extremis* due to severe intoxication. Mortalities occurred at all dose levels, except for the males in the lowest dose group. The bodyweights were lowered during the first week at all dose levels. During the 2nd week, bodyweights began to increase again, and in most cases the animals had nearly regained their initial bodyweights by the end of the study.

Gross pathology: Macroscopic examination of the males and females found dead during the study revealed distinct injection of mesenteric blood vessels. The animals killed at the end of the observation period showed no macroscopically visible changes.

Conclusions

The acute oral LD_{50} of Thiodan in the male rabbit was determined to be 75 mg/kg. In the female rabbit, the oral LD_{50} was determined to be 34 mg/kg. In the sexes combined the oral LD_{50} was determined to be 50 mg/kg.

According to the EU Criteria, Thiodan should be classified with the symbol T (toxic) and the risk expression R25.

B.6.11.2a Percutaneous studies

B.6.11.2.1a Percutaneous study in rats

Ebert,E. 1989c (AgrEvo: IIIA, 7.1.2)

Dates of experimental work: August-September 1989.

The study was performed to a method according to EPA Guidelines (81-2) and OECD nº 402.

GLP: yes

The study is acceptable.

Materials and methods

A group of 30 (15 males and 15 females) Wistar rats, source Hoechst AG, Kastengrund, weighing between 222-293 g the males and 200-241 g the females and 3 dose levels were used for the determination of acute dermal toxicity of material test Hoe 002671 00 EC 33 B317 (Thiodan) After an acclimatization period of 5 days, the animals were divided into 3 groups, each consisting of 5 males and 5 females. The males received 400, 630 and 1250 mg/kg bodyweight and the females 100, 250 and 400 mg/kg bodyweight. The test material was administered undiluted. Before dermal treatment the hair was removed from the dorsal skin of the animals . The material test was applied once as evenly as possible to the shaved and intact dorsal skin. The treated skin area was covered with an aluminium foil, which was held in place with an elastic plaster bandage fixed around the animal's body. After a 24-hour exposure period, the bandage was removed and the treated skin area washed with water in order to remove the test substance.

The animals were housed individually in Makrolon cages under standardised conditions (temperature: 22 ± 3ºC; relative humidity. 50 ± 20%; lighting time: 12 hours daily). The animals had free access to drinking water and food (Altromin 1324 rat diet). After application, the animals were kept under observation for 14 days. Bodyweights were recorded weekly . At the end of the observation period the animals which did not die following treatment were killled by carbon dioxide asphyxiation and examined for macroscopically visible changes.

Findings

In the females, no deaths occurred in the low dose level group. In the middle dose level group, 2 out of 5 animals died and all animals in the high dose level group. In the male test groups, 2 out of 5 animals from the low dose level group and 4 out of 5 animals from each the middle and high dose level groups died. All results are showed in Table 6.11.2.1a-1.

Table 6.11.2.1a-1: Mortalities

Sex	Dose level (mg/kg)	Nº of animals	Mortalities (%)
Females	100	5	0 (0)
	250	5	2 (40)
	400	5	5 (100)
Males	400	5	2 (40)
	630	5	4 (80)
	1250	5	4 (80)

Clinical signs: Straddling of hind legs, irregular respiration, bizarre movements, stilted gait, ataxic gait, uncoordinated gait, twitching, dilated pupil, decreased spontaneous activity, increased startle reflex, diarrhea, straub tail, red coloured salivation, increased sound production, snout blood-coloured incrusted, respiratory sounds, aggressiveness, clonic spasms, tonic spasms, trembling, increased respiratory rate, motor hyperactivity, increased spontaneous activity, increased salivation, hypersensitivity to touch, contraction of flanks, squatting position. The majority of the clinical signs of systemic toxicity occurred during the first 3 days of the study. The test substance proved to be irritating to the treated skin area, in particular during the first 4 days of the study The following dermal findings emerged during the observation period: Erythema, the skin was dry and rough, chapped, incrusted, red, covered with scab, with fine and coarse scales and peeling of fine scales. There were no disturbances of bodyweight gains.

Gross pathology: Macroscopic examination of the males and females found dead during the study revealed the following abnormalities: intestinal tract filled with gas and reddish mucus, liver and spleen discoloured dark, congestion of lungs, small intestine filled with reddish mucus and the kidneys showed patches. In some cases, macroscopic examination of the animals killed at the end of the observation period revealed the following abnormalities: mesenteric vessels of the large intestine distinctly injected, congestion and discolouration of lungs, the spleen was discoloured light and the spleen surface uneven.

Conclusions

The acute dermal median lethal dose (LD_{50}) of Thiodan for the male rat was determined to be 412 mg/kg. For the female rat, the LD_{50} was approximately 266 mg/kg.

According to the EU Criteria Thiodan should be classified with the symbol T (Toxic) and the risk expression R24.

B.6.11.2.2a Percutaneous study in rabbits

Ebert, E. 1990b (AgrEvo: IIIA, 7.1.2)

Dates of experimental work: January 1990.

The study was performed according to the OECD Guidelines for Testing of Chemicals Products nº 402 and EPA Guideline 81-2.

GLP: yes

The study is acceptable.

Materials and methods

Five males and five females New Zealand albino rabbit, source Hoechst AG, Kastengrund, weighing between 2303-2879 g the males and 2552-3034 g the females, received a single topical dose of 400 mg/kg of test substance Hoe 002671 00 EC33 B317 (Thiodan). Before dermal treatment the hair was removed from the dorsal skin of the animals. The undiluted test substance was applied once as evenly as possible to the shaved and intact dorsal skin. The treated skin area was covered with an aluminium foil, which was held in place with an elastic bandage fixed around the animal's body. After a dermal exposure period of 24 hours the bandage was removed and the treated skin area washed with warm water.

The animals were housed individually under standardised conditions (temperature: 20 ± 3ºC; relative humidity: 50 ± 20%; lighting time: 12 hours per day). They had free access to drinking water and food (Altromin 2123 maintenance diet – rabbits).

After application, the animals were weighed weekly and kept under observation for 15 days. And the end of the observation period the surviving animals were killed and examined for macroscopically visible changes.

Findings

Two males and one females died, as noted in Table 6.11.2.2a-1.

Table 6.11.2.2a-1: Mortalities

Sex	Dose Level (mg/kg)	Nº of animals	Mortalities (%)
Male	400	5	2 (40)
Female	400	5	1 (20)

Clinical signs: Periodic generalized convulsions, biting convulsions and salivation. The symptoms started to emerge shortly after application of the test substance. In addition, increased respiratory rate, hyperactivity, bizarre circular movements and nasal red-coloured discharge were observed. After a few

days paresis of the hindlimbs occurred in a few animals; in one male and one female this had not completely receded by the end of the observation period.

The test substance, Thiodan caused severe dermal irritation on the application site. The treated skin showed slight to severe erytheam and slight to moderate oedema. The skin surface was dry, rough, covered with fine and coarse scales, indurated, lumpy and showed desquamation of fine and coarse scales. The skin was also encrusted, chapped and scabbed. The new skin was pink coloured. The body weight was lowered during the first week, but had returned to normal by the end of the study.

Gross pathology: Macroscopic examination of the males and females found dead during the study revealed general autolysis and light-coloured livers. The animals killed at the end of the observation period were free of macroscopically visible changes.

Conclusions

The acute dermal median lethal dose (LD_{50}) of Thiodan was greater than 400 mg/kg.

According to the EU Criteria, Thiodan should be classified with the symbol Xn (harmful) and the risk expression R21.

B.6.11.3a Inhalation

Hollander, H. 1984 (AgrEvo: IIIA, 7.1.3)

Dates of experimental work: February-July 1984.

The study was performed according to US EPA Pesticide Assessment Guidelines, Subdivision F,section 81, 81-3.

GLP: yes.

The study is acceptable.

Materials and methods

35 males and 30 females SPF Wistar rats, source Hoechst AG, Kastengrund, weighing between 168-195 g the males and 171-200 g the females received a four-hour nose only exposure to atmosphere containing aerosol of Endosulfan-emulsifiable concentrate (500 g/l) (code: Hoe 002671 0I EC 43 A103). The concentration levels are: 0.010 (5 females), 0.067 (5 females), 0.083 (5 males and 5 females), 0.132 (5 males and 5 females), 0.153(5 males and 5 females), 0.164 (5 males and 5 females), 0.194 (5 males), 0.347 (5 males) and 1.042 (5 males) mg/l of endosulfan emulsifiable concentrate (500 g/l). More than 70% of the particles were smaller than 1.5 µm, while particles sizes larger than 5 µm amounted in all cases to less than 7% of the total aerosol exposure. The exposure chamber itself consisted of a stainless steel and glass cylinder with a volume of 60 l, standing in a vent pipe of approx.

4 m^3. Air was pressed into the nozzle at 4 bar through a special nozzle with welded tube for adding the preparation by a compressor via oil separation and air filters. Air supply was maintained constant at 800 l/hour. The test material was injected into the nozzle by a permanent infusion apparatus at a constant rate. A suction device at the bottom of the inhalation chamber drew off the aerosol at a rate of 1100 l/hour through a cotton-wool filter and 10% sodium hydroxide solution. The difference in rate between the introduction of 800 l air per hour through the nozzle and the extraction of 1100 l air per hour ensured slight underpressure in the exposure chamber.

The animals were housed in fully air-conditioned rooms in Makrolon cages, in groups of 5 animals/sex (temperature: 22 ± 2ºC; relative humidity: 50 ± 20%, lighting time: 12 hours daily). They had free acces to drinking water and food (Altromin 1324 rat diet). After exposure, the animals were kept under observation for a further 14 days, weighed on days 2, 3, 4, 7 and 14 post inhalation. Lethally intoxicated animals were dissected and examined macroscopically. The surviving test animals were killed by CO_2 gas, dissected and also examined macroscopically.

Findings

The mortality rates showed in Table 6.11.3a-1.

Table 6.11.3a-1: Mortalities

Exposure Level (mg Hoe 002671-emulsifiable concentrate/l air)	**Mortality**		
	Males	**Females**	**Total**
1.042	5/5	-	5/5
0.347	2/5	-	2/5
0.194	2/5	-	2/5
0.164	2/5	5/5	7/10
0.153	1/5	3/5	4/10
0.132	2/5	4/5	6/10
0.083	0/5	4/5	4/10
0.067	-	3/5	3/5
0.010	-	0/5	0/5

Clinical signs: Irregular respiration, red crusted nose, blood-coloured lacrimation, trembling, tonoclonic spasms, mydriasis, squatting position, ataxic gait, prone position and reduced reaction of examined reflexes (corneal, placing reflexes and paw-reflex to pinching).

The body weight gains were not reduced.

Gross Pathology: Pinhead-sized dark red foci were found on the lungs of males and females in individual cases. The animals killed at the end of the observation period showed no macroscopically visible changes.

Conclusions

The acute inhalation median lethal concentration (LC_{50}) of Endosulfan-emulsifiable concentrate (500 g/l) in the male rat was determined to be 0.263 mg/l. In the female rat, the inhalation LC_{50} was determined to be 0.0594 mg/l. According to the EU Criteria, Endosulfan-emulsifiable concentrate (500 g/l) should be classified with the symbol T+ (very toxic) and the risk expression R26.

B.6.11.4a Skin irritation in rabbits

Ebert, E. 1989d (AgrEvo: IIIA, 7.1.4)

Dates of experimental work: August-September 1989.

The study was performed according to OECD Guidelines for Testing of Chemicals nº 404 and EPA Guidelines (81-5).

GLP: yes

The study is acceptable.

Materials and methods

A group of six (3 males and 3 females) New Zealand albino rabbits, origin Hoechst AG, Kastengrund, weighing 3.0-3.9 kg , received a single topical dose of 0.5 ml of test material Hoe 002671 00 EC33 B317 (Thiodan). The material test was applied directly to the skin using a piece of surgical plaster with a 2.5 x 2.5 cm cellulose patch and was fixed on to the shaved skin area of each animal. The areas was then covered with a semi-occlusive bandage. The exposure period was 4 hours. After the exposure period all remnants of the test substance were carefully removed from the skin with water. The animals were individually housed under standardised conditions (temperature: 20 ± 3ºC; relative humidity: 50 ± 20%; lighting time: 12 hours per day). They had free access to drinking water and food (Altromin 2123 maintenance diet- rabbits). After application, the animals were kept under observation for 72 hours. Additional observations of persistent effects were made 7, 14 and 21 days after treatment.

Findings

Clinical signs: 30-60 minutes after removal of the plaster, very slight to well-defined erythema was observed in all animals. In addition, five animals showed very slight oedema which was completely reversible after 7 days. After 24 to 48 hours, all animals exhibited well-defined erythema, 72 hours after application, the signs of irritation had increased in 3 animals, 7 days after treatment, two animals only showed well-defined to moderate erythema. Very slight erythema was observed in four animals. 14 days after application, very slight erythema was observed in 3 animals, one animal showed well-defined erythema. 21 days after application, well-defined erythema was observed in 3 animals.

Very slight oedema was observed up to 7 days after treatment.

All animals were fre of oedema after 14 days and of erythema after 28 days. The means of erythema and oedema values recorded 24, 48 and 72 hours after treatment were 2.2 and 0.4 respectively.

In addition to the clinical signs, the surface of the treated skin was dry and rough, with fine and coarse scales, and showed peeling of fine and coarse scales. The treated skin surface was also chapped and discoloured light brown.

No clinical signs of systemic toxicity were observed.

Conclusions

The test material Thiodan was considered to be irritant to rabbit skin. According to the EU Criteria, Thiodan should be classified with the symbol Xi (irritant) and the risk expression R38.

B.6.11.5 Eye irritation in rabbits

Ebert, E. 1989e (AgrEvo: IIIA, 7.1.5)

Dates of experimental work: August-September 1989.

The study was performed according to OECD Guidelines for Testing of Chemicals nº 405 and EPA Guidelines (81-4).

GLP: yes.

The study is acceptable.

Materials and methods

A group of 6 females New Zealand albino rabbit, origin Hoechst AG, Kastengrund, received a single instillation of 0.1 ml of the test material Hoe 002671 00 EC33 B317 (Thiodan) into the conjunctival sac of the left eye. The right eye remained untreated and served as a control. The treated eyes of the animals were left unwashed for 24 hours. If there were signs of irritation 72 hours after treatment, the test substance was applied to the conjunctival sac of the left eye of 3 other animals, which were left unwashed for only 2 minutes. The animals were housed individually under standardised conditions (temperature: 20 ± 3ºC; relative humidity: 50 ± 20%; lighting time: 12 hours per day). They had free access to drinking water and food (Altromin 2123 maintenance diet-rabbits). The eyes were examined 1, 24, 48 and 72 hours after application of the test substance. The study was terminated 21 days post-treatment.

Findings

Clinical signs: Among the animals whose eyes were washed out after 24 hours, a moderate clear colourless discharge, slight to moderate chemosis and slight to diffuse crimson redness of the

conjuctiva were observed in all animals 1 hour after treatment. In three animals, the iris showed hyperaemia, but reaction to light was still possible.

24 hours after treatment, chemosis was generally moderate. All animals showed moderate to severe, white-yellowish and viscous discharge and diffuse crimson to diffuse beefy red colouring of the treated eye. Inflammation of the iris and corneal opacity with clearly visible iris were observed in the treated eyes of nearly all animals involving more than three quarters of the eye.

48 to 72 hours after treatment, the animals showed almost the same signs of irritation as 24 hours after treatment except for corneal opacity, i.e. in most cases the cornea showed easily discernible translucent areas and details of the iris were slightly obscured.

7 days after treatment, the signs of irritation in 4 of the animals all remained the same as before. One animal had only a diffuse crimson coloured redness of the treated eye and one animal showed no signs of irritation.

14 days after treatment 3 animals still showed signs of irritation. In one animal, the cornea showed nacrous area of opacity, no details of the iris were visible and the size of pupil was barely discernible. Two animals were free of signs of irritation.

21 days after treatment 3 animals still showed signs of irritation nearly the same as those 14 days after treatment.

Details of reactions scores are given in Table 6.11.5a-1.

Table 6.11.5a-1: Individual Animal Irritation Grades

Observation Time	Animal Nº/Sex	Conjunctiva Redness	Conjunctiva Chemosis	Corneal Opacity	Iritis
One hour	41/F	2	1	0	1
	55/F	1	2	0	0
	57/F	2	2	0	1
	85/F	1	2	0	1
	86/F	1	3	0	0
	87/F	1	3	0	0
24 hours	41/F	3	2	1	1
	55/F	3	1	1	1
	57/F	3	3	1	1
	85/F	2	2	1	1
	86/F	2	2	1	1
	87/F	2	2	2	1
48 hours	41/F	3	2	2	1
	55/F	3	2	2	1
	57/F	3	1	2	1
	85/F	3	2	2	1
	86/F	3	3	1	1
	87/F	3	2	2	1
72 hours	41/F	3	2	2	1
	55/F	3	1	2	1
	57/F	2	1	1	1
	85/F	3	2	2	1
	86/F	3	3	1	1
	87/F	3	2	2	1
7 days	41/F	3	1	2	1
	55/F	2	1	2	1
	57/F	2	0	0	0
	85/F	0	0	0	0
	86/F	2	2	2	0
	87/F	2	2	3	1
14 days	41/F	2	2	2	1
	55/F	0	0	2	0
	57/F	0	0	0	0
	85/F	0	0	0	0
	86/F	3	2	2	1
	87/F	1	1	3	1
21 days	41/F	2	1	2	1
	55/F	0	0	0	0
	57/F	0	0	0	0
	85/F	0	0	0	0
	86/F	2	2	2	1
	87/F	1	0	2	1

Mean Ocular Irritation Scores (24, 48 and 72 hours)

Corneal Opacity: 1.55

Iris Lesion: 1.00

Conjunctival Redness: 2.77

Conjunctival Chemosis: 1.94

Conclusions

According to the EU Criteria, Thiodan should be classified as irritant (Xi)with the risk expression R41 (Risk of serious damage to the eyes).

B.6.11.6a Skin sensitization

Ullmann, L. 1986 (AgrEvo: IIIA, 7.1.6)

Dates of experimental work: August-October 1986.

The study was performed according to US EPA Pesticide Assessment Guidelines Subdivision F, 81-6 and OECD Guidelines for Testing of Chemicals nº 406.

GLP: yes

The study is acceptable with some reservations: 2 animals (10%) in the treated group died during the study.

Materials and methods

The potential of Hoe 002671 0I EC33 B310 (Thiodan) to cause delayed contact hypersensitivity in guinea-pig was assessed by the Buehler method.

40 (20 as test and 20 as control) male and female Himalayan white spotted guinea pig, source Institut of Biomedical Research, Switzerland, weighing 412-513 g the males and 380-525 g the females were used for the pre-test and the main study. The study consisted of an induction phase followed by a challenge phase. The study design included a pre-test which imposed limits on the concentration of test material used during the main study. Based on the results of pre-test, a dosage of 100% (undiluted) was selected for the main study. One flank of each of 10 test and control animals were shaved and an adhesive patch containing 0.5 ml of the test article was applied to the skin. The animals were patched for six hours, three times a week for three consecutive weeks (totally 9 applications/animal). No patches were applied during the ensuing two weeks. At the beginning of the fifth week, the untreated flanks of each animal were depilated and 24 hours later identical challenge patches were applied for 6 hours, as used during induction period. Approximately 24 hours and 48 hours later, the treated skin sites were graded. A second challenge was applied 2 weeks after the first. The same procedure was used as described above.

The animals were housed individually in Makrolon cages, under standardised conditions (temperature: 22 ± 3ºC; relative humidity: 40-70%; lighting time: 12 hours per day; ventilation: 10-15 air changes per hour) and they had free access to drinking water and food: pelleted standard Nafag and guinea pig breeding/maintenance diet ("Nafag", Nafag AG, 9202 Gossau/SG, Switzerland).

Findings

Clinical signs: No positive reactions were observed in the animals of the test article treated group after the first and second challenge application, either after the 24-hour nor the 48-hour reading.

One male and one female in the test article treated group died spontaneously on days 15 and 18 of test respectively.

The following local findings were observed in the animals treated with the test article: discoloration (day 12, 13, 16, 18, 20); desiccation (day 12, 13, 16, 18, 20); scale formations (day 13, 16, 18, 20); exfoliation (day 16, 18, 20)

Starting with day 16 of test all animals showed a leather-like skin on the application site. This was observed until day 20 of test.

The bodyweight gain of all animals was not affected during the test procedure.

Conclusions

According to the EU Criteria, Thiodan should not be classified as skin sensitizer.

B.6.11b Acute toxicity including irritancy and skin sensitization of preparations

Applicant: Calliope, S.A.
Manufacturer: Calliope, S.A.
Trade name: Callistar

Callistar Endosulfan 35 EC has been thoroughly tested for acute toxicity (oral and dermal), primary irritation and sensitization potential. Results obtained in these studies are summarised in Table 6.11b-1. All studies were undertaken with a single lot (lot. 1 del 10.01.91), and were performed according procedures of the OECD (except skin sensitization which are performed according to an adaptation of Magnusson Kligman method) and in compliance with GLP.

The acute oral median lethal dose (LD_{50}) of Callistar is approximately 50 mg/kg for male and female rats (the mortality rates indicate that the LD_{50} will be situated between 30 and 80 mg/kg). According to the EU Criteria, Callistar should be classified with the symbol T (toxic) and the risk expression R25.

The acute dermal median lethal dose (LD_{50}) of Callistar for female rats alone is situated below 2000 mg/kg. Therefore, because 60% mortality occurred in the female group, a complete study should be performed.

Material test, Callistar, was considered to be irritant and corrosive in rabbits. According to the EU Criteria, Callistar should be classified with the symbol C (corrosive) and the risk expression R34 and with the symbol Xi (irritant) and the risk expression R38.

The acute eye irritation/corrosion test with Callistar in rabbits were irritant and due of duration of effects and according to the EU Criteria, Callistar must be considered as causing irreversible eye damage.

A skin sensitization study in guinea pig using a modified version of Magnusson Kligman method demostrated that Callistar is not considered to be a skin sensitizer.

Table 6.11b-1

Species/strain	Sex	Route/Method	Result	Reference
Rat/S-D	Both	Oral	LD_{50} approx. = 50 mg/kg	Halaviat. 1991
Rat/Wistar	Both	Dermal	LD_{50}(male)>2000mg/kg LD_{50}(female)<2000mg/kg	Pinon 1991
		Inhalation	Test not conducted	
Rabbit/NZW	n.a.	Dermal	Irritant and corrosive to skin	Halaviat 1991
Rabbit/NZW	n.a.	Eye	Causing irreversible eye damage	Halaviat 1991
Albino Guinea pig/Hartley	Both	Sensitization (modified Magnusson /Kligman)	Not Sensitizing	Pinon 1991

n.a: not available.

B.6.11.1b Oral study in rats

Halaviat, B. 1991a (Calliope: IIIA, 7.1.1/01)

The study was performed according to OECD Guidelines nº 401.

GLP: yes

The study is acceptable.

Materials and methods

A group of 40 (20 males and 20 females) OFA Sprague-Dawley rats, source IFFA Credo (69210 L'Arbresle), weighing between 172.5-195.6 g the males and 162.2-180.0 the females were used for the determination of acute oral toxicity of material test Callistar 350 g/l d'Endosulfan Lot 1.(Callistar). After an acclimatization period of 6 days, the animals were divided into 4 groups of 10 animals (5 males and 5 females). The dose levels are: 20, 30, 50 and 80 mg/kg. Each animals received by force feeding a single dose of the test product freshly prepared in distilled water at a constant volume of 10 ml/kg. The dose levels for this test were selected according to a preliminary study that consisted of a limit test (dosage at 2000 mg/kg), in which all animals (2/sex) died within 30 minutes following dosing, and pre-test to establish the dose levels for the main study.

The animals were housed by sex in makrolon cages (5/sex) and under standardised conditions (temperature: 21,5ºC ± 1,5ºC; relative humidity: 67% ± 5%; air changes per hour: 14; lighting time: 12 hours per day). They had free access to drinking water and food (UAR A 04 C) except for a period of 16 hours prior to dosing and for 4 hours post-dosing.

After dosing the animals were kept under observation for 14 days. Bodyweights were recorded at dosing, and on days 4, 7 and 14. On day 14 the rats were sacrificed by exanguination. All animals, including those that died during the study, were subjected to a gross necropsy.

Findings

7 animals (2 males and 5 females) at 80 mg/kg, 5 animals (1 male and 4 females) at 50 mg/kg and 4 females at 30 mg/kg died, as noted in Table 6.11.1b-1.

Table 6.11.1b-1: Mortalities

Dose Level (mg/kg)	Mortalities (males)	Mortalities (females)	%Mortalities (males)	%Mortalities (females)	%Mortalities Combined
20	0/5	0/5	-	-	-
30	0/5	4/5	-	80	40
50	1/5	4/5	20	80	50
80	2/5	5/5	40	100	70

Clinical signs: Loss of coordination, reduced motor activity, clonic convulsions, piloerection, tremors, and loss of righting reflex were observed with a 30mg/kg dose and above. A decreased growth rate was noted in the survivors (males only) of the highest dose group for approximately 4 days.

Gross necropsy: At necropsy pulmonary congestion was observed in all decedents.

Conclusions

No statistical method was used for the determination of the LD_{50}, however, the mortality rates indicate that the LD_{50} will be situated between 30 and 80 mg/kg. According to the EU Criteria, Callistar should be classified with the symbol T (toxic) and the risk expression R25.

B.6.11.2b Percutaneous study in rats

Pinon, J.-F. 1991a (Calliope: IIIA, 7.1.2/01)

Dates of experimental work: January-February 1991.

The study was performed according OECD Guidelines (nº 402).

GLP: yes

The study is acceptable.

Materials and methods

Five male and five female OFA Sprague-Dawley strain rats, source IFFA Credo (69210 L'Arbresle), weighing between 185.1-207.8 g the males and 177.1-196.1 g the females received a single topical dose of 2000 mg/kg of the test substance Callistar 350 g/l d'Endosulfan (Callistar). After an acclimatization period of 6 days and on the day before dosing, the dorsum of the animals was clipped free of hair. ´24 hours later, the test material was applied to the skin (approximately 10% of the body surface area). The treated skin was covered by a semi-occlusive bandage. The dressings were removed 24 hours after

administration. During the 24 h exposure period, the animals were housed individually and after a 24 hour exposure period the animals were housed 5/cage.

Five animals of the same sex were housed in stainless steel cages, under standardised conditions (temperature: 21ºC ± 1ºC; relative humidity: 65,5 ± 4,5%; 12 h light/dark cycle, with 14 air changes per hour). They had free access to drinking water and food (UAR A 04 C). After dosing, the animals were kept under observation for 15 days. Bodyweights were recorded three days (-3) before dosing, on day of dosing (0), and on days 4, 7 and 14 after dosing. At the end of the study, the animals were killed by exanguination. All animals were subjected to a gross necropsy.

Findings

Three females died as showed in Table 6.11.2b-1.

Table 6.11.2b-1: Mortalities

Sex	Dose Level (mg/kg)	Nº of animals	Mortalities (%)
Male	2000	5	0 (0)
Female	2000	5	3 (60)

Clinical signs: Slight erythema in 3 animals (sex not specified). Bodyweights in 2 female survivors is decreased as compared to normal values.

Gross pathology: Due to advanced autolysis it was not possible to determine the death cause for the 3 female decedentes.

Conclusions

The acute dermal median lethal dose of material test Callistar for female alone is situated below 2000 mg/kg. Therefore, because 60% mortality occurred in the female group, a complete study should be performed.

B.6.11.3b Inhalation

Test no conducted.

B.6.11.4b Skin irritation in rabbits

Halaviat, B. 1991b (Calliope: IIIA, 7.1.4/01)

Dates of experimental work: January-February 1991

The study was performed according to OECD Guidelines (nº 404).

GLP: yes

The study is acceptable.

Materials and methods

A New Zealand White albino rabbit, source L'élévage Feuilletas, 40- Campet Lamolere, France, received a single topical dose of 0.5 ml of material test Callistar (350 g/l d'Endosulfan). The test material was applied to intact skin on a 2.5 x 2.5 cm^2 patch of hydrophylic gauze of the right flanc. The left flanc was treated the same way using distilled water and served as a control. The pad with test substance or water was kept in place for 4 hours using a semi-occlusive dressing, after which period the dressing was removed. The treated skin was not rinsed. The rabbit was housed in stainless steel cage, under standardised conditions (temperature: 21ºC ± 1ºC; relative humidity: 52 % ± 4%; 12 h light/dark cycle, with 14 air changes per hour). It has free access to drinking water and food (UAR 112). The rabbit was observed at 1, 24, 48 and 72 hours and on every subsequent day up to a total of 10 days.

Findings

Clinical signs: Along with the observed erythema and oedema formation, skin necrosis was observed. The lesions developed into a scar and a crust and new epithelium was formed. Detail of reaction scores are given in Table 6.11.4b-1.

Table 6.11.4b-1

Rabbit Nº	Erythema											Oedema										
	1 h	24 h	48 h	72 h	96 h	5 d	6 d	7 d	8 d	9 d	10 d	1 h	24 h	48 h	72 h	96 h	5 d	6 d	7 d	8 d	9 d	10 d
2724A	2	3	3	2	2	2	*	*	*	*	0	2	2	2	2	1	1	0	0	0	0	0

*= Due to crust formation no grading of erythema was possible.

Conclusions

According to the EU Criteria, Callistar should be classified with the symbol C (corrosive) and the risk expression R34 and with the symbol Xi (irritant) and the risk expression R38.

B.6.11.5b Eye irritation in rabbits

Halaviat, B. 1991c (Calliope: IIIA, 7.1.5/01)

Dates of experimental work: January-February 1991.

The study was performed according OECD Guidelines (nº 405).

GLP: yes

The study is acceptable.

Materials and methods

A New Zealand White albino rabbit, source L'élévage Feuilletas, 40-Campet Lamolere, France, received a single instillation of 0.1 ml of material test Callistar (350 g/l d'Endosulfan) into the conjunctival sac of the right eye. The left eye remained untreated and served as control. The eyelids were kept closed for a few seconds and the eye was not rinsed. The animal was kept restrained for one hour when the first examination was done. Then it was returned to its cage. The rabbit was housed in stainless steel cage, under standardised conditions (temperature: 21ºC ± 1ºC; relative humidity: 52 % ± 4%; 12 h light/dark cycle, with 14 air changes per hour). It has free access to drinking water and food (UAR 112). The rabbit was observed at 1, 24, 48 and 72 hours. To determine the reversibility of eye reactions, the animal was observed also on days 4, 7-11, 14-18 and on day 21. The study was terminated 21 days post-treatment.

Findings

Clinical signs: Corneal opacity was observed up to day 21. Effects on the iris were seen after one hour, had disappeared at 24 hours, but reappeared at day 7 to persist until the end of the observation period. Conjunctival effects included a persisting redness and chemosis, and, on occasions, lacrimation. Detail of reaction scores are given in Table 6.11.5b-1.

Table 6.11.5b-1

Rabbit Nº	Observation Time	Conjunctiva		Corneal Opacity	Iritis
		Redness	Chemosis		
	1 hour	1	1	2	1
	24 hours	1	1	2	0
	48 hours	1	1	2	0
	72 hours	1	1	2	0
	4 days	1	1	2	0
	7 days	1	1	2	1
	8 days	1	1	2	1
2721 A	9 days	1	1	2	1
	10 days	1	1	2	1
	11 days	1	1	2	1
	14 days	1	1	2	1
	15 days	1	1	2	1
	16 days	1	1	2	1
	17 days	1	1	2	1
	18 days	1	1	2	1
	21 days	1	1	2	1

Conclusions

According to the EU Criteria, Callistar must be considered as causing irreversible eye damage.

B.6.11.6b Skin sensitization

Pinon, J.-F. 1991b (Calliope: IIIA, 7.1.6/01)

Dates of experimental work: April-June 1991.

The study was performed according to a modified method of the Magnusson and Kligman.

GLP: yes

The study is acceptable with some reservations:

- The highest non-irritant dose for cutaneous applications has not been used (the test material is not irritant at a concentration of 20%).
- The challenge phase must be start on day 20 instead on day 32.
- When the application is topic the patches must be occlusive.
- In the induction phase there is not information regarding if animal's fur was eliminated or not.

Materials and methods

30 (20 as test and 10 as control) male and female Albino Guinea pigs (Hartley), source Gaillard (22700 Pleudaniel France) were used in the main study. Three animals were housed into makrolon cages according to sex under standardised conditions (temperature: 22ºC ± 3ºC; relative humidity: 70% ± 12; 12 h light/dark cycle; 14 air changes per hour). Free access to drinking water and food (UAR 106) was allowed throughout the study.

The animals were sensitized with complete Freund's adjuvant. The test material (Callistar) was injected intradermally and further topically applied. The study consisted of an induction phase followed by a challenge phase.

- **INDUCTION PHASE**

Dose Ranging for Induction

A preliminary dose ranging test was carried out to assess suitable concentrations for injections and topical application. Based on the results of this test, concentrations of 5% Callistar in distilled water were selected for the injection and topical phases of the main study.

Injection phase-Main Test

The test group guinea pig were each given 6 intradermal injection as follows:

0.1 ml Freund's Complete Adjuvant

0.1 ml test material

0.1 ml of a 50:50 of test material in Freund's Complete Adjuvant.

The test material was injected at a concentration of 5% w/w in saline solution, Freund's complete adjuvant was injected at 50%. The 10 control guinea pigs were similarly treated, but with the vehicle, saline solution, replacing the test material.

Topical Induction Phase-Main Test

Ten days after the injection phase, the animals test and control were dosed with 10% solution of sodium lauryl sulphate (1 ml). After 24 h, a patch charged with 0.5 ml of the test material at a concentration of 5% was applied to the pretreated area of each of the test group animals. The patches were left in place for 48 h before removal. The control group guinea pigs were similarly treated, but with the vehicle, saline solution, replacing the test material.

- **CHALLENGE PHASE**

Day 32: Both, the test and control group guinea pigs were challenged with the test material, Callistar, at a concentration of 5% and 2.5% by cutaneous application on the shaved posterior part of the back on either side of the vertebral column. The exposure time was 24 hours under semi-occlusive dressing.

Findings

No abnormal clinical findings were reported. No signs of erythema or oedema were seen during the challenge phase. One animal (control group) died during the resting period.

Conclusions

Callistar was not a sensitiser under the conditions of this study.

B.6.11c Acute toxicity including skin irritancy of preparations

Endosulfan 35% EC has been tested for acute toxicity (oral and dermal) and skin irritation. Results obtained in these studies are summarised in Table 6.11c-1. All studies were undertaken with a single batch of formulation (F94/-/113) and were performed according procedures of the OECD and EC and in compliance with GLP.

The acute oral median lethal dose (LD_{50}) of Endosulfan 35% EC in rats was 69 mg/kg for the sexes combined. Estimated oral LD_{50} values for the males alone were 96 mg/kg and for females alone 28 mg/kg. According to the EU Criteria, Endosulfan 35% EC should be classified with the symbol T (toxic) and the risk expression R25.

The acute dermal median lethal dose (LD_{50}) of Endosulfan 35% EC in rats was 1006 mg/kg for the sexes combined. Estimated dermal LD_{50} values for the males were 1450 mg/kg and for females 449

mg/kg. According to the EU Criteria, Endosulfan 35% EC should be classified with the symbol Xn (harmful) and the risk expression R21.

Material test (Endosulfan 35% EC) was considered to be irritant and corrosive to rabbit skin. According to the EU Criteria, Endosulfan 35% EC should be classified with the symbol C (corrosive) and the risk expression R34 and with the symbol Xi (irritant) and the risk expression R38.

In conclusion, Endosulfan 35% EC might be considered toxic by oral route, harmful by dermal route and irritant and corrosive to rabbit skin.

Table 6.11c-1

Species/strain	Sex	Route/Method	Result	Reference
Rat/Wistar	Both	Oral	LD_{50} combined = 69mg/kg LD_{50} approx. (male)= 96 mg/kg LD_{50} approx.(female)=28 mg/kg	Pels Rijcken 1994
Rat/Wistar	Both	Dermal	LD_{50} combined = 1006mg/kg LD_{50} approx. (male)=1450 mg/kg LD_{50} approx.(female)=449mg/kg	Pels Rijcken 1994
		Inhalation	Test not conducted	
Rabbit/NZW	Male	Dermal	Irritant and corrosive to skin	Pels Rijcken 1994
		Eye	Test not conducted	
		Sensitization	Test not conducted	

B.6.11.1c Oral study in rats

Pels Rijcken, W.R. 1994b (Excel: IIIA, 7.1.2/01)
Dates of experimental work: October 1994.

The study was performed according to OECD Guideline 401 and the EEC Directive 92/69 Part B; B.1.

GLP: yes

The study is acceptable with some reservations. The test material must be prepared at the appropriate concentration to permit administration at a constant volume. In this study the test substance was dosed at 0.278, 1.852 and 10 ml/kg and in two groups (300, 2000 mg/kg),the test substance was dosed undiluted and in two groups (25, 85 mg/kg) the material test was prepared using distilled water.

Materials and methods
A group of 35 (20 males and 15 females) Wistar rats, source BRL Ltd., Basel, Switzerland, weighing between 204-240g the males and 141-182 g the females were used in order to assess the toxicity of Endosulfan 35% EC (Batch F94/-/113) when administered to rats.

After an acclimatization period of 5 days, the females were divided into 3 groups of 5 animals and the males were divided into 4 groups of 5 animals. The dose levels used are: 25, 85, 300 and 2000 mg of the test material Endosulfan 35% EC per kg bodyweight for males and 25, 85 and 2000 mg of Endosulfan 35% EC per kg bodyweight for females. The test material was administered by single dose gavage. The test substance was dosed undiluted in dose groups of 300 (0.278 ml/kg) and 2000 mg/kg (1.852 ml/kg). Formulations (w/w) were prepared in dose groups 25 and 85 mg/kg using distilled water (10 ml/kg). The animals were housed 5 per sex in polycarbonate cages in an air-conditioned room with approximately 15 air changes per hour, temperature: 21ºC, relative humidity: 50% and 12 hours light/12 hours dark. The animals had free access to drinking water and food (Kliba 343, Klingentalmühle AG, Kaiseraugst, Switzerland) except overnight prior to dosing until approximately 3-4 hours after administration of the test substance. After dosing, the rats were kept under observation until day 15. Each animal was weighed the day of treatment and on days 8 and 15. All animals surviving to the end of the observation period were sacrificed by oxygen/carbon dioxide asphyxiation. All animals assigned to the study were subjected to necropsy.

Findings

One female at 25 mg/kg, one male and five females at 85 mg/kg, all males at 300 mg/kg and all males and females at 2000 mg/kg died as noted in Table 6.11.1c-1.

Table 6.11.1c-1: Mortalities

Dose Level (mg/kg)	Males	Females	Sexes Combined
25	0/5	1/5	1/10
85	1/5	5/5	6/10
300	5/5		
2000	5/5	5/5	10/10

Clinical signs: Clinical signs observed during the study period were as follows:

25 mg/kg: Lethargy, hunched posture, uncoordinated movements, piloerection, ptosis.
85 mg/kg: Tremors, lethargy, uncoordinated movements, piloerection (males only).
300 mg/kg: Convulsions.
2000 mg/kg: Convulsions, hunched posture.

The bodyweight gain shown by the surviving animals over the study period was considered to be similar to that expected of normal untreated animals.

Gross pathology: Macroscopic post mortem examination of the animals that died during the study revealed:

25 mg/kg: No abnormalities noted.
85 mg/kg: No abnormalities noted.
300 mg/kg: Pale colouration of the glandular mucosa in the stomach, dark red colouration of the thymus.

2000 mg/kg: Pale colouration of the glandular mucosa in the stomach, dilation of the renal pelvis.

Macroscopic post mortem examination of the surviving animals at termination did not reveal any abnormalities.

Conclusions

The acute oral median lethal dose (LD_{50}) of Endosulfan 35% EC in rats was 69 mg/kg bodyweight for the sexes combined. Due to the mortality distribution, only estimated oral LD_{50} values of Endosulfan 35% EC could be obtained for the males alone and females alone. These were 96 mg/kg for the males and 28 mg/kg for females. According to the EU Criteria, Endosulfan 35% EC should be classified with the symbol T (toxic) and the risk expression R25.

B.6.11.2c Percutaneous study in rats

Pels Rijcken, W.R. 1994

Dates of experimental work: October 1994.

The study was performed according to OECD Guideline 402 and EEC Directive 92/69, Part B; B.3.

GLP: yes

The study is acceptable.

Materials and methods

A group of 30 (15 males and 15 females) Wistar rats, source BRL ltd., Basel Switzerland, weighing between 215-257 g the males and 168-213 g the females and 3 dose levels were used in order to assess the toxicity of Endosulfan 35% EC (Batch F94/-/113) when administered to rats as a single dermal application. Initially one group of five males and five females was treated at 4000 mg/kg (3.704 ml/kg). Nine animals died on day 1 or 2. The tenth animals was killed 24 hours after treatment. A lower dose group of five animals was selected and treated at 400 mg/kg (0.370 ml/kg). No males died, therefore five females were also treated at 400 mg/kg. Subsequently, a mid dose group was selected at 1300 mg/kg (1.20 ml/kg) to complete the full study. After an acclimatization period of five days, and one day before exposure, an area of approximately 5x7 cm on the back of the animal was clipped. 24 hours later, the test material was applied to an area of approximately 25 cm^2 for males and 18 cm^2 for females by application on a gauze patch fixed successively to aluminium foil and flexible bandage. After the 24 hour contact period, the dressing were removed and residual test substance removed using a tissue moistened with water. The animals were housed individually in polycarbonate cages under standardised conditions (12 hours light/12 hours dark; temperature: 21ºC; relative humidity: 50%; ventilation: 15 air changes per hour). They had free access to drinking water and food (Kliba 343, Klingentalmühle AG, Kaiseraugst, Switzerland). After application, the animals were kept under observation for 15 days. Bodyweights were recorded prior to application of the test material as well as on days 8 and 15 and at

death (if found dead after day 1). All animals killed or animals surviving were sacrificed by oxigen/carbon dioxide asphyxiation. All animals were subjected to a gross necropsy examination.

Findings

One female at 400 mg/kg, one male and five females at 1300 mg/kg and all animals at 4000 mg/kg died as showed in Table 6.11.2c-1.

Table 6.11.2c-1: Mortalities

Dose Level (mg/kg)	Males	Females	Sexes Combined
400	0/5	1/5	1/10
1300	1/5	5/5	6/10
4000	5/5	5/5	10/10

Clinical signs: The clinical signs observed during the study period were as follows:

400 mg/kg: Lethargy (males only)

1300 mg/kg: Lethargy, clonic spasms, tremors, ventro-lateral recumbency, uncoordinated, movements and erythema, scales and scabs on the treated skin.

4000 mg/kg: Aggression, clonic spasms, tremors and erythema and pale appearance on the treated skin.

The clinical signs had disappeared in all surviving animals within 48 hours, whilw treated skin abnormalities persisted in one male of the 1300 dose group until day 8.

Bodyweight loss or low bodyweight gain was noted in all surviving animals over the first week of the study period. Improved bodyweight gain was noted in all animals over the second week.

Gross necropsy: Macroscopic post mortem examination of the animals that died or were killed *in extremis* during the study revealed pale colouration of the mucosa of the stomach and a spleen reduced in size in the males of the 4000 mg/kg dose group only. Macroscopic post mortem examination of the surviving animals at termination did not reveal any abnormalities.

Conclusions

The acute dermal median lethal dose (LD_{50}) of the test material Endosulfan 35% EC in rats was 1006 mg/kg for the sexes combined. Due to the mortality distribution, only estimated dermal LD_{50} values of Endosulfan 35% EC could be obtained for males alone and females alone. These were 1450 mg/kg for males alone and 449 mg/kg for females alone. According to the EU Criteria, Endosulfan 35%EC should be classified as harmful (Xn) and the risk expression R21.

B.6.11.4c Skin irritation in rabbits

Pels Rijcken, W.R. 1994 (Excel: IIIA, 7.1.4/01)

The study was performed according to OECD Guideline 404 and the EEC Directive 92/69, Part B; B.4.

GLP: yes

The study is acceptable.

Materials and methods

A group of three male New Zealand White rabbits, source Broekman Institute, Someren, The Netherlands, received a single topical dose of 0.5 ml of the test material Endosulfan 35% EC (Batch F94/-/113)). Approximately 24 hours before treatment, the dorsal fur was shaved. On test day 1, 0.5 ml of the test substance was applied to the intact skin of the shaved area on one flank, using a gauze patch. A similar patch, but without test substance was applied to the contralateral flank, to act as a procedural control. Both patches were secured with elastic bandage. The dressings were removed after four hours exposure; the treatment sites were washed with tap-water to remove excess test material. The animals were individually housed. They had free access to drinking water and food (LKK-20, Hope Farms, Woerden, The Netherlands). The rabbits were kept under standardised conditions (12 hours light/12 hours dark; temperature: 21ºC; relative humidity: 50%; ventilation: 15 air changes per hour). After application the animals were kept under observation for 21 days. The skin reactions were assessed at approximately 1, 24, 48 and 72 hours and 7, 14 and 21 days after treatment.

Findings

Clinical signs: One hour after 4 hours exposure to 0.5 ml of Endosulfan 35% EC, well defined erythema and very slight or slight oedema were observed in the treated skin areas of the three animals. Within 24 hours, grey/brown discolouration of the skin became apparent, indicating signs of necrosis. During the course of the study, eschar formation and fissuring of the skin developed in all animals, resulting in the presence of scar tissue in the treated skin-areas of all animals 14 days after exposure. The skin irritation had resolved within 21days in one animal and signs of erythema and oedema persisted in two animals at termination (21 days after exposure). A bald treated skin was seen in all animals at termination. Detail of reaction scores are given in Table 6.11.4c-1.

Table 6.11.4c-1:

Rabbit Nº	Erythema							Oedema						
	1h	24h	48h	72h	7 d	14d	21d	1h	24h	48h	72h	7d	14d	21d
1445	2	4	4	4	4	0	2	2	2	4	4	*	1	1
1447	2	4	4	4	4	0	0	1	2	3	3	*	1	0
1451	2	4	4	4	4	0	2	1	2	3	3	*	1	1

The group mean 24, 48 and 72 hours scores for erythema and edema were 4 and 2.88 respectively.

Conclusions

Based on the signs of necrosis and the formation of scar tissues, it was concluded that corrosion of the skin had occurred in the animals. According to the EU Criteria, Endosulfan 35% EC should be

classified with the symbol C (corrosive) and the risk expression R34 and with the symbol Xi (irritant) and the risk expression R38.

B.6.12 Dermal absorption (IIIA, 7.3)

Summary

The four dermal absorption studies evaluates in this point are the same evaluates in the B.6.1 points, nevertheless have been evaluted again from the point of view according with this paragraph by the coresponding expert.

The first study (Craine, 1986; cited by Department of Health &Family Services (DHFS, 1997), treated male rats with 14C-endosulfan formulated similarly to 3 EC final spray mix at doses of 0.10 mg/kg (LD), 0.76 mg/kg (MD) and 10.13 mg/kg (HD) on 10.8 CM2 shaved dorsal skin. Doses were equivalent to 0.002, 0.018 and 0.244 mg/cm . Animals were sacrificed at 0.5, 1, 2, 4, 10 or 24 h after dose application and the distribution of radioactivity was assessed by routine procedures. At 24 h, systemic absorption was 21.5%, 21.5% and 8.4% for the LD, MD and HD, respectively. Between 57-67% remained at the application site.

Craine (1988; cited by DHFS, 1997) extended these findings using doses of 0.09 mg/kg (LD), 0.98 mg/kg (MD) and 10.98 mg/kg (HD) on female rats, under similar routine conditions to above. The experiment included a skin wash at 10 h and sacrifice of animals at 24, 48, 72 or 168 h after dose application. Doses were equivalent to 0.002, 0.022 and 0.244 Mg/CM2. Recovery of label was 84-115%. Skin penetration increased with time and skin residues declined over time. By 168 h, systemic absorption accounted for 45, 46 and 20% of the LD, MD and HD, respectively. Most was excreted in the urine and faeces. Only a small proportion of the initial dose remained in the skin, 2% (LD and MD) and 1% (HD).

Lachmann (1987; cited by DHFS, 1997) treated two Rhesus monkeys on the shaved neck and back with an aqueous suspension of 19.025 mg 14 C-endosulfan (equivalent to 2.2 and 3.0 mg/kg). Treated skin was washed after 10 h. Blood, urine and faeces were collected during the experiment and tissue distribution was determined at termination (96 h after the end of exposure). Recovery of label was 50%. Twenty-two per cent was found in carcass, tissue and excreta, 11 % was bound to the skin surface and 17% was not absorbed. The main metabolite in the urine was endosulfan-diol.

The final study (Noctor and John, 1995; cited by DHFS, 1997) investigated *in vitro* penetration of 14C-endosulfan in a formulation similar to the 352 g/L EC, through human and rat skin. Skin samples included intact epidermis and a portion of dermis. Test substance was applied at 0.01 mg/cm^2 (LD), 0.1 mg/cm^2 (MD) and 1 mg/cm^2 (HD) to eight preparations each of rat and human skin. Extra skin preparations treated with HD were washed at 1 0 h. Samples of receptor fluid were collected at 1, 2, 4, 8, 10, 16, 24, 48 and 72 h (termination). Recovery ranged from 110.8-67.8%. The per cent penetrated

across all doses was higher for rat than human skin. At 72 h, the comparisons are indicated in table 6.12-1.

Table 6.12-1

Dose mg/cm^2	% penetrated rat : human
0.01	95.8:60.6 = 1.6
0.1	75.9:29.4 = 2.6
1	40.2:20.0 = 2.0
1 (with wash at 1 Oh)	9.1: 4. 0 = 2.3

The per cent remaining in the skin membrane increased with increasing dose, 13.3-30.7% (rat, unwashed) and 7.4-49.3% (human, unwashed). There was not a reliable trend difference between species.

The study identified endosulfan metabolites in receptor fluid. Rat fluid contained a high percentage of unchanged endosulfan (81.4% total radioactivity). In contrast, human fluid contained less endosulfan (27.3%) and higher amounts of the sulphate (8.3% vs 2.9%), diol (34.0% vs 8.8%) and OH-ether (2.7% vs none detected) metabolites.

The dermal absorption studies suggest that initial absorption is dose related, movement through skin is slow (occurring over 168 h in the rat *in vivo* study), endosulfan continues to be absorbed from skin reservoirs after skin washing and penetration as per cent and rate is lower in human skin than rat skin. The optimum *in vivo* study for assigning a dermal absorption factor is Craine (1988), as it investigates absorption over the longest time period, includes a skin wash and recovery of label is acceptable. Rat: human data is derived from Noctor and John (1995). Dose selection depends upon the most likely occupational exposure. Predicted hand exposures from POEM for mixer/loaders wearing gloves, indicate an endosulfan concentration of 0.02-0.17 mg/cm^2, at standard hand size of 820 cm^2 (US EPA, 1996).

This corresponds to the *in vivo* study MD-HD range with maximum absorption of 46% (MD) at 168 h. In the *in vitro* study, the dose range corresponds to LD->MD range. The rat:human at the LD (maximum absorption) at termination at 72 h is 1.6. The ratio does not change if skin residues are included. Data from the skin wash is not applicable because ¡t relates to the HD only (1 mg/em 2).

The most appropriate dermal absorption factor to use in the risk assessment is 46% / 1.6 29%.

B.6.12.1 Primates

Lachman G (1987)

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

Material and methods

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

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

Blood samples were taken from each monkey at 1, 2, 4, 8, 12, 24, 3 6, 48, 72 and 96 h after the end of exposure to the test compound. Urine and faeces samples were collected over the 10 h exposure period, and for each of the 24 h periods following the end of administration (up to 96 hours). Tissue distribution was determined 96 h after the end of exposure. The monkeys were euthanised, and the liver, kidneys, brain, fat and treated skin were removed. After weighing, tissues and the residual carcasses were stored until preparation. The skin was prepared immediately. After initial determination of radioactivity, additional tissues were collected for determination, including total muscle below the treated skin, muscle of the hind limb (back - to compare with muscle under treated skin), skin at the inner side of the hind limbs and skin at the back of the hind limbs. The hands of the animals were detached, and the carcasses were separated into smaller portions for determination.

The pattern of metabolites in the urine and faeces were determined, using the 0-24h samples of urine, and the 72-96-h sample of faeces. The samples were chromatographed on silica gel plates, after which the plates were exposed to x-ray films. The autoradiographs were examined qualitatively. For the quantification of metabolites in urine, the urine of monkey 2 was used, as this was more concentrated. For metabolites in faeces, equal volumes of samples from both animals were pooled. As the radioactivity of samples was low, they were concentrated prior to chromatography. Three samples of each were used, after being lyophilised. The first sample was dissolved in methanol and used for chromatography. The second was dissolve in acetate buffer with the addition of glucuronidase and arylsulfatase. The third sample was dissolved in sodium hydroxide and allowed to stand overnight. All samples were then separated by HPLC.

Results

The administered dose was 2.2 mg/kg for monkey 1 and 3.0 mg/kg for monkey 2. The radioactive dose was 11.1 MBq for monkey 1 and 13.6 MBq for monkey 2. Blood and plasma levels of endosulfan increased during the first 24 - 36 hours, after which a steady state level was reached. The ratio of blood levels to plasma levels was approximately 0.65 for the first 8 - 12 h, after which ¡t increased to 0.75 – 0,8.

Total recovery of the radioactive dose applied was 50%(table 6.12.2-1). The distribution of this material is indicated in Table 6.12.1-2

Table 6.12.1-1: Absorption profile following single dermal dose in monkey

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

Table 6.12.1-2: Tissue distribution profile for endosulfan in monkey following dermal dose

Tissue	Residue at 96 h post application ng/g	Residue at 96 h post-application % total recovery
Blood	26	
Brain	8.1	0.01
Liver	478	0.40
Kidneys	83	0.02
Fat	233	
Muscles	159	0.21
Skin	1379	0.04

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

Conclusions

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

B.12.2 *In vitro* dermal studies

Noctor J and John SA (1995) (AgrEvo)

Report Date 10 May 1995.

The subject was to determine the: Rate of penetration of ^{14}C-Endosulfan hrough human and rat skin determined using an *in vitro* system.

CY GLP:UK, OECD. (Hoechst Schering AgrEvo, 11482) Report A54103.

Material and Methods

Alpha and beta isomers of radiolabelled endosulfan were obtained from the sponsor. The alpha isomer (batch no 2202211) had a specific activity of 3.051 MBq/mg, a radiochemical purity of 99%; 20.3 mg was received. The beta isomer (batch no 22023II) had a specific activity of 2.935 MBq/mg, a radiochemical purity of 99%; 10.4 mg was received.

Additionally 1 g each of alpha and beta non-labelled endosulfan was received, with chemical purities of 99.8 and 99.4%, respectively. The radioactive purity and specific radioactivity of the material was determined by the testing laboratory prior to use.

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

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

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

Radiolabelled endosulfan was dissolved in a formulation vehicle (emulsifiable concentrate) to produce a concentration of 352 g endosulfan per litre, with a ratio of alpha:beta of 2:1 The formulation was then diluted in PLC grade water to 40, 4.0 and 0.4 mg/mL, providing a nominal application volume of 0.064 mL/preparation, or 0.025 mL/cm^2. Immediately before dose application, the receptor chamber was filled with a known volume of acidified ethanol/water (1: 1 v/v). The test substance was applied at 1, 0.1 and 0.01 mg/cm^2 to 8 preparations each of human and rat skin. Additionally, 4 preparations from each species were treated at the highest dose and the skin surfaces washed 1 0 h after application. Samples of the receptor fluid were taken 1, 2, 4, 8, 10, 16, 24, 48 and 72 h after application. At 72 h post application the receptor fluid was removed. The epidermal skin surface was washed, rinsed and dried. The skin section was removed and weighed.

Results

Recovery in the human system was lower than in the rat. The penetration of endosulfan in the rat was an average of 4.3 times the penetration of the human skin. The metabolites present in the receptor fluid differed for rats and humans. These are detailed in the table 6.12.2-1

Table 6.12.2-1: Endosulfan products present in the Receptor Fluid after 72 h (as % of total radioactivity)

Metabolite	Rat	Human
α-endosulfan	3.1	-
β-endosulfan	81.4	27.3
Endosulfan-sulphate	2.9	8.3
Endosulfan-diol	8.8	34.0
Endosulfan-OH-ether	-	2.7
unknown		17.2

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

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

Conclusion

The penetration of endosulfan through rat and human skin was studied *in vitro.* The test material consisted of radiolabelled endosulfan formulated as an emulsifiable concentrate (containing 353 g/L endosulfan), which had been diluted to concentrations ranging from 0.4 to 4.0 mg/mL in water. The test material was applied at nominal doses of 0.0 1, 0. 1 and 1 mg/cm^2 to rat and human skin mounted in dermal penetration cells, the rate of penetration was determined. The penetration rate for rats was, on average, 4.3 times that of humans. The percentage of the applied dose varied with concentration with 61 % of the lowest dose applied to human skin penetrating (96% in the rat) and 20% of the highest dose penetrating (40% in the rat). When the skin was washed 10 h after application, the amount of endosulfan penetrating decreased to 4% in the human and 9% in the rat. Endosulfan passing through human skin was metabolised or degraded to a greater extent than that passing through rat skin. (Noctor & John, 1995).

B.6.13 Toxicological data on non active substances (IIIA, 7.4 and point 4 of the introduction)

Special toxicological studies for non-active substances are not available. The Safety Data Sheet had been provided.

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.

The following assumptions have been used in calculation operator exposure:

The treated area in one day is:	20 ha/day for field crops
	1 ha/day for hand held spreyers
Worst case is used:	2 l/ha

AOEL = 0.006 mg/kg/bw

Estimates of operator exposure-German model

Endocel 35 EC, calculation of exposure for mixer/loader and spray application by hand held outdoors low level application 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)
$I^*_M = 0.05$ $D^*_{M(H)} = 205$	$I^*_A = 0.001$ $D^*_{A(C)} = 0.06$ $D^*_{A(H)} = 0.38$ $D^*_{A(B)} = 1.6$	1

Expected Inhalation Exposure:

$I_M = I^*_M \times R \times A = 0.05 \times 0.7 \times 1 = 0.035$ mg/person/day

$I_A = I^*_A \times R \times A = 0.001 \times 0.7 \times 1 = 0.0007$ mg/person/day

Expected Dermal Exposure:

$D_{M(H)} = D^*_{M(H)} \times R \times A = 205 \times 0.7 \times 1 = 143.5$ mg/person/day

$D_{A(H)} = D^*_{A(H)} \times R \times A = 0.38 \times 0.7 \times 1 = 0.266$ mg/person/day

$D_{A(C)} = D^*_{A(C)} \times R \times A = 0.06 \times 0.7 \times 1 = 0.0042$ mg/person/day

$D_{A(B)} = D^*_{A(B)} \times R \times A = 1.6 \times 0.7 \times 1 = 1.12$ mg/person/day

Inhalation exposure = 0.035 + 0.0007 mg ai/person = **0.0357 mg ai/person**

Total dermal exposure = **144.89 mg ai/person**

Total dermal exposure based on dermal absorption in humans of 29% = 42.018 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 42.0538 mg ai/person

Total systemic exposure for a 70 kg person = **0.6008 mg ai/kg/day**

With PPE (Gloves during mixing/loading 1%, gloves during aplication 1% and protection clothes during aplication 5%)

Dermal exposure:

$D_{M(H)}$ = 1.435 mg/person/day (1%)
$D_{A(H)}$ = 0.00266 mg/person/day (1%)
$D_{A(C)}$ = 0.0042 mg/person/day
$D_{A(B)}$ = 0.056 mg/person/day (5%)

Total dermal exposure = 1.4979 mg ai/person

Total dermal exposure based on dermal absorption in humans of 29% = 0.4344 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 0.4701 mg ai/person

Total systemic exposure for a 70 kg person = 0.0067 mg ai/kg/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:

$$I_M = I^*_M \times R \times A = 0.0006 \times 0.7 \times 20 = 0.0084 \text{ mg/person/day}$$
$$I_A = I^*_A \times R \times A = 0.001 \times 0.7 \times 20 = 0.014 \text{ mg/person/day}$$

Expected Dermal Exposure:

$$D_{M(H)} = D^*_{M(H)} \times R \times A = 2.4 \times 0.7 \times 20 = 33.6 \text{ mg/person/day}$$
$$D_{A(H)} = D^*_{A(H)} \times R \times A = 0.38 \times 0.7 \times 20 = 5.32 \text{ mg/person/day}$$
$$D_{A(C)} = D^*_{A(C)} \times R \times A = 0.06 \times 0.7 \times 20 = 0.84 \text{ mg/person/day}$$
$$D_{A(B)} = D^*_{A(B)} \times R \times A = 1.6 \times 0.7 \times 20 = 22.4 \text{ mg/person/day}$$

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 29% = 18.0264 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 18.0488 mg ai/person

Total systemic exposure for a 70 kg person **= 0.2578 mg ai/kg/day**

With PPE (Gloves 1% mixing/loading 1%; gloves during aplication 1%; protection cloth during aplication 5% and hear protection 10%):

Dermal exposure:

$D_{M(H)}$ = 0.336 mg/person/day (1%)
$D_{A(H)}$ = 0.0532 mg/person/day (1%)
$D_{A(C)}$ = 0.084 mg/person/day (10%)
$D_{A(B)}$ = 1.12 mg/person/day (5%)

Total dermal exposure = 1.5932 mg ai/person

Total dermal exposure based on dermal absorption in humans of 29% = 0.462 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 0.4844 mg ai/person

Total systemic exposure for a 70 kg person **= 0.0069 mg ai/kg/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, UK POEM 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:

$I_M = I^*_M \times R \times A = 0.0006 \times 0.61 \times 20 = 0.00732$ mg/person/day

$I_A = I^*_A \times R \times A = 0.001 \times 0.61 \times 20 = 0.0122$ mg/person/day

Expected Dermal Exposure:

$D_{M(H)} = D^*_{M(H)} \times R \times A = 2.4 \times 0.61 \times 20 = 29.28$ mg/person/day

$D_{A(H)} = D^*_{A(H)} \times R \times A = 0.38 \times 0.61 \times 20 = 4.636$ mg/person/day

$D_{A(C)} = D^*_{A(C)} \times R \times A = 0.06 \times 0.61 \times 20 = 0.732$ mg/person/day

$D_{A(B)} = D^*_{A(B)} \times R \times A = 1.6 \times 0.61 \times 20 = 19.52$ 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 29% = 15.7087 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 15.7282 mg ai/person

Total systemic exposure for a 70 kg person **= 0.2247 mg ai/kg/day**

With PPE (Gloves during mixing/loading1%; gloves during aplication 1%; protection cloth during aplication 5% and hear protection 10%):

Dermal exposure:

$D_{M(H)}$ = 0.293 mg/person/day (1%)
$D_{A(H)}$ = 0.04636 mg/person/day (1%)
$D_{A(C)}$ = 0.0732 mg/person/day (10%)
$D_{A(B)}$ = 0.976 mg/person/day (5%)

Total dermal exposure = 1.3886 mg ai/person

Total dermal exposure based on dermal absorption in humans of 29% = 0.4027 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 0.4222 mg ai/person

Total systemic exposure for a 70 kg person **= 0.006 mg ai/kg/day**

B.6.14.1c AgrEvo applicant

Operator exposure

The exposure to endosulfan in Thiodan-EC35 is predicted according to the German BBA-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 29% = 27.0396 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 27.0732 mg ai/person

Total systemic exposure for a 70 kg person **= 0.3868 mg ai/kg/day**

With PPE (Gloves 1% during mixing/loading/aplication, protection cloth during aplication 5% and hear protection 10%):

Dermal exposure:

$D_{M(H)}$ = 0.504 mg/person/day (1%)
$D_{A(H)}$ = 0.0798 mg/person/day (1%)
$D_{A(C)}$ = 0.126 mg/person/day (10%)
$D_{A(B)}$ = 1.68 mg/person/day (5%)

Total dermal exposure = 2.3898 mg ai/person

Total dermal exposure based on dermal absorption in humans of 29% = 0.693 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 0.7266 mg ai/person

Total systemic exposure for a 70 kg person **= 0.010 mg ai/kg/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 29-% = 33.8604 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 34.0166 mg ai/person

Total systemic exposure for a 70 kg person **= 0.486 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 10%):

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%)
$D_{A(C)}$ = 1.008 mg/person/day (10%)
$D_{A(B)}$ = 4.032 mg/person/day (5%)

Total dermal exposure = 5.3004 mg ai/person

Total dermal exposure based on dermal absorption in humans of 29% = 1.537 mg ai/person

Total systemic exposure = inhalation + dermal exposure = 1.568 mg ai/person

Total systemic exposure for a 70 kg person **= 0.0224 mg ai/kg/day**

It is imposible to obtaine a exposition < AOEL

B.6.15 References relied on

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5/01; 5.1/01	1968	Maier-Bode, H. properties, effect, residues and analitycs of the insecticide endosulfan. Residue review, vol. 22, item III	No	Yes	Publ.	No
IIA, 5/02; 7/03; 8.0/01	1979	Gupta, P.K.; Gupta, R.C. Pharmacology, toxicology and degradation of endosulfan, a review. Toxicology, 13, 115-130	No	Yes	Publ.	No
IIA, 5.1		See IIA, 6.2				
IIA, 5.1; 5.2/01	1984	WHO Environmental Health Criteria 40 Worlf Health Organization, Geneva, Item 5	No	Yes	Publ.	No
IIA, 5.1/01	1966	Beck, E.W.; Woodham, D.W.; Johnson, Jr., J.C.; Leuck, D.B.; Dawsey, L.H.; Robbins, J.E.; Bowman, M.C. Residues of endosulfan in meat and milk of cattle Fed treated forages. J. Econ. Entomol., vol. 59, no. 6: 1444-1450	No	Yes	Publ.	No
IIA, 5.1/02; 5.2.1/01; 5.8.1/01	1978	Wyman; Dorough, H.; Huhtanen, K.; Marshall, T.C.; Bryant, H.E. Fate of endosulfan in rats and toxicological considerations of apolar metabolites. Pest. Biochem. Physiol, vol. 8: 241-252	No	Yes	Publ.	No
IIA, 5.1.1	1983a	Kellner, H.M.; Eckert Hoe 02671-^{14}C pharmacokinetics and residue determinations after oral and intravenous administration to rats. A49475	No	No	Hoe	No
IIA, 5.1.1/01	1981	Rao, V.R. Acute Oral Toxicity Study of Endosulfan Technical in Mice. [redacted]	No	No	Excel	No
IIA, 5.1.1/02; 5.1.2/01	1977	Srimal, R.C. Test report on acute toxicity of the two samples of pesticide (I) endosulfan technical and (2) endocel 35% None [redacted]	No	No	Excel	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.1.1/03		Dikshith, T.S.S. Acute Oral Toxicity of Endosulfan Technical in chicken or Pigeon. None [redacted]	No	No	Excel	No
IIA, 5.1.1/04		Dikshith, T.S.S. Acute Oral Toxicity of Endosulfan Technical in Pigeon. None [redacted]	No	No	Excel	No
IIA, 5.1.1/05; 5.1.2/04	1969	Gaines, T.B. Acute Toxicity of Pesticides Toxicology and Applied Pharmacology 14. 515-534	No	Yes	Publ.	No
IIA, 5.1.1.2 / 5.1.1.3	1968	Christ; Kellner Investigations with endosulfan-^{14}C in mice [redacted] A53842	No	No	AgrEvo	No
IIA, 5.1.1.3	1968	Gorbach, S. G.; Christ, O. E.; Kellner, H. M.; Kloss, G.; Boerner, E. Metabolism of ENDOSULFAN in Milk Sheep Generated by: Hoechst AG, Germany. Report No.: A14216 J. Agr. Food Chem. Vol. 16, No. 6. page 950. 1968	No	Yes	Publ.	No
IIA, 5.1.2/02		Bhide, M.B. The acute dermal toxicity LD_{50} of Excel Industries Ltd's endosulfan technical No. 1 to the albino rabbits. None [redacted]	No	No	Excel	No
IIA, 5.1.2/03	1975	Gupta, P. K.; Chandra, S.V. The Toxicity of endosulfan in rabbits. Bull. Environm. Cont. & Toxc. Vol. 14 No. 5	No	Yes	Publ.	No
IIA, 5.1.2.2	1987	Leist, K.-H.; Mayer, D. Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003), 30-Day Feeding Study in Adult Male Wistar Rats [redacted] A37112	Yes	No	AgrEvo	No
IIA, 5.1.2.4	1965	Gorbach, S. Investigations on Thiodan in the Metabolism of Milk Sheep [redacted] GermanyReport No.: A14209	No	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.1.2.5	1993	Indranignsih, McSweeney, C.S., Ladds, P.W. Residues of endosulfan in the tissues of lactating goats [redacted] Report No.: A51447 Australian Vet. Journal. Vol. 70. pages 59 - 62. 1993	No	Yes	Publ.	No
IIA, 5.1.2.6	1959	Bowman, James S. Subacute Feeding - Dairy Cows, preliminary report [redacted] A14205	No	No	AgrEvo	No
IIA, 5.1.2.6	1959b	Keller, John G. Subacute Feeding Study - Dairy Cows. (Supplement to Report dated March 20, 1959) [redacted] A14206	No	No	AgrEvo	No
IIA, 5.1.3.1.1	1986	Craine, E.M. A Dermal Absorption Study in Rats with ^{14}C-Endosulfan [redacted] A35730	No	No	AgrEvo	No
IIA, 5.1.3.1.1; IIIA, 7.3	1988	Craine, Elliott M. A Dermal Absorption Study in Rats with 14C-Endosulfan with Extended Test Duration [redacted] A39677	Yes	No	AgrEvo	No
IIA, 5.1.3.1.2; IIIA/7.3	1987	Lachmann, G.; Siegemund, B. Hoe 002671-(5a,9a-14-C). Dermal Absorption of 14C-Endosulfan in Rhesus Monkeys [redacted] A36685	Yes	No	AgrEvo	No
IIA, 5.1.3.1.3	1995	Noctor, J. C.; John, S. A. (14C)-Endosulfan: Rates of penetration through human and rat skin determined using an in vitro system [redacted] Report No.: A54103	Yes	No	AgrEvo	Yes
IIA, 5.1.3.2; IIA, 5.1.2	1981	Robacker, Karen M.; Kulkarni, Arun P.; Hodgson, Ernest Pesticide Induced Changes in the Mouse Hepatic Microsomal Cytochrome P-450-Dependent Monooxygenase System and Other Enzymes [redacted] A35754 J. Environ. Sci. Health. Vol. B16, No. 5. pages 529-545. 1981	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.1.3.2	1968	Schuphan, I,; Ballschmiter, K.; Toelg, G. Zum Metabolismus des Endosulfans in Ratten und Maeusen [redacted] A14215 Z. Naturforsch. Vol. 23b. No. 5. pages 701 - 706. 1968	No	Yes	Publ.	No
IIA, 5.1.5/01		Dikshith, T.S.S. Irritation of the mucose membrane in female rabbits None [redacted]	No	No	Excel	No
IIA, 5.1.5/02		Dikshith, T.S.S. Primary skin irritation test of endosulfan technical (female) None	No	No	Excel	No
IIA, 5.1.5/02		Dikshith, T.S.S. Primary skin irritation test of endosulfan technical (male) None	No	No	Excel	No
IIA, 5.1.6/01	1986	Lisi, P.; *et. al.* A test series for pesticide dermatitis Contact dermatitis, 15; 266-269	No	Yes	Publ.	No
IIA, 5.2/02/03; 5.3/02; 5.5/03; 5.6/03; 5.8.2.1; 6.02	1986	Banerjee, B.D.; Hussain, Q.Z. Effect of sub-chronic endosulfan exposure on humoral and cell-mediated immune responses in albino rats [redacted] Report No.: A43391 Arch. Toxicology. Vol. 59. pages 279-284. 1986	No	Yes	Publ.	No
IIA, 5.2.1	1988b	Diehl, K.-H.; Leist, K.-H. Hoe 002671 - active ingredient technical (Code: Hoe 002671 0I ZD96 0002) - Testing for acute oral toxicity in the male and female Wistar rat [redacted] A39680	Yes	No	AgrEvo	No
IIA, 5.2.1; 5.2.2	1957	Elsea, John R. Progress Report: Acute Oral Administration, Acute Dermal Application, Acute Eye Application [redacted] A13683	No	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.2.1	1958	Elsea, John R. Acute Oral Administration [redacted] A13686	No	No	AgrEvo	No
IIA, 5.2.1	1958	Keller, John G. Final Report - Acute Oral Administration - Dogs [redacted] A13831	No	No	AgrEvo	No
IIA, 5.2.1	1971	Kretchmar, Beverly; Mastri, Carmen; Keplinger, M.L. Acute Oral Toxicity Studies with Two Samples of Endosulfan in Male Albino Rats [redacted] A13713	No	No	AgrEvo	No
IIA, 5.2.1	1957	Lindquist, Donald A.; Dahm, Paul A. Some Chemical and Biological Experiments with Thiodan Lowa State College, Ames, Iowa, United States. Report No.: A13684 J. Econ. Entomol. Vol.50, No.4. 483{Abs}486. 1957	No	Yes	Publ.	No
IIA, 5.2.1	1970	Nogami, K. (Translator) Testing Report on the Toxicity of Endosulfan (Malix) to Dogs through Acute Oral Administration (LD 50) Not mentioned, obviously in Japan. Report No.: A13834	No	No	AgrEvo	No
IIA, 5.2.1	1975c	Reno, Frederick E. Acute Oral Toxicity Study in Rats - Endosulfan Technical. Final Report [redacted] A33732	No	No	AgrEvo	No
IIA, 5.2.1	1971a	Scholz; Weigand Acute Oral Toxicity of Thiodan Technical to the Male Sherman-Rat [redacted] A16757	No	No	AgrEvo	No
IIA, 5.2.1	1971b	Scholz; Weigand Acute Oral Toxicity of Thiodan Technical to the Female Sherman-Rat [redacted] A16758	No	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.2.2	1957	Elsea, John R. Progress Report: Acute Oral Administration, Acute Dermal Application, Acute Eye Application [redacted] A13683	No	No	AgrEvo	No
IIA, 5.2.2	1988a	Diehl, K.-H.; Leist, K.-H. Endosulfan - active ingredient technical (Code: Hoe 002671 0I ZD96 0002) Testing for acute dermal toxicity in the male and female Wistar rat [redacted] A39397	Yes	No	AgrEvo	No
IIA, 5.2.2/01	1981	Gupta, P.K.; *et. al.* Toxicity of endosulfan and Maganese, Chloride: Cumulative Toxicity Rating. Toxicology Letters, 7; 221-227	No	Yes	Publ.	No
IIA, 5.2.3	1983	Hollander, H.; Weigand, W. Hoe 002671 - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003). Testing for Acute Aerosol Inhalation Toxicity in Male and Female SPF Wistar Rats. 4 Hours - LC50 [redacted] A32087	Yes	No	AgrEvo	No
IIA, 5.2.4	1975b	Reno, Frederick E. Primary Skin Irritation Study in Rabbits - Endosulfan Technical. Final Report [redacted] A33731	No	No	AgrEvo	No
IIA, 5.2.5	1975a	Reno, Frederick E. Acute Eye Irritation Potential Study in Rabbits - Endosulfan Technical. Final Report [redacted] A33730	No	No	AgrEvo	No
IIA, 5.2.6	1983	Jung; Weigand Hoe 002671 – Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003). Test for Sensitizing Properties in Female Pirbright-White Guinea Pigs According to the Method of BUEHLER [redacted] A27248	No	No	AgrEvo	No
IIA, 5.3/01	1989	Singh, S.K.; Pandey, R.S. Differential effects of chronic endosulfan exposure to male rats in relation to hepatic drug metabolism and androgen biotransformation. Indian Journal of Biochemistry & Biophysics. Vol. 26; 262-267	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.3.1/01	1978	U.S. Departament of health, education and welfare Bioassay of endosulfan for possible carcinogenicity. National Cancer Institute Carcinogenesis.	No	Yes	Publ.	No
IIA, 5.3.1/02	1981	Reuber, M.D. The role of toxicity in the carcinogenicity of endosulfan. The Science of the total environment, 20; 23-47	No	Yes	Publ.	No
IIA, 5.3.2.1	1985	Barnard, A.V.; Jones, D.R.; Powell, L.A.J. Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) 13 Week Toxicity Study in Rats Followed by a 4-Week Withdrawal Period (Final Report) [redacted] No.: A30700	Yes	No	AgrEvo	No
IIA, 5.3.2.1	1979	Khanna, R.N.; Misra, D.; Anand, M.; Sharma, H. K. Distribution of Endosulfan in Cat Brain Industrial Toxicology Research Centre, India. Report No.:A19001 Bull. Environm. Contam Toxicol. Vol.22. pages 72-79. 1979	No	Yes	Publ.	No
IIA, 5.3.2.1	1989	Muellner, H. Effects of Endosulfan and Aldicarb on rat brain Acetylcholinesterase [redacted] Germany. Report No.: A43395	No	No	AgrEvo	No
IIA, 5.3.2.3	1967	Baran, John; Kodras, Rudolph; Faucher, Otis E. Two-Year Chronic Oral Toxicity of Thiodan Technical - Beagle Dogs Bio-Test, United States. Report No.: A13914	No	No	AgrEvo	No
IIA, 5.3.2.3	1989	Brunk, R. Endosulfan - substance technical (Code: Hoe 002671 0I ZD96 0002) Testing for toxicity by repeated oral administration (1-year feeding study) to Beagle dogs [redacted] Report No.: A40441	Yes	No	AgrEvo	No
IIA, 5.3.2.3	1959a	Keller, John G. Thiodan Technical, Final Report, Repeated Oral Administration - Dogs [redacted] A13924	No	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.3.2.4	1984	Barnard, A. V.; Atkinson, J. S.; Heywood, R. et al. Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) 13-Weeks Toxicity Study in Mice (Final Report) [redacted] No.: A29663	Yes	No	AgrEvo	No
IIA, 5.3.2.5	1985	Donaubauer, H.H.; Leist, K.-H.; Kramer, M. Endosulfan - Substance Technical (Code: Hoe 00267 0I ZD97 0003) 42-day Feeding Study in Mice [redacted] A38104	Yes	No	AgrEvo	No
IIA, 5.3.3.1	1988	Dikshith, T.S.S.; Raizada, R.B.; Kumar, S.N. Effect of repeated dermal application of Endosulfan to rats [redacted] A41365 Vet. Human Tox. Vol. 30. pages 219 - 224. 1988	No	Yes	Publ.	No
IIA, 5.3.3.1	1985a	Ebert, E.; Leist, K.-H.; Kramer, M. Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) Testing for Subchronic Dermal Toxicity (21 Applications over 30 Days) in Wistar Rats [redacted] A30753	Yes	No	AgrEvo	No
IIA, 5.3.3.1	1985b	Ebert, E.; Leist, K.-H.; Kramer, M. Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) Testing for Subchronic Dermal Toxicity (21 Applications over 30 Days) in SPF Wistar Rats [redacted] A30754	Yes	No	AgrEvo	No
IIA, 5.3.3.1	1985c	Ebert, E.; Leist, K.H.; Kramer, M. Toxicological review of studies 721 and 729 (reports 84.0321 and 84.0223) [redacted] A30755	Yes	No	AgrEvo	No
IIA, 5.3.3.2	1984	Hollander, H., Weigand, W., Kramer, M. Endosulfan-Active Ingredient Technical. (Code: Hoe 002671 0 I ZD97 0003) Testing for Subchronic Inhalation Toxicity - 21 Exposures in 29 Days - in SPF Wistar Rats [redacted] A29823	Yes	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.4/01	1995a	Dighe, R.P. *Salmonella Typhimurium* reverse mutation assay of endosulfan technical. OECD guideline 471 IIT No.: 1395 Indian Institute of Toxicology 98/A10, Pune 411 013	Yes	No	Excel	No
IIA, 5.4/02a	1995	Dighe, R.P. In vivo mammalian mouse bone marrow micronucleus test of endosulfan technical of Excel Industries Ltd., Bombay. Study Plan. Excel Industries, Ltd. [redacted]	Yes	No	Excel	No
IIA, 5.4/02	1995b	Dighe, R.P. In vivo mammalian mouse bone marrow micronucleus test of endosulfan technical of Excel Industries Ltd. IIT No.: 1396 [redacted]	Yes	No	Excel	No
IIA, 5.4/03	1990	Pandey, N.; *et. al.* Studies on the genotoxicity of endosulfan, an organochlorine insecticide, in mammalian germ cells. Mutation Research 242, 1-7	No	Yes	Publ.	No
IIA, 5.4/04; 5.4.3/02/03	1984	Velázquez, A.; *et. al.* Mutagenicity on the insecticide endosulafan in *Drosophila melanogaster.* Mutation Research, 136, 115-118	No	Yes	Publ.	No
IIA, 5.4/06	1980	Rani, M.V.U., *et. al.* Mutagenicity studies involving Aldrin, endosulfan, dimethoate, phosphamidon, carbaryl and ceresan Bull. Environm. Contam. Toxicol. 25, 277-282	No	Yes	Publ.	No
IIA, 5.4/07	1986	Dzwonkowska, A.; Hübner, H. Induction of chromosmal aberrations in the Syrian hamster by insecticides tested in vivo. Arch. Toxicology 58, 152-156	No	Yes	Publ.	No
IIA, 5.4/08	1993	Paul, V.; *et. al.* Effect of Chronic endosulfan treatment on pharmacological actions of diazepam in rats. Bull. Environm. Contam. Toxicol. 51, 18-23	No	Yes	Publ.	No
IIA, 5.4/09; 5.4.1/01	1983	Sobti, R.C.; *et. al.* Cytokinetic and Cytogenetic effect on agricultural chemicals on human lymphoid cells in vitro Arch. Toxicol. 52, 221-231	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.4.1/02	1987	Pednekar, M.D.; Gandhi, S.R.; Netrawali, M.S. Evaluation of mutagenic actvivities of endosulfan, phosalone, malathion and permethrin before and after metabolic activation in the Ames Salmonella Test. Bull. Environ. Contam. Toxicol. 38/925-933	No	Yes	Publ.	No
IIA, 5.4.1.1	1978	Shirasu, D.V.M.; Moriya, M.; Ohta, T. Microbial Mutagenicity Testing on ENDOSULFAN Inst.Environ.Toxicol., Japan. Report No.: A21215	No	No	AgrEvo	No
IIA, 5.4.1.2	1989	Asquith, J. C.; Baillie, J. H. Endosulfan substance technical (Code Hoe 002671 0I ZD95 0005) Metaphase Analysis of Human Lymphocytes Toxicol Lab. Ltd., United Kingdom. Report No.: A40411	Yes	No	AgrEvo	No
IIA, 5.4.1.2	1986	Pirovano, R.; Millone, M.F. Chromosome Aberration in Human Lymphocytes Cultured "in vitro" [redacted]A33127	Yes	No	AgrEvo	No
IIA, 5.4.1.3	1984b	Cifone, Maria A.; Myhr, Brian C. Mutagenicity Evaluation of Hoe 002671 - Substance Technical in the Mouse Lymphoma Forward Mutation Assay. Final Report [redacted] A29801	Yes	No	AgrEvo	No
IIA, 5.4.1.3	1984a	Mellano, Diego; Milone, Marco Ferro Study of the Mutagenic Activity `in vitro` of the Compound Endosulfan - Technical (Code Hoe 002671 0I ZD97 0003) with Schizosaccharomyces pombe [redacted] A29312	Yes	No	AgrEvo	No
IIA, 5.4.2/01	1978	Dikshith, T.S.S.; Datta, K.K. Endosulfan: lack of cytogenetic effects in male rats. Bull. Environ. Contam. Toxicol. 20: 826-833	No	Yes	Publ.	No
IIA, 5.4.2/02; 5.4.3/01	1980	Usha Rani, M.V.; Reddi, O.S.; Reddy, P.P. Mutagenicity studies involving aldrin, endosulfan, dimethoate, phosphamidon, carbaryl and ceresan. Bull. Environ. Contam. Toxicol. 25: 277-282	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.4.2.1	1988a	Mueller, W. Endosulfan - Substance, technical (Code: Hoe 002671 0I ZD95 0005) Micronucleus Test in Male and Female NMRI Mice After Oral Administration [redacted] A38059	Yes	No	AgrEvo	No
IIA, 5.4.2.2	1984a	Cifone, Maria A.; Myhr, Brian C. Evaluation of Hoe 002671 - Substance Technical in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay. Final Report [redacted] A29800	Yes	No	AgrEvo	No
IIA, 5.4.2.2	1984b	Mellano, D.; Milone, M., F. Study of the Mutagenic Activity of the Compound Endosulfan - Technical (Code Hoe 002671 0I ZD97 0003) with Saccharomyces cerevisiae. Gene Conversion - DNA Repair Test RBM, Italy. Report No.: A29313	Yes	No	AgrEvo	No
IIA, 5.4.2.2	1988b	Mueller, W. Evaluation of Endosulfan substance, technical (Code: Hoe 002671 0I ZD95 0005) in the unscheduled DNA Synthesis Test in Mammalian Cells in Vitro [redacted] A38445	Yes	No	AgrEvo	No
IIA, 5.5/01	1989	Singh, S.K.; Pandey, R.S. Gonadal toxicity of short term chronic endosulfan exposure to male rats. Indian Journal of Biology. Vol. 27; 341-346	No	Yes	Publ.	No
IIA, 5.5/01/02	1978	Metrek, a team of authors. Bioassay of endosulfan for possible carcinogenicity, cas no. 115-29-7, NCI-TR-62 National Cancer Institute, Carcinogenesis, Technical Report Series. No.: 62	No	Yes	Publ.	No
IIA, 5.5/02	1990	Singh, S.K.; Pandey, R.S. Effect of sub-chronic endosulfan exposure on plasma, gonadotrophins, testosterone, testicular testosterone and enzymes of androgen biosynthesis in rat. Indian Journal of Experimental Biology. Vol. 28; 953-956	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.5.1	1990	Gopinath, C.; Cannon, M. W. J. Endosulfan, Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) Combined Chronic Toxicity/Carcinogenicity Study (104-week feeding in rats). Photomicrographic Addendum to Histopath. Report HST/289 [redacted] No.: A44604	No	No	AgrEvo	No
IIA, 5.5.1/01; 5.6.2.1	1978	Gupta, P.K.; Chandra, Satya V.; Saxena, D.K. Teratogenic and Embryotoxic Effects of Endosulfan in Rats Ind.Toxicol.Res.Cent., India. Report No.: A17149 Acta Pharmacol. Toxicol. Vol. 42. pages 150-152. 1978	No	Yes	Publ.	No
IIA, 5.5.1/2/3	1995	Hack R.; Ebert E.; Leist K.-H. Chronic toxicity and carcinogenicity studies with the insecticide Endosulfan in rats and mice [redacted] No. A55880 [redacted] Toxicology, Germany. Fd Chem. Toxic, 1995, 33, 11, 941-950	Yes	Yes	Publ.	No
IIA, 5.5.1	1959	Keller, John G. Thiodan Technical, Final Report, Two Year Chronic Feeding Study - Rats [redacted] A14037	No	No	AgrEvo	No
IIA, 5.5.1	1989a	Leist, K.-H. Amendment to Report No. HST 289/881067 (Doc No. A40440) Endosulfan, active ingredient technical (Code: Hoe 002671 0I ZD97 0003) combined chronic toxicity / carcinogenicity study (104-week feeding in rats) Residue Determination [redacted] .: A41265	Yes	No	AgrEvo	No
IIA, 5.5.1	1989	Ruckman, S.A.; Waterson, L.A.; Crook, D. Endosulfan, active ingredient technical (code: Hoe 002671 0I ZD97 0003) Combined chronic toxicity / cancerogenicity study (104-week feeding in rats) (Final report). [redacted] No.: A40440	Yes	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.5.1	1978	Thomas, L.W.; Kornreich, M.R.; Walker,P. Bioassay of ENDOSULFAN for Possible Carcinogenicity [redacted] A14117 Natl. Cancer Inst., Carcinogenesis, Techn. Rep. Series. No 62. 1978	No	Yes	Publ.	No
IIA, 5.5.2	1988a	Donaubauer, H.H. Endosulfan - substance technical (Code: Hoe 002671 0I ZD97 0003), Carcinogenicity study in mice, 24 months feeding study [redacted] A38008	Yes	No	AgrEvo	No
IIA, 5.5.2	1988b	Donaubauer, H.H. Endosulfan - substance technical (Code: Hoe 002671 0I ZD97 0003) Carcinogenicity study in mice 24 months feeding study. Amendment to Document A38008 [redacted] A38884	Yes	No	AgrEvo	No
IIA, 5.5.2	1989	Donaubauer, H.H. Amendment to the Report No.88.0278 Endosulfan-substance technical (Code: Hoe 002671 0I ZD97 0003) Carcinogenicity study in mice 24 months feeding study [redacted] A41617	Yes	No	AgrEvo	No
IIA, 5.5.2	1989b	Leist, K.-H. Endosulfan - substance technical (Code: Hoe 002671 0I ZD97 0003) Carcinogenicity study in mice 24 months feeding study - Residue Determination - Amendment to Report No. 88.0278 of April 6, 1988 [redacted].: A41264	Yes	No	AgrEvo	No
IIA, 5.5.3	1987	Flodstroem, S.; Waerngard, L.; Hemming, H.; Fransson, R.; Ahlborg, U. G. Tumour Promotion Related Effects by the Cyclodiene Insecticide Endosulfan Studied in Vitro and in Vivo [redacted] A43389 Pharmacol.Toxicol. Vol. 62. Pages 230 - 235. 1987	No	Yes	Publ.	No
IIA, 5.6/01	1984	WHO Environmental Health Criteria 40 Worlf Health Organization, Geneva, Item 4	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.6/02	1992	Gilbert, M.E. A characterization of chemical kindling with the pesticide endosulfan. Neurotos. And Tertology, Vol. 14, 151-158	No	Yes	Publ.	No
IIA, 5.6.1	1982	Edwards, James A.; Hughes, Elizabeth W.; Almond, Richard H. Preliminary Investigation of the Effect of Endosulfan (Code, Hoe 02671 0I AT 209) on Reproduction of the Rat [redacted] No.: A29563	No	No	AgrEvo	No
IIA, 5.6.1	1984	Edwards, J. A.; Reid, Y. J.; Offer, J. M., Almond, R. H., Gibson, W. A. Effect of Endosulfan-Technical (Code: Hoe 02671 0I AT209) on Reproductive Function of Multiple Generations in the Rat [redacted] No.: A29428	Yes	No	AgrEvo	No
IIA, 5.6.1	1965	Kennedy, Gerald; Calandra, J.C. Three-Generation Reproduction Study in Albino Rats on Thiodan [redacted] A14054	No	No	AgrEvo	No
IIA, 5.6.1	1985	Offer, John M. Addendum to HST 204 Effect of Endosulfan-Technical (Code: Hoe 02671 0I AT209) on the Reproductive Function of Multiple Generations in the Rat Histopathological Review of the Kidneys in Adult Rats of th F1B Generation. [redacted] Report No.: A30757	No	No	AgrEvo	No
IIA, 5.6.1/01, 5.1.1.1	1978	Dorough, H.W.; Huhtanen, K.; Marshall, T.C.; Bryant, H.E. Fate of ENDOSULFAN in Rats and Toxicological Considerations of Apolar Metabolites U[redacted] A14276 Pesticide Biochem. Physiol. Vol. 8. pages 241 - 252. 1978	No	Yes	Publ.	No
IIA, 5.6.1/02	1982	Wali, R. K.; *et. al.* Effects of a single oral dose of endosulfan on intestinal uptake of nutrients and on brush-border enzymes in rats. Toxicology letters, 12; 7-12	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.6.2/01; IIIA, 7.1/03		Gorbach, S. Fate of pesticides in environment. Terminal residues of endosulfan. Fate of pesticides in environment, vol. VI, 283-285	No	Yes	Publ.	No
IIA, 5.6.2.1	1993	Albrecht, M.; Baeder, Ch. Hoe 002671 - substance technical (Code: Hoe 002671 00 ZD98 0005) Testing for embryotoxicity in the Wistar rat after oral administration [redacted] t[redacted]: A51695	Yes	No	AgrEvo	Yes
IIA, 5.6.2/01; 5.6.2.1	1978	Gupta, P.K.; Chandra, Satya V.; Saxena, D.K. Teratogenic and Embryotoxic Effects of Endosulfan in Rats [redacted] A17149 Acta Pharmacol. Toxicol. Vol. 42. pages 150-152. 1978	No	Yes	Publ.	No
IIA, 5.6.2.1	1972	Haley, S.; Plank, J. B.; Wright, P. L.; Keplinger, M. L. Teratogenic Study with Thiodan Technical in Albino Rats [redacted] A14053	No	No	AgrEvo	No
IIA, 5.6.2.1	1980	MacKenzie, Karen M.; Rao, G.N.; Thomson, Gordon M. Final Report, Teratology Study with FMC 5462 in Rats [redacted] A21393	Yes	No	AgrEvo	No
IIA, 5.6.2.2	1981	Dickie, S. M.; MacKenzie, K. M.; Rao, G. N. Teratology Study with FMC 5462 in Rabbits [redacted] A23192	Yes	No	AgrEvo	No
IIA, 5.7	1983	Roberts, Nicholas L.; Phillips, Christine N.K. Acute Delayed Neurotoxicity Study with Endosulfan -Technical (Code: Hoe 002671 0I ZD97 0003) in the Domestic Hen [redacted] No.: A32153	Yes	No	AgrEvo	No
IIA, 5.7/02	1976	Gupta, P.K. Endosulfan-induced neurotoxicity in mice and rats. Bull. Environm. Contam. Vol. 15, 708-713	No	No	Excel	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.8.1.1	1991b	Ehling, G.; Leist, K.-H. Hoe 051329; substance technical (Code: Hoe 051329 00 ZD98 0001) Testing for acute oral toxicity in the male and female Wistar rat [redacted] Germany. Report No.: A45783	Yes	No	AgrEvo	No
IIA, 5.8.1.1	1991d	Ehling, G.; Leist, K.-H. Hoe 051327; substance, pure (Code: Hoe 051327 00 ZB99 0002) Testing for acute oral toxicity in the male and female Wistar rat [redacted] Germany. Report No.: A46286	Yes	No	AgrEvo	No
IIA, 5.8.1.1	1975a	Hollander; Kramer ENDOSULFAN Lactone. Acute Oral Toxicity in Male SPF-Wistar-Rats (Vehicle: Starch Suspension) [redacted] Germany. Report No.: A06964	No	No	AgrEvo	No
IIA, 5.8.1.1	1975b	Hollander; Kramer ENDOSULFAN Sulphate = NIA 7985. Acute Oral Toxicity in Male Beagle Dogs (Vehicle: Starch Suspension) [redacted] Germany. Report No.: A06965	No	No	AgrEvo	No
IIA, 5.8.1.1	1975c	Hollander; Kramer ENDOSULFAN Sulphate = NIA 7985. Acute Oral Toxicity in Female SPF-Wistar-Rats (Vehicle: Starch Suspension) [redacted] Germany. Report No.: A06966	No	No	AgrEvo	No
IIA, 5.8.1.1	1975d	Hollander; Kramer 1-Hydroxy ENDOSULFAN Ether. Acute Oral Toxicity in Female SPF-Wistar-Rats (Vehicle: Starch Suspension) [redacted] Germany. Report No.: A06967	No	No	AgrEvo	No
IIA, 5.8.1.1	1975e	Hollander; Kramer Comperative Test on the Acute Oral Toxicity of ENDOSULFAN Ether and ENDOSULFAN Alcohol in Female SPF-Wistar-Rats (Vehicle: Suspension) [redacted] Germany. Report No.: A07170	No	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.8.1.1	1975f	Hollander; Kramer ENDOSULFAN LACTONE Acute Oral Toxicity in Female SPF-Wistar-Rats (Vehicle:Starch Suspension) [redacted] Germany. Report No.: A07171	No	No	AgrEvo	No
IIA, 5.8.1.1	1971	Kramer; Weigand Endosulfan-lactone (Vehicle: Sesame Oil). Acute Oral Toxicity to the Male and Female SPF-Wistar-K-Rat [redacted] Germany. Report No.: A18276	No	No	AgrEvo	No
IIA, 5.8.1.1	1982a	Weigand Akute orale Toxizitaet von Hoe 51329 an weiblichen Ratten [redacted] Germany. Report No.: A23296	No	No	AgrEvo	No
IIA, 5.8.1.1	1982b	Weigand Akute orale Toxizitaet von Hoe 51330 an weiblichen Ratten [redacted] Germany. Report No.: A23297	No	No	AgrEvo	No
IIA, 5.8.1.2	1991a	Ehling, G.; Leist, K.H. Hoe 051329; substance, technical (Code: Hoe 051329 00 ZD98 0001) Testing for acute dermal toxicity in the male and female Wistar rat [redacted] Germany. Report No.: A45829	Yes	No	AgrEvo	No
IIA, 5.8.1.2	1991c	Ehling, G.; Leist, K.-H. Hoe 051327, substance, pure; (Code: Hoe 051327 00 ZB99 0002) Testing for acute dermal toxicity in the male and female Wistar rat [redacted] Germany. Report No.: A 46130	Yes	No	AgrEvo	No
IIA, 5.8.1.3	1992	Stammberger, I. Hoe 051329 substance technical (Code: Hoe 051329 00 ZD99 0001) Study of the Mutagenic Potential in Strains of Salmonella typhimurium (Ames test) and Escherichia coli [redacted] Toxicol., Germany. Report No.: A49396	Yes	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.8.1.3	1993a	Stammberger, I. Evaluation of Hoe 051329; Substance technical (Code: Hoe 051329 00 ZD99 0001) in the Unscheduled DNA Synthesis Test in Mammalian Cells in Vitro [redacted] A49781	Yes	No	AgrEvo	Yes
IIA, 5.8.1.3	1993b	Stammberger, I. Hoe 051329; substance, technical micronucleus test in male and female NMRI mice after oral administration [redacted] A50772	Yes	No	AgrEvo	Yes
IIA, 5.8.1.4	1996a	Hammerl, R. Hoe 051329; substance technical (Code: Hoe 051329 00 ZD99 0002) Testing for sensitizing properties in the Pirbright-White guinea pig in a maximization test [redacted] A57234	Yes	No	AgrEvo	Yes
IIA, 5.8.1.4	1996b	Hammerl, R. Hoe 051329; substance technical (Code: Hoe 051329 00 ZD99 0002) Testing for sensitizng properties in the Pirbright-White guinea pig according to the technique of BUEHLER [redacted] A57233	Yes	No	AgrEvo	Yes
IIA, 5.8.1.4	1996c	Hammerl, R. Hoe 051329; substance technical (Code: Hoe 051329 00 ZD99 0002) Testing for primary dermal irritation in the rabbit [redacted] A56247	Yes	No	AgrEvo	Yes
IIA, 5.8.1.4	1996d	Hammerl, R. Hoe 051329; substance technical (Code: Hoe 051329 00 ZD99 0002) Testing for primary eye irritation in the rabbit [redacted] A56248	Yes	No	AgrEvo	Yes
IIA, 5.8.1.5	1964	Cervanka, Hildegard; Kay, John H.; Calandra, J.C. Ninety-Day Subacute Oral Toxicity of Thiodan Sulfate - Beagle Dogs [redacted] A14328	No	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.8.1.5	1996a	Ebert, E., Hack, R. Supplement to report no. 95.0692 Hoe 051329, substance technical (Code: Hoe 051329 00 ZD99 0001) Subchronical oral toxicity (13-week feeding study) in the Wistar rat Neurotoxicity screening [redacted] A57068	Yes	No	AgrEvo	Yes
IIA, 5.8.1.5	1996b	Ebert, E.; Hack, R. Hoe 051329, substance technival, (Code: Hoe 051329 00 ZD99 0001) Subchronic oral toxicity (13-week feeding study) in the Wistar rat [redacted].: A57069		No	AgrEvo	Yes
IIA, 5.8.1.5	1994	Stammberger, I. Hoe 051329 - substance technical (Code: Hoe 051329 00 ZD99 0001) Testing for toxicity by repeated oral administration to Beagle dogs (3-month feeding study) [redacted] A53046	Yes	No	AgrEvo	Yes
IIA, 5.8.1.5	1965	Wolf Claude, B. S.; Calandra, J.C. 90-Day Subacute Oral Toxicity of Thiodan Sulfate - Albino Rats [redacted] No.: A14329	No	No	AgrEvo	No
IIA, 5.8.1.6	1968	Schuphan, I,; Ballschmiter, K.; Toelg, G. Zum Metabolismus des Endosulfans in Ratten und Maeusen Univ.Mainz, Germany. Report No.: A14215 Z. Naturforsch. Vol. 23b. No. 5. pages 701 - 706. 1968	No	Yes	Publ.	No
IIA, 5.8.2.1	1986	Banerjee, B.D.; Hussain, Q.Z. Effect of sub-chronic endosulfan exposure on humoral and cell-mediated immune responses in albino rats National Inst. of Communicable Diseases, India. Report No.: A43391 Arch. Toxicology. Vol. 59. pages 279-284. 1986	No	Yes	Publ.	No
IIA, 5.8.2.1	1987	Banerjee, B.D.; Hussain, Q.Z. Effects of Endosulfan on Humoral and Cell-Mediated Immune Responses in Rats National Inst. of Communicable Diseases, India. Report No.: A43390 Bulletin Environ. Contam. Toxicol. Vol. 38. pages 435-441. 1987	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.8.2.1	1982	Vos, J. G.; Krajnc, E. I.; Beekhof, P. K.; van Logten, M. J. Methods for Testing Immune Effects of Toxic Chemicals: Evaluation of the Immunotoxicity of Various Pesticides in the Rat National Inst. of Public Health, Netherlands. Report No.: A31625 Pesticide Chemistry: Human Welfare and the Environment (Proc. 5th Int. Congr. Pestic. Chem. pages 497-504. 1982	No	Yes	Publ.	No
IIA, 5.8.2.2	1995	Ahlborg, U.G., Lipworth, L., Titus-Ernstoff, L., Chung-Cheng, H., Hanberg, A., Baron, J., Trichopoulos, D. and Adami, H.-O. Organochlorine Compounds in Relation to Breast Cancer, Endometrial Cancer, and Endometriosis: An Assessment of the Biological and Epidemiological Evidence Institute of Environmental Medicine, Karolinska Institute, Sweden. Report No.: A57353 Critical Reviews in Toxicology, 1995, 25, 6, 463 - 531	No	Yes	Publ.	No
IIA, 5.8.2.2	1996	ECPA ECPA Position Paper - Adequacy of Required Regulatory Hazard Testing for the Detection of Potential Hormonal Activity of Crop Protection Chemicals European Crop Protection Association, ECPA, Belgium. Report No.: A57354 European Crop Protection Assocation	No	Yes	Publ.	No
IIA, 5.8.2.2	1991	Raizada, R. B., Srivastava, M. K., Dikshith, T. S. S. Lack of estrogenic effects of endosulfan : An organochlorine insecticide in rat [redacted] Report No.: A57355 Nat. Acad. Sci. Letters, 1991, 14, 2, 103 - 107	No	Yes	Publ.	No
IIA, 5.8.2.2	1994	Soto, A. M., Chung, K. L., Sonnenschein, C. The Pesticides Endosulfan, Toxaphene, and Dieldrin Have Estrogenic Effects on Human Estrogen-Sensitive Cells [redacted] Report No.: A57357 Environmental Health Perspectives, 1994, 102, 4, 380 - 383	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.8.2.2	1995	Soto, A. M., Sonnenschein, C., Chung,K. L. et al. The E-SCREEN Assay as a Tool to Identify Estrogens: An Update on Estrogenic Environmental Pollutants University of Boston, USA. The paper was presented at the Symposium on Estrogens in the Environment, 9 - 11 Jan 1994, Washington, DC. Environmental Health Perspect 103, 1995, (Suppl 7):113 - 122,	No	Yes	Publ.	No
IIA, 5.8.2.2	1996	Stevens, J. T., Tobia, A., Lamb, J. C. et al. FIFRA Subdivision F Testing Guidelines: Are these tests adequate to detect potential hormonal activity for crop protection chemicals? Not stated. Report No.: A57358 submitted to J. Toxicol. Environ. Health	No	Yes	Publ.	No
IIA, 5.9.2	1987	Bernardelli, Brenno C.; Gennari, Maurizio C. Death caused by indigestion of endosulfan. Case report Univ. Parma, Italy. Report No.: A43387 Journal of Forensic Sciences. Vol. 32, No. 4. pages 1109-1112. 1987	No	Yes	Publ.	No
IIA, 5.9.2	1989	Geissbuehler, J.; Schlatter, I.; Schaffner, T. Cases of fatal poisoning with Endosulfan Pathol. Institut, Universitaet Bern, Switzerland. Report No.: A57045 Schweiz. med. Wschr. Vol. 119, Suppl. 28. page 33. 1989	No	Yes	Publ.	No
IIA, 5.9.2	1989	Sauer, W.; Jacober, B; Luft, D. Suizidale Intoxikation mit dem Insektizid Endosulfan Generated by: Medizin.Universitaetsklinik Tuebingen, Germany. Report No.: A43388 Intensivmed. Vol. 26. pages 35-37. 1989	No	Yes	Publ.	No
IIA, 5.9.2	1988	Shemesh, Y.; Bourvine, A.; Gold, D.; Bracha, P. Survival after acute Endosulfan intoxication Barzilai Medical Center, Israel. Report No.: A40161 Clinical Toxicology. Vol. 26, No. 3/4. pages 265-268. 1988	No	Yes	Publ.	No
IIA, 5.9.3	1990	Maddy, K.T.; Edmiston, S.; Richmond, D. Illness,Injuries, and Deaths from Pesticide Exposures in California 1949-1988 California Dep. Food Agric., Sacramento, USA. Report No.: A54105 Rev. Environm. Contam. Toxicol. Vol. 114. Pages 57 - 123. 1990	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.9.3	1988	Volger, B. Potentially Related Illnesses / Injuries of Exposure to Endosulfan in California. A Brief Summary 1976-1986 Hoechst-Roussel Agri-Vet, Sommerville, USA. Report No.: A54106	No	No	AgrEvo	No
IIA, 5.9.4 / 5.9.5.3	1984	Ebert; Weigand Testing of the Therapeutic Effect of Diazepam (Valium R) and Phenobarbital (Luminal R) in the Event of Acute Poisoning with Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) in Wistar Rats [redacted] A29211	Yes	No	AgrEvo	No
IIA, 5.9.5.3	1988	Shemesh, Y.; Bourvine, A.; Gold, D.; Bracha, P. Survival after acute Endosulfan intoxication Barzilai Medical Center, Israel. Report No.: A40161 Clinical Toxicology. Vol. 26, No. 3/4. pages 265-268. 1988	No	Yes	Publ.	No
IIA, 5.9.6	1989	Geissbuehler, J.; Schlatter, I.; Schaffner, T. Cases of fatal poisoning with Endosulfan Pathol. Institut, Universitaet Bern, Switzerland. Reoprt No.: A57045 Schweiz. med. Wschr. Vol. 119, Suppl. 28. page 33. 1989	No	Yes	Publ.	No
IIA, 5.10/01		Report on field monitoring studies on human volunteers and livestock with endocel (Endosulfan) 35 EC	No	No	Excel	No
IIA, 5.10/02		Medical data of endosulfan	No	No	Excel	No
IIA, 5.10/03		Health record of factory workers	No	No	Excel	No
IIA, 5.10/04	1967	Ely, T.S., *et. al.* Convulsions in thiodan workers Journal of occupational medicine, Vol. 9, No. 2	No	Yes	Publ.	No
IIA, 5.10.2/01	1988	Shemesh, M.D. Survival after acute Endosulfan intoxication Clinical Toxicology. Vol. 26, No. 3/4. pages 265-268. 1988	No	No	Excel	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA, 5.10.7/01		Excel Industries Medical data	No	Yes	Publ.	No
IIIA, 7.1.1	1989a	Ebert, E.; Leist, K.-H. Endosulfan; emulsifiable concentrate; 352 g/l (Code: Hoe 002671 00 EC33 B317). Testing for acute oral toxicity in the male and female Wistar rat [redacted] A42355	Yes	No	AgrEvo	No
IIIA, 7.1.1	1989b	Ebert, E.; Leist, K.-H. Endosulfan; emulsifiable concentrate; 352 g/l, (Code: Hoe 002671 00 EC33 B317). Testing for acute oral toxicity in the male and female NMRI mice [redacted] A42359	Yes	No	AgrEvo	No
IIIA, 7.1.1	1990a	Ebert, E.; Leist, K.-H. Endosulfan; Emulsifiable Concentrate; 352 g/l (Code: Hoe 002671 00 EC33 B317), testing for acute oral toxicity in the male and female rabbit [redacted] A43165	Yes	No	AgrEvo	No
IIIA, 7.1.1/01	1991a	Halaviat, B. Evaluation de la Toxicité Aigue chez le rat par voie orale Callistar 350 g/l d' endosulfan lot 1 [redacted]	Yes	No	Calliope	No
IIIA, 7.1.1/01	1994a	Rijcken, W.R. Pels Assesment of acute oral toxicity with endosulfan 35% EC in the rat. Referenca No.: 127946 [redacted]	Yes	No	Excel	No
IIIA, 7.1.2	1989c	Ebert, E.; Leist, K.-H. Endosulfan; emulsifiable concentrate; 352 g/l (Code: Hoe 002671 00 EC33 B317). Testing for acute dermal toxicity in the male and female Wistar rat [redacted] A42278	Yes	No	AgrEvo	No
IIIA, 7.1.2	1990b	Ebert, E.; Leist, K.-H. Endosulfan; Emulsifiable Concentrate; 352 g/l (Code: Hoe 002671 00 EC33 B317). Testing for acute dermal toxicity - limit test 400 mg/kg body weight - in the male and female New Zealand albino rabbit [redacted] A43164	Yes	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIIA, 7.1.2/01	1991a	Pinon, J.F. Evaluation de la Toxicité Aigue chez le rat par voie dermique Callistar 350 g/l d' endosulfan Report No.: END/R0002 [redacted]	Yes	No	Calliope	No
IIIA, 7.1.2/01	1994b	Rijcken, W.R. Pels Assesment of acute oral toxicity with endosulfan 35% EC in the rat. Referenca No.: 127957 [redacted]	Yes	No	Excel	No
IIIA, 7.1.3	1984	Hollander, H.; Weigand, W. Endosulfan - emulgierbares Konzentrat (500 g/l). Code: Hoe 002671 0I EC43 A 103. Akute Aerosolinhalation an maennlichen und weiblichen SPF-Wistar Ratten. 4 Stunden - LC 50 [redacted] A29562	Yes	No	AgrEvo	No
IIIA, 7.1.4	1989d	Ebert, E.; Leist, K.-H. Endosulfan; emulsifiable concentrate; 352 (g/l) (Code: Hoe 002671 00 EC33 B317). Testing for primary dermal irritation in the rabbit [redacted] A42356	Yes	No	AgrEvo	No
IIIA, 7.1.4/01	1991b	Halaviat, B. Evaluation de la Tolérance Cutanée chez le Lapin Callistar 350 g/l d' endosulfan lot 1 du 10.01.91 Report No.: END/R0003 [redacted]	Yes	No	Calliope	No
IIIA, 7.1.5/01	1991c	Halaviat, B. Evaluation de la Tolérance oculaire chez le Lapin Callistar 350 g/l d' endosulfan lot 1 du 10.01.91 Report No.: END/R0004 [redacted]	Yes	No	Calliope	No
IIIA, 7.1.4/01	1994c	Rijcken, W.R. Pels Primary skin irritation/corrosion study with endosulfan 35% EC in the rabbit (4 hour semi-occlusive application) Referenca No.: 127946 [redacted]	Yes	No	Excel	No
IIIA, 7.1.5	1989e	Ebert, E.; Leist, K.-H. Endosulfan; emulsifiable concentrate; 352 g/l (Code: Hoe 002671 00 EC33 B317), testing for primary eye irritation in the rabbit Hoechst L Toxikologie, Germany. Report No.: A42223	Yes	No	AgrEvo	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIIA, 7.1.6	1986	Ullmann, L.; Sachsse, K. Delayed Contact Hypersensitivity to Endosulfan-Emulsifiable Concentrate 352 (g/l) (Code: Hoe 002671 0I EC33 B310) in Albino Guinea Pigs. Buehler Test [redacted]A34194	Yes	No	AgrEvo	No
IIIA, 7.1.6/01	1991b	Pinon, J.F. Recherche du pouvoir seneibilisant sur le cobaye albinos test de maximisation selon magnusson et kligman Produit Callistar 350 g/l d´endosulfan lot 1 du 10.01.91 Report No.: END/R0005 [redacted]	Yes	No	Calliope	No
IIIA, 7.2	1994	Wolf, R. Working-hours required for application of plant protection products Report No: A53404	No	No	AgrEvo	No
IIIA, 7.2.1.2	1991	Idstein, H.; Wolf, R.; Merz, H.D. Endosulfan; emulsifiable concentrate; 352 g/l (Hoe 002671 0I EC33 B313); Endosulfan; oil in water emulsion; 350 g/l (Hoe 002671 0I EW31 A104); Comparative Examination of the user exposure during handling and application of an emulsifiable concentrate (352 g/l) and an oil in water emulsion (350 g/l) of Thiodan Hoechst ; Produktentwicklung Oekologie II, Germany. Report No.: A49473	No	No	AgrEvo	No
IIIA, 7.2.1.2	1992	Idstein, H.; Wolf, R.; Merz, H.D. Endosulfan; emulsifiable concentrate; 352 g/l (Hoe 002671 0I EC33 B313); Examination of the user exposure during handling and application of ®Thiodan 35 liquid, using a knapsack sprayer Hoechst ; Produktentwicklung Oekologie II, Germany. Report No.: A49146	Yes	No	AgrEvo	No
IIIA, 7.2.2	1993	Gilbert, A. J. Europoem - Guidance for bystander exposure data gathering Central Science Laboratory, MAFF; Hatching Green, Harpendon, Herfordshire, AL52BD, U.K.Report No. 53757	No	No	AgrEvo	No
IIIA, 7.2.3.1	1994	Siebers, J.; Smolka, S.E.; Nolting, H.G. Investigations on dichlofluanid and endosulfane in greenhouse air after pesticide application in cucumbers and chrysanthemums Federal Biological Research Centre Braunschweig. Report No.: A53509 Nachrichtenbl. Deut. Pflanzenschutzd. 1994, 46 (12), p282-286	No	Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIIA, 7.3	1986	Craine, E.M. A Dermal Absorption Study in Rats with ^{14}C-Endosulfan Wil Res.Lab., United States. Report No.: A35730	Yes	No	AgrEvo	No
IIIA, 7.3	1995	Noctor, J. C.; John, S. A. (14C)-Endosulfan: Rates of penetration through human and rat skin determined using an in vitro system [redacted] Report No.: A54103	Yes	No	AgrEvo	Yes
		British Crop Protection Council The pesticide manual incorporating The Agrochemicals Handbook, Tenth Edition		Yes	Publ.	No
	1982	Goebel, H.; Gorbach, S.; Knauf, W.; Rimpau, R.H.; Hüttenbach, H. Properties, effects, residues and analyticcs of the insecticide endosulfan. Reidue reviews, Vol. 83		Yes	Publ.	No
	1968	FAO/WHO Evaluations of some pesticide residues in food, endosulfan. Rome, Food and Agriculture Organisation of the United Nations		Yes	Publ.	No
	1969	Evaluations of some pesticide residues in food, endosulfan. Rome, Food and Agriculture Organisation of the United Nations		Yes	Publ.	No
	1984	World Health Organisation IPCS (International Programme on Chemical Safety) Environmental Health Criteria, 40, Endosulfan World Health Organisation, Geneva		Yes	Publ.	
	1995	Pluigmen, M.H.M. Endosulfan/Callistar predictive operator exposure calculations using the UK predictive operator exposure model. Calliope, S.A.		No	Calliope	Yes
References quoted by the Rapportuer						
	1997	Wade, M.G., Desaulniers, D., Leingartner, K., Foster, W.G. Interactions between endosulfan and dieldrin on estrogen-mediated proccesses in vitro and in vivo. Reprod Toxicol Nov. 1997; 11(6) : 791-798		Yes	Publ.	No

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	1996	Vonier, P.M.; Crain, D.A.; McLachlan, J.A.; Guillette, L.J. Jr.; Arnold, S.F. Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. Environ Health Perspect 1996 Dec; 104 (2) : 1318-1322		Yes	Publ.	No
	1994	Soto, A.M.; Chung, K.L.; Sonnenschein, C. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on humen estrogen-sensitive cells. Environ Health Perspect 1994 Apr.; 102 (4) : 380-383		Yes	Publ.	No
	1993	Colborn, T.; vom Saal, F.S.; Soto, A.M. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 1993 Oct.; 101 (5) : 378-384		Yes	Publ.	No
	1996	Banerjee, B.D.; Koner, B.C.; Ray, A. Immunotoxicity of pesticides: perspectives and trends. Indian J. Exp. Biol. 1996 Aug.; 34 (8) : 723-733		Yes	Publ.	No

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