ANNEX B

ENDOSULFAN

ADDENDUM B-6: TOXICOLOGY AND METABOLISM

This addendum, corresponding to Mammalian Toxicology (Section 6) has been prepared by the Toxicology Evaluation Group of the Instituto de Salud Carlos III, in order to clarify the position of the RMS with respect to the main open point issues.

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Data requirement 4.3: RMS to evaluate new genotoxicity data

Background

RMS observed that published studies concluded that Endosulfan present genotoxicity to germ cells. A convincing case to explain the reported genotoxicity to germ cells or results from an additional assay on chromosomal aberrations in mouse germ cells was required to the notifier.

The notifier submitted a position paper (C016123), but the case presented in the report was not considered convincing for the RMS. New information has been submitted by the notifier: a new position paper (C019321), an external review conducted by an internationally recognized independent expert (C026050), and a genotoxicity study about chromosome aberration in spermatogonial cells of the mouse (C032454).

All these documents had been evaluated and summarized below.

Summary

The position papers (C019321 and C016123) had been evaluated, and in them, the notifier claims for published test with positive results to be omitted, based on the fact that observed effects were seen with a non-defined test substance or may possibly due to the clearly defined toxic potential of epichlorohydrine. Nevertheless, dominant lethal mutations were induced in spermatogonia from mice treated with endosulfan of known purity (97.03%).

The notifier claims also that for all established chemical known to have genotoxic effects on germ cells also produce positive results in bone marrow assays (Shelby, 1996). There is no evidence for genotoxicity of endosulfan to somatic cells. However, two chemicals, cyclohexylamine and monomeric acrylamide, did not induce chromosomal aberrations in bone marrow cells but clastogenic in spermatogonia (Preston et al, 1981).

The notifier was requested to provide a mouse spermatogonial chromosome aberration assay (OECDE 483-test guideline), which could be include the analysis for chromosome-type aberrations at diakenesis-metaphase I when the treated cells become spermatocytes, might be appropriated.

In this sense, the notifier has submitted on May 2003 the *In vivo* chromosome aberration assay in mouse spermatogonial cells.

The genotoxicity study conducted was to the GLP and regulatory guideline 483 concurrent at the time the study was performed.

In conclusion, under conditions of this study, Endosulfan did not induce chromosome aberrations in spermatogonial cells of the mouse.

Individual study evaluation

B.6.4 Genotoxicity (IIA, 6.4)

B.6.4.3. *In vivo* studies in mammalian germ cells

B.6.4.3.1. *In vivo* chromosome aberration assay in mouse spermatogonial cells

Honarvar, N., 2003 (C032454)

Date of experimental work: 01 November 2002 to 31 January 2003. Date of report: 15 April 2003.

Objective: To evaluate the clastogenic potential of Endosulfan in the spermatogonial cells of treated

mice.

Guidelines: Cited OCDE 483 (1997).

Deviations from OECD 483 (1997): None.

Comments: Chemical analysis of the test item formulation demonstrated that the nominal dose levels were not achieved with the analysed concentration and homogeneity values. It was due to inadequate sampling procedure.

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan, batch nº AAPC10026 with purity of 98.1%. The vehicle was 1% (w/v) methyl cellulose (negative control). The positive control was Adriblastin. The study was conducted using young adult male NMRI mice.

In the chromosomal aberration test, male mice were treated once orally with the vehicle or test substance. Six animals were treated once intraperitoneally with the positive control. Clinical signs were observed at intervals of 1 h, 2-4 h, 6 h and 24 h.

Five hours prior to sampling, animals were injected intraperitoneally with colchicine. The groups of mice treated at highest dose level with Endosulfan were sacrificed at 24 and 48 h after dose. Mice treated with the positive control, vehicle and the remaining dose levels were sacrificed 24 h after treatment. Animals were sacrificed by cervical dislocation.

Five animals per group were evaluated in order to determine the analysis of metaphase cells. Testicles samples from each animal were taken, tubes were centrifuged, and cells were isolated, fixed and stained. Slides were prepared, rinsed, dried, and mounted with cover slips. Slides were examined for mitotic index (groups means and individual animal data) and structural chromosome aberrations. For mitotic index 1000 cells/animal were analysed. For structural chromosomal aberrations 100 cells/animal were analysed. Structural chromosome aberrations were classified as: gaps, deletions, exchanges, breaks, fragments, multiple aberrations and chromosomal disintegrations. The frequency of aberrant metaphases expressed as the percentage of damaged cells (cells with aberrations excluding gaps) in the total population of cells was calculated.

A nonparametric Mann-Whitney test was used to evaluate the results. The proportion of cells with aberrations in vehicle control is compared with the normal historical range. A test article is considered positive if it induces either a dose-related increase in the number of aberrant cells or a relevant positive response for at least one of the test points.

Four preliminary toxicity range finder studies were performed using groups of two mice. Clinical signs were recorded at intervals of around 1h, 2-4h, 6h, 24h, 30h and 48h. The dose levels selected for each experiment were 40, 20, 15 and 10 mg/kg respectively according with the DL_{50} calculated by the sponsor, it was between 10-110 mg/kg. The animals treated at 40 and 20 mg/kg died in the first hour after treatment. At dose level of 10 and 15 mg/kg, reduction of the spontaneous activity and ruffled fur were observed in the animals treated during the first 30 h post treatment, in mice treated at 15 mg/kg

these symptoms continued until 48 h. Abdominal position was recorded in the first hour in animals treated at 10 mg/kg and in the first 6h in one animal treated at 15 mg/kg.

Accordingly, dose levels of 3.75, 7.5 and 15 mg/kg were tested in the chromosomal aberration test. Six of the nine animals receiving the highest dose died within the first six hours after treatment. A second main experiment was performed, the dose levels selected were 2.5, 5 and 10 mg/kg, nevertheless eight of the nine animals receiving the highest dose died within the first six hours after treatment.

Finally, a third main experiment was performed, three groups were treated at 5, 2.5 and 1.25 mg/kg (12 animals treated at the highest dose level and 6 animals treated at medium and low dose level). For the chromosome analysis five animals/group were used.

Findings

Chemical analysis of the test item formulation demonstrated that the nominal dose levels were not achieved with the analysed concentration and homogeneity values. The detected levels of the test item were between 1.3-36% of the nominal values. Results are summarised on table 6.4.3.1-1.

Table 6.4.3-1: Concentration and homogeneity of Test Item in Vehicle

Nominal Concentration (mg/ml)	**Concentration found**			
	mg/ml	**% of nominal**	**Mean % of nominal**	**± Dev. In % of mean**
Date of sampling 20 Nov 02				
0.125	0.012 0.035	9.5 27.8	18.7	-49/+49
0.25	0.016 0.059	6.4 23.7	15.1	-58/+57
0.5	0.034 0.176	6.8 35.2	21.0	-68/+68
Date of sampling 25 Nov 02				
0.125	0.048 0.042	38.2 33.8	36	-6/+6
0.25	0.069* 0.028*	27.4 11.1	19.3	-42/+42
0.5	0.007* n.d*	0.007 ---	1.3	---

* result was confirmed by reanalysis; only repeated analysis is presented
n.d. no counts detected

The notifier attribute these results to inadequate sampling procedure.

No animal died during the study. Toxicological signs were dose-related increased, and time-related decreased. Reduction of the spontaneous activity and ruffled fur were observed in all treated groups. Abdominal position and eye closure were observed at 5 and 2.5 mg/kg.
Mitotic indices were not affected at any dose tested.
No statistically significant results were recorded but there was a dose-related increase in the percentage of cells with chromosomal aberrations but these results fell within the range of historical negative controls. Positive control gave a satisfactory response. Results are summarised on table 6.4.3.1-2.

Table 6.4.3.1-2: Chromosome aberration rates

Experimental group	Dose (mg/kg)	Sampling time	Total number of cells scored	% aberrant cells excl. gaps	Statistics
Vehicle	0	24	500	0.2	
Endosulfan	1.25	24	500	0.4	0.5
	2.50	24	500	1.0	0.14
	5	24	500	1.2	0.08
	5	48	500	1.0	0.14
Adriblastin	5	24	500	6.0	0.004*

* Statistical significant ($p<0.05$)

Conclusion

Endosulfan showed a dose-related increase (but not statistically) in the percentage of aberrant cells but these results fell within historical negative controls.

In conclusion, under conditions of this study, Endosulfan did not induce chromosome aberrations in spermatogonial cells of the mouse.

Data requirement 4.4: The data submitter was requested to provide genotoxicity studies performed with the sulfate metabolite

Background

Endosulfan-sulphate was considered a toxicologically relevant metabolite according to results from rat toxicity studies (oral and dermal acute, and oral subchronic toxicity studies). Genotoxicity data are necessary. The notified had submitter two studies: Ames test (**C017165)** and chromosomal abberation in cultured human blood lymphocytes (**C017169).** The evaluation of these studies is included below.

Summary

Endosulfan-sulfate is not genotoxic under the conditions of the studies.

Individual studies evaluation

B.6.8.2.3 Genotoxicity of Endosulfan-sulfate

Ballantyne M., 2001 c (Aventis CropScience C017165)

Dates of experimental work: April 27, 2001 to May 25, 2001. Date of report: July 31, 2001.

The objective of this study was to evaluate the mutagenic activity of Endosulfan-sulfate by examining its ability to revert four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 471 (1997), EEC Annex V Tests B13 and B14 (1993), UKEMS (1990), Japanese MHW (1989) and MAFF (1985), ICH Harmonised Tripartite (1997) and EPA-OPPTS 870.5100 (1998).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan-sulfate, batch number CIW999 with purity 99.3%. It was dissolved in DMSO. Appropriate positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline-1-oxide, benzo[a]pyrene and 2-amino-anthracene) were included. The study was conducted using four *Salmonella typhimurium* strains (TA1535, TA1537, TA98 and TA100) and two *Escherichia coli* strains (WP2 pKM101 and WP2 uvrA pKM101). The mammalian metabolic activation system was S9 (10%) from Aroclor 1254-induced male Sprague Dawley rats. The plate incorporation procedure was used. The m-statistic was calculated to check that the data were Poisson-distributed, and Dunnett's test was used to compare the counts of each dose level with the control. The presence or otherwise of a dose level response was checked by linear regression analysis. The test article is considered to be mutagenic if: 1) Dunnett's test give a significant response ($p \leq 0.01$) and the data set shows a significant dose level correlation; and 2) the positive responses described before are reproducible.

An initial toxicity range-finder experiment was carried out in TA100 strain only, using Endosulfan-sulfate at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls, with and without S9. Following these treatments, there was no clear evidence of toxicity (diminution of the background bacterial lawn or reduction in revertant numbers). Precipitation of test article was observed on all plates treated at the two highest concentrations during the treatment, but only at 5000 µg/plate following 3 days incubation.

Endosulfan-sulfate was assayed for mutation in two separate experiments. Negative and positive controls were included for all strains in each experiment.

In the first mutation experiment, Endosulfan-sulfate was tested at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, in the presence and absence of S9, in all strains except TA100. Data from the range-finder experiment were considered acceptable for mutation assessment, and presented in the report as the experiment 1 mutation data for TA100 strain. A diminution of the background bacterial lawn was observed, in the absence of S9, at 5000 µg/plate in TA1537, and, in the presence of S9, at 1000 and 5000 µg/plate in TA98, and at 200 µg/plate and above in TA1537. Some small reductions in revertant numbers were also observed at the highest dose levels in several strains, in the absence and/or in the presence of S9. There was precipitation of test article on all plates treated at 1000 µg/plate and above. Only treatments of WP2 uvrA pKM101 strain in the presence of S9 resulted in an increase (not dose level related) in revertant numbers that was statistically significant at 1.6 and 200 µg/plate. The maximum increase in revertant numbers above control (1.21) was obtained at 1.6 µg/plate.

Due to the appearance of toxic effects within the precipitating dose level range with at least some strain treatments in experiment 1, it was considered that the lower limit of precipitation was not an appropriate dose level-limiting factor to be employed for this study. Therefore, in the second mutation experiment, Endosulfan-sulfate was tested at concentrations of 51.2, 128, 320, 800, 2000 and 5000 µg/plate in the presence and absence of S9, in all strains. In addition, treatments in the presence of S9 were modified by the inclusion of a pre-incubation step. A diminution of the background bacterial lawn was observed, only in the absence of S9, from 320 µg/plate in TA100 and TA1537, and from 800 µg/plate in TA98. Some small reductions in revertant numbers were also observed at the highest dose levels in several strains, in the absence and/or in the presence of S9. Precipitation of test article was observed on all plates treated at 800 µg/plate and above. When mutagenicity data were analysed at the 1% level using Dunnett's test, the only statistically significant increase in revertant numbers (1.77 above control) was obtained at 320 µg/plate in TA1537 strain in the presence of S9.

Endosulfan-sulfate was considered non mutagenic because the only statistically significant increases in revertant numbers, obtained in WP2 uvrA pKM101 strain (experiment 1) and in TA1537 (experiment 2), both in the presence of S9, were extremely small in magnitude (< 2 above control), and no concentration related.

Positive controls gave a satisfactory response in all strains used in each experiment except 2-amino-anthracene that did not provided the expected increase in revertant numbers in WP2 pKM101 strain, when tested in the presence of S9. Nevertheless, metabolic activity of the *E. coli* S9 mix was established from the strain WP2 uvrA pKM101 data.

Conclusion

Endosulfan-sulfate showed no mutagenic potential under the conditions of this study.

Whitwell J., 2001 (Aventis CropScience C017169)

Dates of experimental work: May 17, 2001 to July 23, 2001. Date of report: August 22, 2001.

The objective of this study was to evaluate the clastogenic potential of Endosulfan-sulfate by examining its effects on the chromosomes of the lymphocytes of human donors, cultured *in vitro* and treated in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 473 (1997), ICH Harmonised Tripartite (1995) and EPA-OPPTS 870.5375 (1998).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan-sulfate, batch number CIW999, with purity 99.3%, and LOT 1059X, with purity 97.4%. It was dissolved in DMSO. The test article solutions were used within 2.75 hours of initial formulation. Appropriate positive controls, 4-nitroquinoline 1-oxide and cyclophosphamide, were included. Blood from three healthy, non-smoking male volunteers was used for each experiment of this study. The mammalian metabolic activation system was S9 (2%) from Aroclor 1254-induced male Sprague Dawley rats.

For each experiment, an appropriate volume of whole blood was drawn from the peripheral circulation within one day of culture initiation. Blood was stored refrigerated prior use. Cultures were set up placing heparinised blood in tubes containing culture medium, foetal calf serum, gentamycin and PHA. Blood cultures were incubated for 48 hours before treatment. All cultures received colchicine 2 hours before harvesting. Slides were examined for mitotic index (at least 200 cells were counted per concentration tested, i. e. 100 cells per replicate culture). The highest concentration used for chromosome analysis was in general that produced a suppression of the mitotic activity of about 50%. Slides from the highest selected concentration and two lower dose levels were taken for microscope analysis. Where possible, 200 metaphases were examined at each level of treatment for chromosome aberrations. The classification system used in scoring aberrations was based on the scheme described by ISCN (1995) and is detailed in Appendix 2 of this report. The aberrant cells in each culture were categorised as follows: 1) cells with structural aberrations including gaps; 2) cells with structural aberrations excluding gaps; and 3) polyploid, endoreduplicated or hyperdiploid cells. A test article is considered as positive if: a) the proportions of cells with structural aberrations at one or more concentrations exceeded the historical negative control range in both replicates, and b) a statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) occurs at these concentrations. The statistical method used was the Fisher's exact test ($p \leq 0.05$).

Preliminary solubility data indicated that Endosulfan-sulfate dissolved in DMSO precipitated into culture medium at concentrations down to approximately 544 µg/mL. Therefore, a concentration of 400 µg/mL was chosen as a suitable maximum for the chromosome aberration study. Osmolality measurements on post-treatment media were not performed because the highest concentration tested (400 µg/mL) was less than 10 mM (molecular weight of Endosulfan-sulfate = 422.9). The test article had no observed effect on

the pH of culture medium. Two mutation experiments were carried out with Endosulfan-sulfate along with concurrent negative (solvent) and positive controls.

In experiment 1, trial 1, treatment of blood cultures from donor 1 in the absence and presence of S9 was for 3 hours followed by a 17-hour recovery period prior to harvest. Endosulfan-sulfate was tested at concentrations of 9.007, 11.26, 14.07, 17.59, 21.99, 27.49, 34.36, 42.95, 53.69, 67.11, 83.89, 104.9, 131.1, 163.8, 204.8, 256, 320 and 400 µg/mL. Precipitation of test article was observed at treatment from 256 µg/mL (without and with S9), and at harvest from 104.9 µg/mL (without S9) or from 131.1 µg/mL (with S9). The mitotic index was determined at concentrations from 42.95 µg/mL to 400 µg/mL (to see Table B.6.8.2.3-1)

Table B.6.8.2.3-1 Mitototic index determination in experiment 1 trial 1 (donor 1)

Treatment (µg/mL) for 3 h	MI[a] (-S9)	MI[a] (+ S9)
42.95	5	0
53.69	15	6
67.11	22	6
83.89	36	21
104.9	69 E	22
131.1	69 E	40 E
163.8	70 E	38 E
204.8	86 E	55 E
256.0	73 PE	62 PE
320.0	69 PE	58 PE
400.0	79 PE	72 PE

[a]MI: Mitotic inhibition (%) = [1-(mean MI_T/mean MI_C)] x 100%
where treatment T= treatment and C= negative control)
P: indicates precipitation observed at treatment
E: indicates precipitation observed at harvest.

Due to the observed toxicity curve of the test article, it was not possible to identify a suitable top concentration for chromosome analysis in the absence of S9. It was therefore necessary to repeat this treatment in a separate trial (Experiment 1, trial 2), using blood cultures from donor 2 and Endosulfan-sulfate concentrations of 30, 40, 50, 60, 70, 80, 85, 90, 95, 100, 105, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200 and 210 µg/mL. Precipitation of test article was observed at treatment from 190 µg/mL, and at harvest from 90 µg/mL. The mitotic index was determined at concentrations from 40 µg/mL to 210 µg/mL (to see Table B.6.8.2.3-2). The mitotic inhibition was 54%, in the absence of S9, at 130 µg/mL (trial 2) and 55%, in the presence of S9, at 204.8 µg/mL (trial 1). Thus, the concentrations selected for chromosome analysis were 40, 80 and 130 µg/mL (- S9), and 67.11, 131.1 and 204.8 µg/mL (+ S9).

Table B.6.8.2.3-2: Mitototic index determination in experiment 1 trial 2 (donor 2)

Treatment (µg/mL) for 3 h	MI[a] (- S9)
40	0
50	9
60	5
70	7
80	27
85	36
90	41 E
95	34 E
100	40 E
105	39 E
110	43 E
120	49 E
130	54 E
140	61 E
150	54 E
160	68 E
170	48 E
180	56 E
190	53 PE
200	62 PE
210	51 PE

[a]MI: Mitotic inhibition (%) = [1-(mean MI_T/mean MI_C)] x 100%
where treatment T= treatment and C= negative control)
P: indicates precipitation observed at treatment
E: indicates precipitation observed at harvest.

In experiment 2, blood cultures from donor 3 were used. The treatment in the absence of S9 was continuous for 20 hours. Treatment in the presence of S9 was for 3 hours only followed by a 17-hour recovery period prior to harvest. Endosulfan-sulfate was tested at concentrations of: 8.047, 9.467, 11.14, 13.10, 15.42, 18.14, 21.34, 25.10, 29.53, 34.74, 40.87, 48.09, 56.57, 66.56, 78.30, 92.12, 108.4, 127.5 and 150 µg/mL (without S9), and 25.25, 29.70, 34.94, 41.11, 48.36, 56.90, 66.94, 78.75, 92.65, 109, 128.2, 150.9, 177.5, 208.8, 245.6, 289, 340 and 400 µg/mL (with S9). Precipitation of test article was observed at harvest from 108.4 µg/mL (without S9) and from 177.5 µg/mL (with S9), and at treatment, only at 400 µg/mL (with S9). The mitotic index was determined at concentrations from 18.14 µg/mL to 150 µg/mL, without S9, and from 92.65 µg/mL to 400 µg/mL, with S9 (to see Table B.6.8.2.3-3). In the absence of S9, the mitotic inhibition was 47% at 56.57 µg/mL. In the presence of S9, the maximum mitotic inhibition (44%) was observed at 245.6 µg/mL, while at the highest concentration tested (400 µg/mL) the mitotic inhibition was only 29%. The concentrations selected for chromosome analysis were 18.14, 29.53 and 56.57 µg/mL (without S9) and 109, 245.6 and 400 µg/mL (with S9).

Table B.6.8.2.3-3: Mitototic index determination in experiment 2 (donor 3)

Treatment (µg/mL) for 20 h (-S9)	MIH[a] (-S9)	Treatment (µg/mL) for 3 h (+ S9)	MI[a] (+ S9)
18.14	0		
21.34	20		
25.10	17		
29.53	25		
34.74	35	92.65	0
40.87	27	109	8
48.09	26	128.2	22
56.57	47	150.9	36
66.56	65	177.5	30 E
78.30	64	208.8	26 E
92.12	62	245.6	44 E
108.4	66 E	289	38 E
127.5	59 E	340	26 E
150.0	61 E	400	29 PE

[a]MI: Mitotic inhibition (%) = [1-(mean MI_T/mean MI_C)] x 100% where treatment T= treatment and C= negative control)
P: indicates precipitation observed at treatment; E: indicates precipitation observed at harvest.

According to the report a difference in toxicity was observed between experiment 1 and 2 for 3 h treatment in the presence of S9. We consider that this difference was also observed between trials 1 and 2 (experiment 1) for 3 h treatment in the absence of S9. The report indicates that reason for this disparity was not clear but that may have had something to do with blood donor variability and/or the presence of precipitate at the beginning and end of the treatment phases at a number of concentration levels. In addition, it should be taken into account the test substance assayed because there were two batches of technical material with different purity but it was not reported which batch was used in each experiment.

Treatment of cultures with Endosulfan-sulfate in the absence and presence of S9 (both experiments) resulted in frequencies of cells with structural chromosome aberrations that were within the historical solvent control range for the majority of concentrations analysed (to see Tables B.6.8.2.3-4, B.6.8.2.3-5, and B.6.8.2.3-6). The only exception was observed at the intermediate concentration (245.6 µg/mL) in the presence of S9 in experiment 2. However, although the aberrant cell frequency was seen to exceed the historical negative control range, this increase was small and present in just one of the two replicate cultures and was not dose related.

Positive controls gave a satisfactory response in each experiment.

Table B.6.8.2.3-4: Cells with structural chromosome aberrations excluding gaps in experiment 1

Without S9 (3 h treatment)				With S9 (3 h treatment)			
Treatment (µg/mL	Replicate	Cells scored	Cells with structural aberrations (%)	Treatment (µg/mL	Replicate	Cells scored	Cells with Structural aberrations (%)
Solvent	A	100	1 (1)	Solvent	A	100	0 (0)
	B	100	3 (3)		B	100	2 (2)
	Total	200	4 (2)		Total	200	2 (1)
40	A	100	0 (0)	67.11	A	100	2 (2)
	B	100	2 (2)		B	100	3 (3)
	Total	200	2 (1)		Total	200	5 (2.5)
80	A	100	0 (0)	131.1	A	100	1 (1)
	B	100	2 (2)		B	100	4 (4)
	Total	200	2 (1)		Total	200	5 (2.5)
130	A	87	2 (2)	204.8	A	100	3 (3)
	B	97	2 (2)		B	100	3 (3)
	Total	184	4 (2)		Total	200	6 (3)
NQO	A	100	16 (16)	CPA	A	100	57 (57)
	B	100	23 (23)		B	100	72 (72)
	Total	200	39 (19.5) *		Total	200	129 (64.5) *

*Statistical significance p≤ 0.01
NQO: 4-nitroquinoline 1-oxide
CPA: cyclophosphamide

Table B.6.8.2.3-5: Cells with structural chromosome aberrations excluding gaps in experiment 2

Without S9 (20 h treatment)				With S9 (3 h treatment)			
Treatment (µg/mL	Replicate	Cells scored	Cells with structural aberrations (%)	Treatment (µg/mL	Replicate	Cells scored	Cells with structural aberrations (%)
Solvent	A	100	1 (1)	Solvent	A	100	0 (0)
	B	100	1 (1)		B	100	2 (2)
	Total	200	2 (1)		Total	200	2 (1)
18.14	A	100	0 (0)	109	A	100	2 (2)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	200	2 (1)
29.53	A	100	2 (2)	245.6	A	100	5 (5)
	B	100	2 (2)		B	100	1 (1)
	Total	200	4 (2)		Total	200	6 (3)
56.57	A	100	3 (3)	400	A	100	2 (2)
	B	100	2 (2)		B	100	1 (1)
	Total	200	5 (2.5)		Total	200	3 (1.5)
NQO	A	100	26 (26)	CPA	A	100	38 (38)
	B	100	23 (23)		B	100	38 (38)
	Total	200	49 (24.5) *		Total	200	76 (38) *

*Statistical significance p≤ 0.01
NQO: 4-nitroquinoline 1-oxide
CPA: cyclophosphamide

Table B.6.8.2.3-6: Historical ranges for solvent controls (cells with structural chromosome aberrations excluding gaps)

Sex and S9 treatment	Total number of cells scored	Mean per 100 cells	Calculated range per 100 cells
Male (-S9)	20600	0.87	0–4
Male (+S9)	16200	0.69	0–4

On the other hand, in the majority of Endosulfan-sulfate treated cultures, the frequencies of cells with numerical chromosome aberrations were within the historical solvent control range (to see Tables B.6.8.2.3-7, B.6.8.2.3-8 and B.6.8.2.3-9). Exceptions to this were observed for treatment in the presence of S9 in experiment 1. Single cultures at the lowest and intermediate concentrations analysed (67.11 and 131.1 µg/mL) had aberrant cell frequencies that exceeded the historical negative control range. However, these increases were marginal, present in just one of the two replicate cultures in each case, and were not dose-related.

Table B.6.8.2.3-7: Cells with numerical chromosome aberrations in experiment 1

Without S9 (3 h treatment)				With S9 (3 h treatment)			
Treatment (µg/mL	Replicate	Cells scored	Cells with numerical aberrations (%)	Treatment (µg/mL	Replicate	Cells scored	Cells with numerical aberrations (%)
Solvent	A	100	0 (0)	Solvent	A	101	1 (1)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	201	1 (0.5)
40	A	100	0 (0)	67.11	A	100	0 (0)
	B	100	0 (0)		B	103	3 (2.9)
	Total	200	0 (0)		Total	203	3 (1.5)
80	A	100	0 (0)	131.1	A	102	2 (2)
	B	101	1 (1)		B	103	3 (2.9)
	Total	201	1 (0.5)		Total	205	5 (2.4)
130	A	88	1 (1.1)	204.8	A	100	0 (0)
	B	98	1 (1)		B	101	1 (1)
	Total	186	2 (1.1)		Total	201	1 (0.5)
NQO	A	100	0 (0)	CPA	A	101	1 (1)
	B	100	0 (0)		B	101	1 (1)
	Total	200	0 (0)		Total	202	2 (1)

NQO: 4-nitroquinoline 1-oxide
CPA: cyclophosphamide

Table B.6.8.2.3-8: Cells with numerical chromosome aberrations in experiment 2

Without S9 (20 h treatment)				With S9 (3 h treatment)			
Treatment (µg/mL	Replicate	Cells scored	Cells with numerical aberrations (%)	Treatment (µg/mL	Replicate	Cells scored	Cells with numerical aberrations (%)
Solvent	A	100	0 (0)	Solvent	A	100	0 (0)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	200	0 (0)
18.14	A	100	0 (0)	109	A	100	0 (0)
	B	101	1 (1)		B	100	0 (0)
	Total	201	1 (0.5)		Total	200	0 (0)
29.53	A	100	0 (0)	245.6	A	100	0 (0)
	B	101	1 (1)		B	100	0 (0)
	Total	201	1 (0.5)		Total	200	0 (0)
56.57	A	100	0 (0)	400	A	100	0 (0)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	200	0 (0)
NQO	A	100	0 (0)	CPA	A	101	1 (1)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	201	1 (0.5)

NQO: 4-nitroquinoline 1-oxide
CPA: cyclophosphamide

Table B.6.8.2.3-9: Historical ranges for solvent controls of cells with numerical chromosome aberrations

Sex and S9 treatment	Total number of cells scored	Mean per 100 cells	Calculated range per 100 cells
Male (-S9)	20686	0.41	0-3
Male (+S9)	16246	0.28	0-2

Conclusion

Endosulfan-sulfate did not exhibit clastogenic activity in cultured human lymphocytes, under the conditions of this study.

Open point 4.5: The meeting discussed the available dermal penetration data. Findings were variable but indicated long lag phase and high depot effect. The meeting concluded that reliable data were needed to address this issue satisfactorily

Background

The submitter's arguments towards a lower skin penetration of Endosulfan than 20% were presented in Wicke and Leist, 2001(Doc No C010955), where they proposed a 6% skin penetration factor to be used for operator risk assessment approach. To support this value, the notifier had perform an *in vitro* human/swine/rat skin absorption study based on new OECD guidelines. In dependence of the outcome, an *in vivo* study in swine is planned.

The in vitro human/swine/rat skin absorption study (C021864) had been received and evaluated.

In the meantime, all animal studies and human biomonitoring studies have being reevaluated (Document Numbers Studies A35730, A36685, A39677, A54103 and AASI Study AA 950305) as well as Documents C008763, C010955 and C016123.

Summary

Re-evaluation of all animal studies and human biomonitoring studies

Three *in vivo* studies have been conducted in male and female rats and in monkeys.

In the first study conducted in male rats (A35730), radiolabelled Thiodan 3EC was applied at three dose levels (0.1, 0.8 and 10 mg/kg b.w of endosulfan) to the skin and exposure periods of 0.1, 1, 2, 4, 10 or 24h were chosen. The treated skin was observed for visible signs of irritation and the rats were sacrificed at the end of each exposure period. The disposition of ^{14}C was determined. The results indicated that although about a mean of 73% and 78% of endosulfan was bound to the skin after 4h and 24h of exposure, respectively, at the three dose levels, only a mean of 1.8% at the three dose levels was absorbed into the body after the exposure period of 4h whereas 21% was absorbed after 24h of exposure with an excretion in faeces and urine of 10%. The data indicated that endosulfan accumulates early in the skin and is absorbed gradually through the skin. Endosulfan equivalents were eliminated in both urine and faeces, but 2 to 3 times more was found in the faeces than in the urine. According to the "Draft Guidance Document of Dermal Absorption" (Sanco/222/2000 rev.4, 11/04/2001), as the experiment was terminated before serial non-detects in excreta were observed and no clear decline in excreta was measured, the amount located in the skin should be considered as being absorbed. In conclusion, after an exposure time of 4h and 24h a dermal absorption in a range of 68-80% should be expected at the three dose levels in male rats.

Another study has been conducted *in vivo* in female rats (A39677). Thiodan 3EC was applied for ten hours at dose levels of 0.09, 0.98 and 10.98 mg/kg b.w. of endosulfan. At time points of 24, 48, 72 and 168h after application of the test material, sub-groups of four rats were sacrificed. The results showed that endosulfan retained in the skin was almost completely absorbed after 168h, indicating that the rate of absorption is low through the skin but almost complete. At 168h, levels of endosulfan are still present in blood, tissues and carcass. Although 94%, 95% and 91% of the absorbed dose, at 0.1, 1 and 10 mg/kg b.w., respectively, is excreted by 168h, this excretion is slow as a strong decline in excretion is not observed within 168h. In conclusion, after an exposure time of exposition of 10h a dermal absorption of 45% should be expected at 0.1 and 1 mg/kg bw, and of 20% at 10 mg/kg bw.

The study in monkeys, with Thiodan 35EC is not acceptable as the total recovery of radioactivity 96h after an exposure period of ten hours was only 47.8% in monkey 1 and 54.6% in monkey 2. Although the study is not reliable, the results indicated that endosulfan binds strongly to the skin (81-84% of the administered dose) and is absorbed gradually, as the levels of endosulfan in blood and plasma evidence it. During the first 24-36h the blood and plasma levels increased, afterwards a steady state level was obtained until the end of the experiment (96h). The excretion of endosulfan after 96h was not complete and high levels of endosulfan were still present in urine and faeces.

One dermal absorption *in vitro* study in human and rat skin has been performed (A54103).

Thiodan 35EC was applied to the epidermal surface at 0.01, 0.1 and 1.0 mg endosulfan /cm^2. For a good estimation of the dermal absorption of endosulfan through rat and human skin, the exposure period as well as the concentration examined should reflect the anticipated occupational exposure conditions. Therefore, for an agrochemical product or its spray strenght dilution, exposure time is recommended to be a 6-8h period. In this study only 4 samples for each group at the higher dose levels have been washed after a 10h exposure period. Total recovery of radioactivity in the human skin study was only of 68%. The loss of radioactivity has not been justified, therefore results are not considered appropriate for evaluation of dermal absorption in humans. Results from rat skin after a 10h exposure period indicated a 30% absorption of endosulfan. Results from human skin after a 10h exposure period indicated a 8.3% absorption, but probably it should be higher as total recovery of radioactivity was only of 68%. Results from this study also indicated that very little detoxification/degradation occurred in rat skin, but it was more extensive in human skin preparations (see below).
From all the results it can be concluded that, although the skin appears to have a depot function, the amount of endosulfan retained in the skin is absorbed gradually and almost completely. The excretion is also gradual, as a strong decline in excretion is not observed. In terms of dermal absorption, and in accordance with "Draft Guidance Document of Dermal Absorption" (Sanco/222/2000 rev.4, 11/04/2001), as no clear decline in excreta is measured, the amount located in the skin should be considered as being absorbed.

On the other hand, it is possible that longer storage time in the skin may be the basis of the higher degree of dermal detoxification. The dermal detoxification has not been well defined. The metabolites present in the skin after the exposure period in the *in vivo* studies have not been evaluated. From the

studies *in vitro* in rat and human skin after an exposure period of 72h, the results indicated a lack of extensive detoxification/degradation within the rat epidermal membrane. Nevertheless the results in human skin indicated that detoxification/degradation was occurring within the human epidermal membrane. The compounds identified in the fluid receptor after an exposure period of 72h were beta-endosulfan (27.33% of total radioactivity), endosulfan diol (34.0%) with some endosulfan sulphate (8.27%), endosulfan hydroxyether (2.67%) and an unidentified component (17.23%). Endosulfan sulphate shows a toxicological profile very similar to that of endosulfan. The metabolites endosulfan diol and endosulfan hydroxyether are less toxic than the parent compound. As the period of exposure was too long, these results can not be used, , to conclude that endosulfan is highly detoxified by the skin and, in consequence, to expect a lower extent of absorption of endosulfan.

A biomonitoring study in workers during mixing/loading and application of Thiodan 35EC for control of coffee berry borers in Colombia has been provided to calculate the absorbed dose. This study is not representative for the uses proposed for Thiodan 35EC in cotton and tomatoes. In this study the preparation has been applied at 0.49 kg a.s./ha and the proposed application rate per treatment for cotton and tomatoes is of 0.84 kg a.s./ha. In this study, two replicates of 6 workers were used instead of the minimum of ten subjects without repeated monitoring of the same individuals recommended by the "Guidance document for the conduct of studies of occupational exposure to pesticides during agricultural application" (OCDE, 1997). On the other hand, a high proportion of urine samples were not well collected by workers.(approx. 50%, 48% and 75% of Day –1, Day 0 and Day +1 Thiodan 35EC samples and 83%, 48% and 83% of Day –1, Day 0 and Day +1 blank samples). These samples are not acceptable for the purposes of this study. In conclusion, the biomonitoring study in coffee berrys is not representative of the GAPs proposed for Thiodan 35EC in cotton and tomatoes. The design of this study has not been well selected and accomplished.

Evaluation of an *in vitro* study with human, pig and rat skin

An *in vitro* study with human, pig and rat skin had been submitted. Epidermal membranes of human, pig and rat skin were treated with a single dose of radiolabelled material at three dose levels, corresponding to the commercially available 350g/l EC formulation and its aqueous dilutions (1/2.06 and 1/333 v/v). Two-exposure time was selected: 8 and 24 hours. The following amounts of Endosulfan are considered available for absorption after 8 and 24 hours exposure:

	8 hours			24 hours		
Species	Human	Pig	Rat	Human	Pig	Rat
350 g/l conc. form. (3.58 mg/cm^2)	0.82	1.30	28.37	0.96	2.06	24.60
1/2.06 v/v aq. dil. (1.71 mg/cm^2)	0.37	7.20	21.48	0.69	5.48	33.11
1/333 v/v aq. dil. (0.01 mg/cm^2)	3.69	28.73	73.70	2.69	44.81	81.70

The results obtained in this study demonstrate that the rate of penetration of endosulfan through human epidermis in vitro is much lower than through pig and rat epidermis.

As a conclusion, rat *in vivo* absorption was 45% for diluted formulation (corresponded to the dose levels of 0.1 and 1 mg/kg), and 20% for the concentrate one (corresponded to the dose level of 10 mg/kg). *In*

vitro rat absorption was 28.37% and 81.70% for concentrate and diluted formulations, and *in vitro* human absorption was 0.96% and 3.69% for concentrate and diluted solutions, respectively.

Using the equation proposed in the EC Draft Guidance on Dermal Absorption:

$$\textit{In vivo}\text{ human absorption} = \textit{in vivo}\text{ rat absorption} \times \frac{\textit{in vitro}\text{ human absorption}}{\textit{in vitro}\text{ rat absorption}}$$

and data summarised in the paragraph above, human absorption is calculated as follows:

$$\textit{In vivo}\text{ human absorption} = \frac{45\% \times 3.69\%}{81.70\%} = \textbf{2.03\% for diluted solution}$$

$$\textit{In vivo}\text{ human absorption} = \frac{20\% \times 0.96\%}{28.37\%} = \textbf{0.67\% for concentrate solution}$$

Individual studies evaluation

B.6.12 Dermal absorption

B.6.12a THIODAN 35EC

B.6.12a.1 Dermal absorption *in vivo* in rats.

Craine, E.M., 1986 (Hoechst, AG; A35730)

Dates of experimental work: April, 1986 – July 1986.

Date of report: 11 December 1986

Objectives: The study was conducted to assess the dermal absorption of ^{14}C-Endosulfan in rats.

Guidelines: U.S. EPA 1983 Federal Register, volume 48, no. 230, 40 CFR Part 160; U.S. EPA 1982 Subdivision F-Hazard Evaluation: Human and Domestic Animals

The following information is unavailable, thus making it difficult to conclude on endosulfan dermal absorption: The preparation used for the study was Thiodan 3EC. The components of the blank formulation E4847:27, code 519, were not indicated. Male rats were used instead of female rats, the more sensitive sex. The amount applied to the skin was greater than 10 µl/cm^2. Each group of animals should be terminated after different time intervals, for example at the end of the exposure period (typically 6 or 24 h) and subsequent occasions (almost at 48 and 72 h)

GLP: Yes

The study is acceptable

Materials and Methods

Three groups of 24 male rats each, were treated dermally with single doses of ^{14}C endosulfan mixed with a blank formulation (E 4847:27, code 519) at ratios to produce the preparation Thiodan 3EC. The mixture was then dispersed in water to produce a suspension similar to a spray mixture which would be used in the field. This suspension was then applied dermally to the rats. Three suspensions were prepared for the study at average dose levels of 0.1, 0.8 and 10 mg/kg b.w. of endosulfan. After exposure periods of 0.1, 1, 2, 4, 10 or 24h, the treated skin was observed for visible signs of irritation and the rats were sacrificed. The disposition of ^{14}C was determined. The amounts of test material present in blood, excreta, carcass and cage washes were measured as well as the amounts of test material washed from the skin after the exposure periods (i.e. combined rubber ring, carcass and skin of the application site) and tissues as liver, kidney, fat, untreated skin and brain.

Findings

After 4h of exposure with endosulfan applied to the skin of rats at the three dose levels, approximately 73% (76%, 74% and 68% at 0.1, 0.8 and 10 mg/kg bw, respectively) bound to the skin with 28% (27%, 26% and 32% at 0.1, 0.8 and 10 mg/kg bw, respectively) being removed by washing with soapy water. After 4 h of exposure, 71% (76%, 72% and 67%) of the applied dose (0.1, 0.8 and 10 mg/kg, respectively) remained bound to the skin and was not absorbed. Approximately 1.8% was absorbed into

the body (1.7%, 2.6% and 1.2% at the three dose levels, respectively) with only 0.07% been excreted in urine (0.04%, 0.1% and 0.08%). Excretion in faeces was not significant (Table 6.12.1, 6.12.2 and 6.12.3).

After a time period of exposure of 24h, rats were sacrificed and the disposition of endosulfan was evaluated. About 78% of the dose applied bound to the skin at the three dose levels. The dose absorbed into the body at the low doses 0.1 and 0.8 mg/kg bw was 21% with an excretion in faeces and urine of 10%. About 57% of the applied dose remained bound to the skin. At the highest dose, the dose penetrated was lower (8.4%) with an elimination in excreta of 3.7% (Table 6.12a.1-1, 6.12a.1-.2 and 6.12a.1-.3). Endosulfan equivalents were eliminated both in urine and faeces, but 2 to 3 times more was found in the faeces than in the urine.

Table 6.12a.1-1: Mean amounts of endosulfan (µg) present in different materials from groups of 4 rats sacrificed after 4 and 24 h

Rat Group	Time (h)	Endosulfan applied (µg)	Urine (µg)	Faeces (µg)	Tissues (µg)	Carcass (µg)	Application (µg)	Adjacent (µg)
I	4	25	0.01	<0.01	0.11	0.31	18.8	0.11
	24	25	0.67	2.0	0.54	2.1	14.1	0.12
II	4	242	0.24	<0.01	1.4	4.6	173	0.30
	24	222	5.3	16	5.0	21	128	0.34
III	4	2566	2.1	<0.01	7.7	21	1711	4.3
	24	2173	24	56	22	81	1449	2.1

Table 6.12a.1-2: Mean amounts of endosulfan (% of applied) present in different materials from groups of 4 rats sacrificed after 4 and 24 h

Rat Group	Time (h)	Endosulfan applied (µg)	Urine (% of applied)	Faeces (% of applied)	Faeces/ Urine	Tissues (% of applied)	Carcass (% of applied)	Skin (Application + adjacent) (% of applied)
I	4	25	0.04	<0.04	-	0.44	1.2	76
	24	25	2.7	8.0	3.0	2.2	8.4	57
II	4	242	0.1	<0.004	-	0.58	1.9	72
	24	222	2.4	7.2	3	2.2	9.4	58
III	4	2566	0.08	<0.0004	-	0.30	0.82	66.8
	24	2173	1.10	2.6	2.4	1.0	3.7	66.8

Table 6.12a.1-3: Mean amounts of endosulfan from groups of 4 rats sacrificed after 4 and 24h

Rat Group	Time (h)	Endosulfan applied (µg)	Endosulfan removed[a] (µg)	Endosulfan removed (% of applied)	Endosulfan penetrated[b] (µg)	Endosulfan penetrated (% of applied)	Endosulfan absorbed[c] (µg)	Endosulfan absorbed (% of applied)	Amounts of Endosulfan measured (% of applied)
I	4	25	6.7	27	19	76	0.43	1.7	102.8
	24	25	5.3	21	20	80	5.3	21	101.2
II	4	242	63	26	179	74	6.2	2.6	100
	24	222	46	21	176	79	47	21	100
III	4	2566	819	32	1746	68	31	1.2	99.9
	24	2173	538	25	1634	75	183	8.4	99.9

[a] The amounts of endosulfan in materials analyzed (paper cover, site wash and ring extract)
[b]The amount penetrated was defined as a sum of the amount present in tissues, in the excreta, in the carcass and bound to the skin of the application site and the skin adjacent to the application site.
[c] The amount absorbed was defined as a sum of the amount present in tissues, in the excreta and in the carcass

Conclusions

The preparation used for the study was Thiodan 3EC instead of Thiodan 35EC. As absorption can vary with solvent used and dilution, the study should have been conducted with the preparation at doses including the undiluted preparation and the preparation as diluted to recommended concentrations for uses in the field, or if not, at the strongest dilution.

The study should have been conducted in female rats, the more sensitive sex.

Animals should have been exposed to the preparation during 6 or 24h. After this exposure period the application site should have been washed three times and different groups of animals should have been terminated after different time intervals, at the end of the exposure period and at least at 48 and 72 h. This would have been relevant, in order to get insight into the fate of the amount located in the skin. The sample time should have been long enough until serial non-detects in excreta were recorded.

In this study, exposure periods of 0.1, 1, 2, 4, 10 or 24h were chosen. The treated skin was observed for visible signs of irritation and the rats were sacrificed at the end of each exposure period. The disposition of ^{14}C was determined.

The results indicated that although about a mean of 73% and 78% of endosulfan was bound into the skin after 4h and 24h of exposure at the three dose levels, only a mean of 1.8% at the three dose levels was absorbed into the body after the exposure period of 4h whereas 21% was absorbed after 24h of exposure with an excretion in faeces and urine of 10%. The data indicated that endosulfan accumulates early in the skin and is absorbed gradually through the skin. Endosulfan equivalents were eliminated in both urine and faeces, but 2 to 3 times more was found in the faeces than in the urine.

According to the "Draft Guidance Document of Dermal Absorption" (Sanco/222/2000 rev.4, 11/04/2001), as the experiment was terminated before serial non-detects in excreta were observed and no clear decline in excreta was measured, the amount located in the skin should be considered as being absorbed.

In conclusion, after an exposure time of 4h and 24h a dermal absorption of 68-80% should be expected at the three dose levels.

B.6.12a.2 Dermal absorption *in vivo* in rats.

Craine, E.M., 1988 (Hoechst, AG; A39677)

Dates of experimental work: 16 June, 1986-30 June 1986.

Date of report: 17 November 1988

Objectives: The study was conducted to assess the dermal absorption of ^{14}C-Endosulfan in rats.

Guidelines: U.S. EPA 1984 Federal Register, volume 48, no. 230, 40 CFR Part 160; U.S. EPA 1982 Subdivision F-Hazard Evaluation: Human and Domestic Animals

The following information is unavailable, thus making it difficult to conclude on endosulfan dermal absorption: The preparation used for the study was Thiodan 3EC. The components of the blank formulation E4847:27, code 519, were not indicated.

GLP: Yes

The study is acceptable.

Materials and Methods

Three groups of 16 female rats each were treated dermally with single doses of ^{14}C endosulfan mixed with a blank formulation (E 4847:27, code 519) at ratios to produce the preparation Thiodan 3EC.

The mixture was then dispersed in water to produce a suspension similar to a spray mixture which would be used in the field. This suspension was then applied dermally to the rats. Three suspensions were prepared for the study at average dose levels of 0.09, 0.98 and 10.98 mg/kg b.w. of endosulfan. After an exposure period of 10 h, the application site was washed with soapy water to remove unabsorbed test material. The rats were placed back into the metabolism units and the disposition of ^{14}C was determined. At time points of 24, 48, 72 and 168h after application of the test material, sub-groups of four rats were sacrificed. The amounts of test material removed from the skin (paper covering the application site, soapy water wash solutions, ring defining application site) after the 10h exposure period were determined. The amounts of endosulfan equivalents present in blood, tissues (liver, kidneys, fat, brain, untreated skin, skin of the application site and of the adjacent site), excreta and carcass taken at sacrifice were determined.

Findings

Endosulfan equivalents which absorbed and passed through the skin, appeared both in urine and faeces within 24 h after exposure to the test material. Although exposure was limited to 10 h, endosulfan equivalents continued to be eliminated through the 7-day experimental period. Consistently there was about twice as much in faeces as in the urine. After 168h, an average of 95% of the dose bound to the skin had been excreted. The amounts absorbed, increased with increasing dose level and time. The increases suggested that the endosulfan equivalents associated with skin continued to be absorbed through the skin after the first 24h. Thus the actual amounts, which were absorbed at 168h, were better indications of the extent of absorption. The average amounts which were absorbed by the skin of the rats of Groups I and II, calculated as a percent of the dose, were about the same (45% and 46%) but were lower for Group III (20%). At this time only 1.7, 1.5 and 1.0 percent of the dose, respectively, was associated with the application site. Therefore, by 168h after exposure, the absorption process was

essentially complete. Excretion iniatilly lags absorption, but by 168h 94%, 95% and 91% of the absorbed dose, at 0.1, 1 and 10 mg/kg, respectively had been excreted. Therefore, it can be concluded that essentially all the absorbed endosulfan had been excreted by 168h. There did not appear to be any accumulation in the major organs or tissues. The concentrations appeared to peak at about 48h, the time of maximum blood concentration.

Table 6.12a.2-1: Mean amounts of endosulfan (% of applied) present in different materials from groups of 4 rats sacrificed at different times after an exposure period of 10h

Rat Group	Time (h)	Endosulfan applied (µg)	Endosulfan in blood (µg)	Urine (% of applied)	Faeces (% of applied)	Urine + Faeces (% of applied)	Urine/ Faeces	Tissues + carcass (% of applied)	Skin (Application + adjacent) (% of applied)
I	24	20.9	0.01	3.5	5.6	9.1	1.6	13.0	41.4
	48	20.7	0.02	7.9	14.8	22.7	1.9	12.6	23.8
	72	21.0	0.01	10.4	21.9	32.3	2.1	6.6	7
	168	21.0	0.01	13.7	28.6	42.3	2.1	2.5	1.7
II	24	236	0.12	2.7	3.2	5.9	1.2	10.2	39.0
	48	237	0.18	7.2	13.6	20.8	1.9	15.3	17.3
	72	238	0.11	7.2	15.2	22.4	2.1	7.4	11.8
	168	237	0.08	13.1	31.1	44.2	2.4	2.3	1.5
III	24	2512	0.30	0.7	0.6	1.3	0.8	2.5	33.0
	48	2482	0.74	2.4	3.2	5.6	1.3	5.5	20.2
	72	2510	0.51	2.8	5.6	8.4	2.0	3.6	13.4
	168	2505	0.41	5.8	13.2	19.0	2.2	1.4	1.0

Table 6.12a.2-2: Mean amounts of endosulfan (% of applied) present in different materials from groups of 4 rats sacrificed at different times after an exposure period of 10h

Rat Group	Time (h)	Endosulfan applied (µg)	Endosulfan removed (% of applied)	Endosulfan penetrated (% of applied)	Endosulfan absorbed[c] (% of applied)	Amounts of Endosulfan measured (% of applied)
I	24	20.9	43.1	63.5	22.1	106.6
	48	20.7	46.2	59.1	35.3	105.3
	72	21.0	38.2	46.0	39.0	84.2
	168	21.0	39.9	46.5	44.8	86.4
II	24	236	60.1	55.1	16.1	115.2
	48	237	64.3	53.5	36.2	117.8
	72	238	56.2	40.5	28.7	96.7
	168	237	54.7	47.9	46.4	102.6
III	24	2512	61.8	36.8	3.8	98.6
	48	2482	78.7	31.3	11.1	110.0
	72	2510	71.7	25.4	12.0	97.1
	168	2505	71.8	21.3	20.3	93.1

[a] The amounts of endosulfan in materials analyzed (paper cover, site wash and ring extract)
[b]The amount penetrated was defined as a sum of the amount present in tissues, in the excreta, in the carcass and bound to the skin of the application site and the skin adjacent to the application site.
[c] The amount absorbed was defined as a sum of the amount present in tissues, in the excreta and in the carcass

Conclusion

Endosulfan retained in the skin was almost completely absorbed after 168h, indicating than the rate of absorption is low through the skin but almost complete. At 168h, levels of endosulfan are still present in blood, tissues and carcass. Although 94%, 95% and 91% of the absorbed dose is excreted by 168h, this excretion is slow as a strong decline in excretion is not observed within 168h.

In conclusion, after an exposure time of exposition of 10h a dermal absorption of 45% should be expected at 0.1 and 1 mg/kg bw, and of 20% at 10 mg/kg bw.

B.6.12a.3 Dermal absorption *in vivo* in monkeys

Lachmann, G., 1987 (Hoechst, AG; A36685)

Dates of experimental work: Not indicated

Date of report: 08 May 1987

Objectives: The study was conducted to assess the dermal absorption of ^{14}C-Endosulfan in monkeys.

Guidelines: Not indicated

The following information is unavailable, thus making it difficult to conclude on endosulfan dermal absorption: The components of the blank formulation E4847:27, code nº Hoe 002671 OI EC 00 A301, were not indicated. The total recovery of radioactivity was only of 50% and no justification was provided

GLP: Yes

The study is not acceptable as the total recovery of radioactivity was only of 50% and no justification was provided**.**

Materials and Methods

Two male Rhesus monkeys were treated dermally with single doses of ^{14}C endosulfan mixed with a blank formulation (E 4847:27, code nº Hoe 002671 OI EC 00 A301) to produce the preparation Thiodan 35EC. The resulting concentration of the test compound in the formulation was 29.48% (w/w). The mixture was then dispersed in water to produce a suspension (the administered dose was 2.2 mg/kg for monkey 1 and 3.0 mg/kg for monkey 2). The suspension was then applied dermally to the monkeys and ten hours after application, the treated skin was washed with a soap solution with the aid of disposable wash-cloths. The remaining radioactivity in application vials, paint-brushes and wash-cloths was measured. After the exposure time, blood and plasma levels, and renal and faecal excretion were measured over a period of 96h. At the end of the experiment the tissue distribution of ^{14}C-endosulfan equivalents was examined in liver, kidneys, brain, fat, residual carcass, muscle below the treated skin, muscle of the hind limb, skin at the inner side of the hind limbs, skin at the back side of the hind limbs, hands and the treated skin. The radioactivity in the samples was measured with a Searle-Mark III liquid scintillation counter with external standardization.

A quantification of the metabolites of urine and faeces was performed for 3 urine (0-24h, 24-48h, 48-72h) and faeces (0-24h, 24-48h, 48-72h) fractions, respectively. The urine and faeces samples were chromatographed on silica gel plates after enzymatic hydrolysis of the glucuronide and sulfate conjugates and as native samples. After chromatography, the silica gel plates were exposed to X-rays films for 20 days. The autoradiographs were examined qualitatively. For the quantification of metabolites of urine only the urine of monkey 2 was used because this animal excreted a more concentrated urine with a higher radioactivity. For the metabolites of faeces equal volumes of the supernatants of the homogenates of both animals were pooled. Since the radioactivity of urine and faeces was very low, the samples were concentrated prior to HPLC analysis by lyophilization.

The disposition of ^{14}C was determined. The amounts of test material present in blood, excreta ,carcass and cage washes were measured as well as the amounts of test material washed from the skin after the

exposure periods (i.e. combined rubber ring, carcass and skin of the application site) and tissues as liver, kidney, fat, untreated skin and brain.

Findings

Blood and plasma levels are shown in Table 6.12a.3-1.

Table 6.12a.3-1: Blood and plasma levels of ^{14}C-endosulfan equivalents (ng/ml)

	Blood		Plasma	
Time (h)	**Monkey 1**	**Monkey 2**	**Monkey 1**	**Monkey 2**
1	6.5	4.3	10.7	6.1
2	8.3	6.2	11.1	9.3
4	8.1	10.3	13.4	16.5
8	14.3	8.1	21.8	13.1
12	18.5	9.9	28.3	14.1
24	24.5	13.7	36.2	19.6
36	28.3	19.2	39.9	26.8
48	28.6	19.5	38.4	26.3
72	26.5	22.8	38.2	30.3
96	27.8	23.8	36.9	30.3

During the first 24-36h of the experiment the blood and plasma levels increased, afterwards a steady state level was obtained until the end of the experiment. This indicates a steady state between uptake and elimination of endosulfan for blood and plasma.

The cumulative excretion of ^{14}C endosulfan equivalents in % of the administered dose in urine, faeces, treated skin, tissues, carcass and wash-clothes over a period of 96h after an exposure of 10h is summarized in Table 6.12a.3-2.

Table 6.12a.3-2: Cumulative excretion of ^{14}C endosulfan equivalents in % of the administered dose

Time (h)	**Monkey 1**	**Monkey 2**
Urine		
-10 – 0	0.05	0.06
24	0.92	2.98
48	1.85	3.52
72	2.52	3.92
96	3.02	4.33
Cage washes (96h)	3.24	2.53
Faeces		
-10 – 0	0.02	0.02
24	0.39	0.27
48	2.17	1.36
72	2.83	2.09
96	5.01	3.65
Treated skin (96h)	8.26	13.90
Tissues (96h)	1.23	1.40
Carcass (96h)	10.85	10.17
Wash-cloth	16.15	18.62
Total recovery (96h)	47.76	54.58

The levels of ^{14}C endosulfan equivalents in brain at the time of necropsy (96h) were very low. They amounted to one third of the blood levels at that time. Therefore an accumulation of endosulfan in brain is not possible. The radioactivity of muscle (hind limb) was lower than the blood levels, too. All the

other tissues showed higher values than the respective blood levels, especially the liver, indicating a high metabolic activity and/or an enterohepatic circulation. Evidence for the latter is given in the relatively high radioactivity of the late faeces fractions (72-96h).

The most important metabolite of the native urine was endosulfan-diol, the maximum amount of which (1.5% of the administered dose) was excreted between 0-24h. Besides, remarkable amounts of radioactivity remained at the origin of the chromatogram, indicating more polar metabolites. Further an unknown metabolite, presumably endosulfan hydroxycarboxylic acid, amounted to 0.6% of the administered dose for the same sampling interval. Only neglegible amounts of α- and β-endosulfan were found in the urine.

In faeces, the unknown metabolite was the most important metabolite (0.4% of the dose, 24-48h and 72-96h sampling intervals). Endosulfan-diol could only be detected for the 24-48h sampling interval. The sum of α- and β-endosulfan amounted to 0.1% of the dose for both the 24-48h and the 72-96h sampling interval.

Conclusions

The total recovery of radioactivity at 96h after an exposure period of ten hours was only 47.8% in monkey 1 and 54.6% in monkey 2. In consequence, the study is not acceptable.

After the ten hour exposure period the application site was washed with a soap solution and the radioactivity measured in the washes was 16.1% and 18.6% of the administered dose in monkey 1 in monkey 2, respectively. These results indicate that 84% and 81% of endosulfan penetrated into the skin of the monkeys 1 and 2, respectively.

According to the "Draft Guidance Document of Dermal Absorption" (Sanco/222/2000 rev.4, 11/04/2001), as no clear decline in excreta was measured 96h after the ten hour exposure period, the amount located in the skin should be considered as being absorbed.

In conclusion, although the study is not acceptable and considereing the results of washes reliable, after an exposure time of exposition of 10h a dermal absorption of 81-84% of the administered dose should be expected after the administration of 2.2 or 3 mg/kg b.w. to the monkeys 1 and 2, respectively.

B.6.12a.4 Dermal absorption *in vitro* in human and rat skin

Noctor, J.C. and John S.A., 1995 (Hoechst, AG; A54103)

Dates of experimental work: 3 February – 10 May 1993 and 2 June-8 November 1993

Date of report: 10 May 1995

Objectives: The study was conducted to determine and compare the rates of absorption of radioactivity following dermal applications of ^{14}C-endosulfan to non-occluded excised human and rat skin at 3 dose levels. Information is required on the comparative rates of penetration between rat and man following dermal exposure to the field formulation, endosulfan 35EC.

Guidelines: Not indicated

The following information is unavailable, thus making it difficult to conclude on endosulfan dermal absorption: The adequate solubility of the test chemical in the receptor fluid as well as the affectation of integrity of the skin by the receptor fluid have not been studied. The components of the formulation vehicle batch nº Hoe 002671 OI EC 00 A212, were not indicated. The amount applied to the skin was greater than 10 µl/cm^2. The total recovery of radioactivity in human skin was lesser than 100 ± 10% at the highest dose level and no justification was provided. Reference compounds to increase the confidence of the results have not been included.
GLP: Yes

The study is not validated

Materials and Methods

Pieces of excised skin (rat and human) were partially thawed and cut to a uniform thickness (0.4 mm) using a dermatome. The resulting section consisted of intact epidermis and a portion of dermis. Skin sections were used immediately or stored flat at –20ºC until used. On the day prior to dose application, the skin sections were thawed and mounted in a "Franz type" static *in vitro* dermal penetration cell. Each section was then assigned a unique experimental number. The integrity of the epidermal barrier of each skin preparation was assessed prior to application of ^{14}C-endosulfan.

The rate of penetration of ^{14}C-endosulfan through isolated human and rat skin preparations was assessed *in vitro* following a single application of radiolabelled formulation at 0.01, 0.1 and 1.0 mg endosulfan/cm^2 to the epidermal surface. The test article was dissolved in formulation vehicle to provide a nominal concentration of 352g active ingredient/L, consisting of alpha-endosulfan:beta-endosulfan in the ratio 2:1. The formulation was then diluted in deionised or HPLC grade water to 0.4, 4 and 40 mg/ml, providing a nominal application volume of 0.064 ml/preparation (25 µl/cm^2).
Immediately prior to dose application, the receptor chamber was refilled with a known volume of acidified ethanol/water (1:1 v/v), pH 5.5. The receptor fluid was mantained at 32 ± 2ºC and stirred constantly on a magnetic stirrer. The test article was applied to the epidermal surface of each skin preparation by syringe as follows:

Table 6.12a.4-1: Study design

Dose group	Species	Dose level (mg/cm^2)	Nº of skin preparations
A	Rat	1.0	12*
B	Rat	0.1	8
C	Rat	0.01	8
D	Human	1.0	12*
E	Human	0.1	8
F	Human	0.01	8

*Four preparations washed at 10h post-application to remove surface test article

Duplicate aliquots (0.05 ml) of receptor fluid were taken at the following time points after dose application: 1, 2, 4, 8, 10, 16, 24, 48 and 72 h. An equal volume of fresh receptor fluid was added to the receptor chamber after each sampling occasion. At 10h post-application the epidermal surface of four skin preparations each from dose groups A and D were washed with Liquid Ivory soap and rinsed with

deionised water. At 72 h post-application the receptor fluid was removed from the receptor chamber. The epidermal surface of the skin was washed with Liquid Ivory soap rinsed with deionised water and dried. The skin section was removed from the cell and weighed prior to solubilisation. Radioactivity in receptor fluid, washings and skin samples was assayed by liquid scintillation counting. The presence of metabolites in the receptor fluid at 72h post-application from groups A and D were studied using an HPLC system.

Findings

Following a single application of the preparation to rat skin preparations, penetration rates of 0.88, 4.36 and 15.91 µg endosulfan/cm^2/h were observed at the low, intermediate and high dose levels, respectively. The extrapolated lag times were 0.072, 0.74 and 0.43 h at the low, intermediate and high dose levels, respectively.

Following a single application of the preparation to human skin preparations, penetration rates of 0.22, 0.76 and 5.16 µg endosulfan/cm^2/h were observed at the low, intermediate and high dose levels, respectively. The extrapolated lag times were 0.64, 1.49 and 1.09 h at the low, intermediate and high dose levels, respectively.

Following a single application of the preparation to rat skin preparations, 95.7, 75.9 and 40.2 of the applied dose (low, intermediate and high dose levels, respectively) was recovered in the receptor fluid after 72h. 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 10h post-application, a mean of 51.05% of the applied dose was recovered in washings, and the residual radioactivity was subsequently recovered from receptor fluid (9.13%), terminal washings (7.47%) and skin (20.61%). Following 72h exposure, chromatographic analysis of the receptor fluid showed that the percentages of total radioactivity were mainly beta-endosulfan (81.4%) with some alpha-endosulfan (3.05%), endosulfan-sulfate (2.91%) and endosulfan diol (8.80%) indicating a lack of extensive detoxification/degradation within the rat epidermal membrane.

Following a single application of the preparation to human skin preparations, 60.5, 29.4 and 19.9% of the applied dose (low, intermediate and high dose levels, respectively) was recovered in the receptor fluid after 72h. 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 10h 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 (4.01%), terminal washings (0.83%) and skin (4.28%). The penetrant was identified by HPLC as mainly beta-endosulfan (27.33% of total radioactivity) and endosulfan diol (34.0%) with some endosulfan sulphate (8.27%), endosulfan hydroxyether (2.67%) and an unidentified component (17.23%), indicating that detoxification/degradation was occurring within the human epidermal membrane.

Table 6.12a.4-2: Findings scheme

Dose group	Species	Dose level applied (mg/cm^2)	Endosulfan in receptor fluid over 72h (% of applied)	Endosulfan removed (% of applied)		Endosulfan in skin over 72h (% of applied)	Endosulfan absorbed (% of applied)	Total recovery radioactivity at 72h
				Washings (10h)	Washings (72h)			
A (10h exposure)	Rat	1.0	9.13	51.05	7.47	20.61	29.74	88.26
A1 (72h exposure)	Rat	1.0	40.23		23.77	30.69	71.02	94.68
B (72h exposure)	Rat	0.1	75.91		3.90	14.29	90.20	94.10
C (72h exposure)	Rat	0.01	95.75		1.73	13.30	109.05	110.8
D (10h exposure)	Human	1.0	4.01	58.71	0.833	4.28	8.29	67.83
D1 (72h exposure)	Human	1.0	19.97		49.32	6.68	26.65	75.97
E (72h exposure)	Human	0.1	29.39		44.28	13.54	42.93	87.21
F (72h exposure)	Human	0.01	60.55		25.59	7.39	67.94	93.53

Conclusion

For a good estimation of the dermal absorption of endosulfan through rat and human skin, the exposure period as well as the concentration examined should reflect the anticipated occupational exposure conditions. Therefore, for an agrochemical product or its spray strenght dilution exposure time is recommended to be a 6-8h period.

In this study only 4 samples for each group A and D have been washed after a 10h exposure period, but total recovery of radioactivity in the human skin study was only of 68%. The loss of radioactivity has not been justified, therefore results are not appropriate for estimation of dermal absorption in humans. Results from rat skin after a 10h exposure period indicated a 30% absorption of endosulfan. Results from human skin after a 10h exposure period indicated a 8.3% absorption, but probably they should be higher as total recovery of radioactivity was only of 68%.

B.6.12a.5 Biomonitoring study (absorbed dose)

Singer, G.M. et al., 1997 (AgrEvo GmbH, AASI Study AA950305)

Date of report: 28 February 1997

Objectives: This biomonitoring study was conducted to quantify the residues of endosulfan and four metabolites in workers for up to 48h after handling Thiodan 35EC for control of coffee berry borers in

Colombia. The presence of endosulfan metabolites in the urine was expected to reflect the actual absorption of endosulfan residues by the workers.
Guidelines: USEPA Pestice Assessement Guidelines, Subdivision U: Applicator Exposure Monitoring, 1987.
Deviations: A high proportion of urine samples were not appropriately collected by workers.
GLP: Yes

The study is not validated as a high proportion of urine samples were not appropriately collected by workers.

Materials and Methods
Thiodan 35EC was open-pour mixed into the tanks of hand-pump-powdered backpack sprayers at the label-specified rate of 0.4 L/100L water (applied at approx. 250-350 L finished spray/ha for plant densities of 5000-10000 trees/ha, or approx. 50 ml finished spray/tree); each tank load consisted of 72 ml of EC in 18 L water (0.49 kg/ha). Water from local sources was used to make up the spray mixture at each test site.

A total of twenty-four replicates of mixing/loading/spraying were performed at five locations in the Department of Caldas. Each replicate consisted of one worker open-pour mixing/loading and applying finished spray for periods of approx. 4h (within 10-min breaks at each mix/load cycle). Approximately 50 ml of finished spray was applied to each plant sprayed. Each of six workers applied two replicates each (3 days apart) using Thiodan 35EC formulation as the test substance: the remaining six workers applied two replicates each using a specially prepared formulation containing no active ingredient. The blank formulation was used to establish whether any observed health effects and residue excretion were actually due to the test product.

The exposure to endosulfan of workers before and after the mix/load/spray task was estimated from the residues of six compounds (endosulfan α and β-isomers, and endosulfan sulfate, lactone, diol and hydroxy ether metabolites) found in the total daily urine output on the day of the task (day 0) and the day after the task (day +1). Each worker collected his total urine output on each day into a fresh, labelled steel can, which was kept in a foam-lined cooler with “blue ice” between voidings. Study personnel collected each can at the end of the collection period, recorded the volume collected, and poured a 200 ml aliquot of the sample into a glass bottle for freezing and shipment to the analytical laboratory. Day –1 (day before application) samples were pooled for aliquoting and shipment, and were used for preparing laboratory-fortified controls. In order to determine whether worker urine samples represented full-day urine outputs, creatinine levels were determined in each sample using a variation of the Jaffe reaction.

While performing each replicate of his monitored tasks, each worker wore a new long-sleeved work shirt and work trousers, chemical-resistant boots, plastic goggles, a billed hat, a disposable half-mask respirator, and fresh chemical resistant gloves.

On each day of monitoring, four mix/load/spray replicates were performed approximately simultaneously in adjacent plots at the test site. Due to Colombian import restrictions, control urine samples could not be fortified with the endosulfan compounds in the field; control samples were fortified at the analytical laboratory.

Residues were extracted from the samples by partinioning with 25:75:3 (v:v:w) acetone:water:NaCl in water and dichloromethane (DCM). The combined, filtered DCM phases were evaporated to 1 ml and mixed with hexane. An aliquot was cleaned up on a silica gel column, and residues were eluted with 90:10 toluene:acetone. The eluate was evaporated to approximately 1 ml. Fifty µl of the extract were derivatized and analyzed by GC with an DB^{TM}-1701 capillary column and electron capture detection. The LOQ for residues of endosulfan isomers and metabolites in urine by this method was 0.01 ppm. Measured residues in worker samples collected each day (days 0 and +1) were adjusted for mean recoveries of endosulfan compounds from field- or laboratory-generated pooled control urine samples analyzed concurrently.

Complete medical evaluations were performed on each applicator prior to and subsequent to the application. A pre-codified medical questionnaire, provided by AgrEvo, was used. It included a total of 47 signs and symptoms that eventually could be presented in the event of intoxication with Thiodan 35EC.

Findings

As shown in Table 6.12a.5-1, workers produced urine volumes of 0.47-1.97 L on Day –1, 0.51-9.85 L on Day 0 and 0.26-2.00 L on day +1. Two particularly large Day 0 samples (9.85 and 4.31 L, collected during blank replicate 18 and EC replicate 17, respectively) were collected on the Monday following a holiday Sunday. Urinary creatinine excretion per day is relatively constant for each person and is primarily dependant on a person's weight and sex. It was intended that urinary creatinine levels would be measured during the course of the field phase of the study to establish the completeness of each worker's 24h urine sample, and, hence, its acceptability for the purposes of this study. The results shown in Table 6.12a.5-1, showed that creatinine levels in approx. 50%, 42% and 25% of Day –1, Day 0 and Day +1 Thiodan 35EC samples and 17%, 42% and 17% of Day –1, Day 0 and Day +1 blank samples were within the range expected for typical adult males based on weight.

Table 6.12a.5-1: Mix/load/spray parameters during study AA950305

Replicate N° and type	Exposure (h)	Urine volume collected (L)			% of expected creatinine level collected		
		Day –1	Day 0	Day +1	Day –1	Day 0	Day +1
Thiodan 35EC							
1	3:58	1.40	1.44	0.31	87	107	45 (a)
3	3:58	0.88	1.92	1.34	82	102	64 (a)
4	3:58	1.06	1.93	0.28	50 (a)	121 (a)	28 (a)
5	3:33	1.13	0.52	2.00	75 (a)	87	145 (a)
8	4:10	0.47	0.79	1.72	55 (a)	110	122 (a)
12	2:00	1.71	1.20	1.71	N/D	N/R	N/R
13 (rep.1)	4:25	1.03	N/C	0.70	101	N/C	89
15 (rep.3)	4:30	1.66	N/C	1.84	93	N/C	107
16 (rep.4)	4:30	1.46	N/C	0.44	114	N/C	44 (a)
17 (rep.5)	3:45	1.62	4.90	2.00	104	194 (a)	92

Replicate Nº and type	Exposure (h)	Urine volume collected (L)			% of expected creatinine level collected		
		Day –1	Day 0	Day +1	Day –1	Day 0	Day +1
20 (rep.8)	4:04	1.97	2.80	0.76	66 (a)	84	59 (a)
24 (rep.12)	4:35	1.27	0.51	0.67	N/D	N/R	N/R
Blank							
2	3:41	0.79	1.05	0.38	N/D	52 (a)	63 (a)
6	4:21	1.14	1.92	1.805	60 (a)	85	72 (a)
7	4:15	1.35	1.07	1.950	84	95	149 (a)
9	2:24	0.72	1.34	0.68	69 (a)	96	37 (a)
10	2:20	1.71	1.33	1.26	106	89	74 (a)
11	2:32	0.62	1.08	0.26	56 (a)	108	33 (a)
14 (rep.2)	4:22	0.90	N/C	1.05	40 (a)	N/C	88
18 (rep.6)	4:25	1.40	9.84	1.41	N/D	277 (a)	74 (a)
19 (rep.7)	2:25	1.94	1.90	1.86	N/D	77 (a)	97
21 (rep.9)	4:47	0.52	0.99	0.26	50 (a)	44 (a)	23 (a)
22 (rep.10)	4:43	0.54	1.36	0.58	49 (a)	77 (a)	35 (a)
23 (rep.11)	4:40	1.34	1.62	0.80	N/D	67 (a)	39 (a)

(a): outside normal range of 80-120% of expected values
N/D: not determined (sample pooled, so creatinine could not be determined)
N/R: weight not recorded
N/C: not collected

Recoveries of endosulfan isomers and metabolites from samples of urine fortified in the laboratory with 0.01 or 0.1ppm and analyzed concurrently with field samples were:

α-isomer: 96 ± 12%, n=17

β-isomer: 98 ± 7%, n=18

sulfate: 101 ± 13%, n=18

lactone: 97 ± 8%, n=18

diol: 97 ± 11%, n=18

OH-ether: 92 ± 8%, n=18

No quantifiable residues of the expected endosulfan metabolites were found in any of 9 urine samples collected from workers between 0 and 24h after they mixed, loaded, and applied Thiodan 35EC to coffee, or in any of 10 samples collected between 24 and 48h after their work (Table 6.12a.5-2). Low levels (at or slightly above the LOQ) of one or both isomers of the parent compound were found in three samples. On day 0, α-endosulfan appeared at 0.014 ppm in a reliable sample (replicate 5). On day +1, α-endosulfan was present in replicates 3 and 4 and β-isomer in replicate 3. Replicates 3 and 4 were partial collections, so results are not reliable.

Table 6.12a.5-2: Residues of endosulfan compounds in Day 0 and Day +1 worker urine samples.

Repli Cate Nº and type	% of expected creatinine level collected		Residues in worker urine (ppm)											
			Day 0						Day +1					
	Day 0	Day +1	α	β	sulf	lact	diol	OH-ether	α	β	sulf	lact	diol	OH-ether
Thiodan 35EC														
1	107	45 (a)	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
3	102	64 (a)	NQ	NQ	NQ	NQ	NQ	NQ	0.014	0.010	NQ	NQ	NQ	NQ
4	121 (a)	28 (a)	NQ	NQ	NQ	NQ	NQ	NQ	0.012	NQ	NQ	NQ	NQ	NQ
5	87	145 (a)	0.014	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
8	110	122 (a)	NQ	NQ	NQ	NQ	NQ	NQ	-	-	-	-	-	-
12	N/R	N/R	NQ	NQ	NQ	NQ	NQ	NQ	-	-	-	-	-	-
13	-	89	-	-	-	-	-	-	NQ	NQ	NQ	NQ	NQ	NQ
15	-	107	-	-	-	-	-	-	NQ	NQ	NQ	NQ	NQ	NQ
16	-	44 (a)	-	-	-	-	-	-	NQ	NQ	NQ	NQ	NQ	NQ
17	194 (a)	92	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
20	84	59 (a)	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
24	N/R	N/R	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ

-: sample not collected or bottle-broken sample
NQ: not quantifiable, <LOQ (0.01 ppm)
N/R: weight not recorded

Pre-application (Day –1) samples were not analyzed individually, but were pooled and used as fortified and/or nonfortified controls. Three urine subsamples pooled from preapplication (Day –1) worker samples contained 0.014-0.017 mg/L (from Reps.1-4) or 0.010-0.011 mg/L (from Reps. 13-16) of the α-isomer. The Rep. 1-4 pooled sample also contained 0.011-0.012 mg/L of the β-isomer. These results probably reflect contamination of those samples, as replicates 1, 3 and 4 showed no quantifiable levels of endosulfan and metabolites on Day 0, indicating than these workers had not been exposed previously to the study to endosulfan.

Hypothetical rates of excretion of endosulfan and its metabolites (in µg residue/kg a.i. handled) were calculated from data from those replicates in which Thiodan was handled, disregarding apparent residues of parent isomers as artifacts. On Day 0, the calculated rate was 501 µg/kg a.i. handled/day, and on day +1, the calculated excretion rate was 731 µg/kg a.i. handled day. The difference between the rates for the two days resulted from the collection of slightly less urine per worker on Day +1. These excretion values are overestimates since all residue levels were assigned the value of 50% of the LOQ for calculation purposes.

After the first application, one applicator (nº4) presented cramps in his upper third left thigh, which were relieved with rest and without medication. During the second day of application, another applicator (nº8) presented wheezes in the lower right lung with no other respiratory symptoms. He did not present any respiratory difficulty, and intermediate medical managing was not required. At the clinical examination during the second application four workers (33.33%) presented some symptoms. During the first day the applicator nº2, replicate 14 presented with a violent intensive global headache with no other symptoms. The headache was relieved with the administration of Nabumetone (AINE). During the second day at the examination, applicator nº5, replicate 17 presented bitemporal severe

headache, which was relieved with the administration of Nabumetone. Applicator nº7 presented with epigastric pain and fatigue with no other abdominal symptoms, which was relieved by the administration of Aluminum Hydroxide Gel plus Cimethacone. Applicator nº8 presented bilateral erithematous conjunctivitis (eye irritation) that was relieved by the application of Prefrin eye drops

Conclusion

The results of this study in coffee berry are not validated for the uses proposed of Thiodan 35EC in cotton and tomatoes. In this study the preparation has been applied at 0.49 kg a.s./ha and the proposed application rate per treatment for cotton and tomatoes is of 0.84 kg a.s./ha.

In this study, two replicates of 6 workers were used instead of the minimum of ten subjects without repeated monitoring of the same individuals recommended by the "Guidance document for the conduct of studies of occupational exposure to pesticides during agricultural application" (OCDE, 1997).On the other hand, a high proportion of urine samples were not appropriately collected by workers .(approx. 50%, 48% and 75% of Day –1, Day 0 and Day +1 Thiodan 35EC samples and 83%, 48% and 83% of Day –1, Day 0 and Day +1 blank samples).. These samples are not acceptable for the purposes of this study.

In addition low levels (near the LOQ) of α- and β-endosulfan have been detected in urine from day 0 and day +1 but the authors of the study justified these results as contamination of the samples.

Studies of dermal absorption *in vivo* in rats (A39677) showed that endosulfan binds to the skin and is absorbed slowly and almost completely over a period of 168h, and is excreted almost completely after 168h. In view of these results, the urine of consecutive days after 48h should be collected and evaluated for amounts of endosulfan and its metabolites.

In conclusion, the biomonitoring study in coffee berries is not representative of the GAPs proposed for Thiodan 35EC in cotton and tomatoes. The design of this study has not been well selected and accomplished.

B.6.12a.6 Dermal absorption *in vitro* in human, rat and pig skin.

Davies DJ, 2002 (Aventis Cropscience No. C021864)

Performing laboratory: Central Toxicology Laboratory (CTL), UK. CTL Study Number:JV1673.

Dates of experimental work: October 31, 2001 to December 13, 2001.

Date of report: March 26, 2002.

Objectives: The study was aimed at determining the *in vitro* absorption of endosulfan through human, pig and rat epidermis as a model to aid the quantitative assessment of the hazard from human skin contact with an emulsifiable concentrate (EC) preparation containing 350g endosulfan/l and its aqueous dilutions (1/2.06 and 1/333 v/v).

Guidelines: OECD Draft Guideline 428 (OECD, 2000) and ECETOC recommendations which provide information on the practical use of this *in vitro* technique.

The following information was not included in the report: the thickness of the epidermal membranes used in the experiments; and reference compounds to increase the confidence of the results were not included in the study. None of these issues are however considered to influence the validity of the study.
GLP: Yes

The study is considered acceptable

Materials and Methods

Epidermal membranes of human, pig and rat skin were obtained by carefully teasing away epidermis from dermis, and stored frozen until used. Previously to the experiments, membranes were thawed, placed in static *in vitro* dermal penetration cells and put in a water bath maintained at 32 ± 1 ºC. The receptor fluid, which consisted of 50% ethanol in water, was selected to ensure solubility of the test substance, and was shown not to affect skin preparation integrity. The receptor chamber contents were continuously stirred. The integrity of the epidermal barrier of each skin preparation was assessed prior to application of test articles by determining the electrical resistance across the skin membranes. Membranes with a measured resistance of < 10 kΩ (human), < 2.5 kΩ and < 2 kΩ were discarded. For each application, 4-6 intact membranes from at least two different specimens were used.

For determining the penetration of [^{14}C]-Endosulfan, a single dose of the radiolabelled material was applied topically for 8 or 24 hours, as the emulsion concentrate (nominally 350g/l, i.e., the concentration of the commercially available EC formulation) and as aqueous dilutions (1/2.06 and 1/333 v/v) of the concentrate. The radiolabelled formulations were prepared by adding [^{14}C]-Endosulfan to the formulations prior to application. These doses were designed to simulate potential human dermal exposure to the formulation during normal use. The actual concentrations achieved were 358, 171 and 1 g endosulfan/l for the concentrate and the two dilutions, respectively, and corresponded to application rates of 3582, 1713 and 9.96 µg/cm^2 endosulfan. The amount of formulation applied was in all cases 10 µl/cm^2. The membranes were left unoccluded throughout the entire exposure period (8 or 24 hours).

Non-radiolabeled test substance: Endosulfan technical, batch No. AAPC10026, purity 98.1%.
Radiolabeled test substance: [^{14}C]-Endosulfan, batch No. Z 31038-0, radiochemical purity 96.3%.
Emulsion concentrate formulation (350g/l EC formulation): batch No. AE F002671 00 EC 33 C7.
Blank formulation (used to prepare concentrate and dilutions): AE F002671 00 EC00 A803

For cells assigned to the 24 h exposure period, samples of the receptor fluid were taken at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. For cells assigned to the 8 hour exposure period, receptor fluid samples were not taken until 8 hours after application. The volume of receptor fluid taken with each sample was immediately replaced with fresh receptor fluid. At the end of the experimental period, the epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3% Teepol®, and with further sponges pre-wetted with water. Sponges were kept for analysis. After drying naturally, human epidermis only was sequentially tape-stripped to remove the *stratum corneum*, and tape strips were kept for analysis. The remaining epidermis was carefully removed from the receptor chamber and digested in Soluene 350® and analyzed. The chambers were also washed and washes collected for analysis and mass balance.

Findings

High dose (350 g/l EC formulation). Following a single 8 or 24 h application of the concentrate formulation, skin permeability to radioalabeled Endosulfan increased in the following order: human < pig < rat (see tables 6.12a.6-1 and 6.12a.6-4).

Table 6.12a.6-1. Distribution of radioactivity following a single topical application of ^{14}C-Endosulfan (concentrate formulation; equivalent to 3.58 mg/cm^2) to epidermal membranes. Results are expressed as percent of the radiochemical dose applied.

	8 hours			24 hours		
Species	**Human**	**Pig**	**Rat**	**Human**	**Pig**	**Rat**
Dose in skin wash (%)	79.0	64.5	64.6	65.7	68.6	65.4
Dose remaining in donor chamber (%)	20.9	36.9	5.13	33.2	32.6	7.92
Dose in tape strips (%)	0.30	-	-	0.35	-	-
Dose remaining in epidermis (%)	0.40	0.54	21.2	0.28	0.73	14.4
Dose in receptor fluid (%) *	0.12	0.76	7.17	0.33	1.33	10.2
Total recovery (%)	*101*	*103*	*98.0*	*99.8*	*103*	*97.9*
TOTAL ABSORBED DOSE (epidermis + tape strips + receptor fluid)	0.82	1.30	28.37	0.96	2.06	24.60

***** The study director considered that only the dose present in the receptor fluid should be regarded as absorbed (systemically available). However, we consider more appropriate to calculate the total absorbed dose as the addition of the amount present in epidermis + receptor fluid + tape strips (if available), and therefore we have included this information in the last row of the table. See discussion below.

Most of the radioactivity (approximately 65%) was detected in the skin washes taken at the end of the respective experimental periods (8 or 24 hours). In the case of human epidermis, this value was even higher at 8 hours (79.0%). A large proportion of the dose was recovered in the donor chambers of human and pig epidermis (between 20.9 and 36.9), whereas this value was much lower in the case of rat epidermis (approximately 5 to 8%). For human epidermis, an additional part of the dose, of 0.30 and 0.35% was removed in the surface tape-strips taken at 8 and 24 hours, respectively. This part of the dose belongs to surface residues following incomplete removal of the dose with the washes, and/or material from the *stratum corneum*. The remaining skin contained an additional part of the dose, which was very small in the case of human and pig epidermis, but significantly higher for rat epidermis. Recovery was in all cases close to 100%. The kinetics of absorption were linear between 0 an 24 hours, with absorption rates of 0.51, 2.06 and 14.8 µg/cm^2/h for human, pig and rat skin, respectively.

Mid dose (1/2.06 v/v aqueous solution). Also at this dose did rat skin prove to be more permeable than pig and human skin to radiolabelled endosulfan, and pig skin more permeable than human skin (tables 6.12a.6-2 and 6.12a.6-4).

Table 6.12a.6-2: Distribution of radioactivity following a single topical application of ^{14}C-Endosulfan (1/2.06 v/v aqueous solution; equivalent to 1.71 mg/cm^2) to epidermal membranes. Results are expressed as percent of the radiochemical dose applied.

	8 hours			24 hours		
Species	**Human**	**Pig**	**Rat**	**Human**	**Pig**	**Rat**
Dose in skin wash (%)	90.8	68.3	88.7	69.2	72.9	58.2
Dose remaining in donor chamber (%)	19.2	23.2	2.66	28.5	20.2	3.11
Dose in tape strips (%)	0.11	-	-	0.08	-	-
Dose remaining in epidermis (%)	0.12	6.29	14.9	0.14	2.64	24.2
Dose in receptor fluid (%) *	0.14	0.91	6.58	0.55	2.84	8.91
Total recovery (%)	*110*	*98.8*	*113*	*98.4*	*98.6*	*94.4*
TOTAL ABSORBED DOSE (epidermis + tape strips + receptor fluid)	0.37	7.20	21.48	0.69	5.48	33.11

***** The study director considered that only the dose present in the receptor fluid should be regarded as absorbed (systemically available). However, we consider more appropriate to calculate the total absorbed dose as the addition of the amount present in epidermis + receptor fluid + tape strips (if available), and therefore we have included this information in the last row of the table. See discussion below.

A similar recovery profile was observed, with a high amount of radioactivity detected in the skin washes (values ranging between 58.2% for rat skin at 24 hours and 90.8% for human skin at 8 hours). Donor chambers that contained human and pig skin retained important amounts of radioactivity, whereas this value was lower in the case of chambers that contained rat skin. Tape-stripping of human epidermis recovered a small proportion of radioactivity, i.e., 0.11 and 0.08 % of the dose at 8 and 24 hours, respectively. The dose that remained within the epidermis was significantly larger for rat than for pig and human skin, and for pig than for human skin (see table B.6.12a.6-2), both at 8 and 24 hours. Finally, the endosulfan contents of the receptor fluid were low for human epidermis, relatively low for pig epidermis, and much higher for rat epidermis (table B.6.12a.6-2). Absorption of the mid-dose endosulfan formulation was also linear, and the absorption rates for human, pig and rat skin were 0.38, 2.04 and 5.83 µg/cm2/h, respectively (table B.6.12a.6-4).

Low dose (1/333 v/v aqueous solution): The recovery profile of this low dose of radiolabeled endosulfan was remarkably different from that observed at higher doses (see table 6.12a.6-3).

Table 6.12a.6-3: Distribution of radioactivity following a single topical application of ^{14}C-Endosulfan (1/333 v/v aqueous solution; equivalent to 0.01 mg/cm^2) to epidermal membranes. Results are expressed as percent of the radiochemical dose applied.

	8 hours			24 hours		
Species	**Human**	**Pig**	**Rat**	**Human**	**Pig**	**Rat**
Dose in skin wash (%)	98.2	68.5	23.3	96.0	48.6	12.2
Dose remaining in donor chamber (%)	0.52	2.6	1.58	2.12	3.66	1.10
Dose in tape strips (%)	1.34	-	-	1.16	-	-
Dose remaining in epidermis (%)	1.17	5.73	30.8	0.79	4.11	15.9
Dose in receptor fluid (%) *	1.18	23.0	42.9	1.90	40.7	65.8
Total recovery (%)	*102*	*99.9*	*98.5*	*102*	*97.1*	*95.1*
TOTAL ABSORBED DOSE (epidermis + tape strips + receptor fluid)	3.69	28.73	73.70	2.69	44.81	81.70

* The study director considered that only the dose present in the receptor fluid should be regarded as absorbed (systemically available). However, we consider more appropriate to calculate the total absorbed dose as the addition of the amount present in epidermis + receptor fluid + tape strips (if available), and therefore we have included this information in the last row of the table. See discussion below.

In the case of human skin, a very high proportion of the dose (96-98%) was found in the skin washes. The donor chambers contained an amount of radioactivity much lower (0.52 – 2.12%) than that found when the high or mid dose formulations were used. Additional parts of approximately 1-2% of the dose were detected in each of the following compartments: tape strips, remaining epidermis and receptor fluid. Regarding pig skin, an important amount of radioactivity was found in skin washes (68.5% and 48.6% at 8 and 24 hours, respectively). The proportion found in the donor chambers was also significantly lower than that collected for the mid and high doses of endosulfan (between 2 and 4%). The remaining epidermis contained between 4 and 6% of the dose, whereas a high amount of radioactivity was found in the receptor fluid (23% at 8 hours and 40.7 at 24 hours). Finally, the low dose of endosulfan penetrated rat skin to a large extent: only 23.3% and 12.2 % of the dose was removed with the washings performed at 8 and 24 hours, respectively, and a further 1.58 (8 hours) and 1.1 % (24 hours) was detected in the donor chamber. Most of the dose was recovered in the remaining epidermis and receptor fluid compartments (see values in table B.6.12a.6-3). Recovery of radioactivity was acceptable in all cases. Absorption was fastest during the first hour of exposure in the case of human and pig skin, and linear afterwards (see table B.6.12a.6-4). The absorption rate through human, pig and rat epidermis increased in this order (see table 6.12a.6-4).

Table 6.12a.6-4: Mean absorption rates (0-24 hours) and interspecies ratios following a single topical application of ^{14}C-Endosulfan to epidermal membranes. Results are expressed as µg/cm^2/h.

	Human	**Pig**	**Rat**	***Ratio pig/human***	***Ratio rat/human***	***Ratio rat/pig***
350 g/l conc. form. (3.58 mg/cm^2)	0.51	2.06	14.8	*4.04*	*29.02*	*7.18*
1/2.06 v/v aq. dil. (1.71 mg/cm^2)	0.38	2.04	5.83	*5.37*	*15.34*	*2.86*
1/333 v/v aq. dil. (0.01 mg/cm^2)	0.01 (0.03*)	0.14 (1.05*)	0.27	*14.0 (35.0*)*	*27.00*	*1.93*

* Between brackets, the absorption rate during the first hour of exposure (Absorption rate (0-1 hour)) is presented. After that time, absorption was linear.

Discussion and conclusion.

This *in vitro* dermal penetration study with human, pig and rat skin has been adequately conducted and reported. Determination of radioactivity after exposure of skin *in vitro* to the radiolabeled test substance showed that rat epidermis is more permeable than pig epidermis, and in turn, pig epidermis is more permeable than human epidermis to radiolabelled Endosulfan when applied for 8 or 24 hours either at a high dose (as the commercially available emulsion concentrate) at a mid dose (1/2.06 v/v aqueous dilution) or at a low dose (1/333 v/v aqueous dilution). These doses and application times simulate human dermal exposure during mixing and loading operations and field use of endosulfan. However, it would have been interesting to include an experimental group with exposure to the test substance for 6, 8 or 10 hours (normal daily exposure time) followed by washing of the epidermis, and, instead of terminating the experiment, keeping the epidermis in the chamber up to 24 hours and sampling afterwards for analysis. This would have allowed determination of the absorption of the test substance retained in the skin after cesation of exposure, and would have reflected normal real use conditions.

As stated in the report, the study director considered that only the amount of endosulfan detected in the receptor fluid should be regarded as absorbed (systemically available), and the data were originally reported in this manner. However, based on the recommendations of the OECD Draft Guideline on Dermal Absorption, nº 428 (2000), and of EC Draft Guidance Document on Dermal Absorption (Sanco/222/2000 rev. 4, April, 2001), "…By including the amount (of test substance) retained in the skin *in vitro*, a more acceptable estimation of skin absorption can be obtained." This is specially recommended for lipophilic substances, as is the case of Endosulfan. The EC guideline does not ignore that this approach may result in a conservative estimate of the amount becoming systemically available *in vivo*. As a solution, it recommends that, if refinement is needed, it should be convincingly demonstrated that the skin dose does not become absorbed at a larger stage. Although the study director correctly suggests in the report that "*in vivo*, the majority of the dose in the epidermis, especially that recovered from the *stratum corneum* (i.e., that found on the tape strips) would eventually be lost by desquamation", this has not been experimentally demonstrated. Therefore, the possibility that a significant part of Endosulfan retained in the epidermis could eventually be absorbed cannot be discarded.

For these reasons, and as mentioned in the table legends, we consider more appropriate to calculate the total absorbed dose as the addition of the amount present in epidermis + receptor fluid + tape strips (if available), as presented in the last row of tables B.6.12a.6-1, B.6.12a.6-2 and B.6.12a.6-3.

An additional interesting point for discussion is the fact that following application of the high and mid doses, a large proportion of the dose (mean of 26.8%) was associated with the donor chambers of the human and pig experiments, in contrast with that found with the rat experiments. It is indicated in the report that the reason might be that the residual dose on the surface of human and pig epidermis was still in liquid form, an effect which might have led to flow of a part of the dose to the edges of the chamber across the surface of the less densely haired human and pig epidermis. This had no effect on the actual dose levels to which epidermis was exposed, because the test substance was in liquid form, and therefore available for absorption. It is suggested that, under normal exposure conditions, this proportion of the dose would be removed as part of the skin wash. This effect was not apparent in the low dose formulation, because it had a higher proportion of water. The report mentions that rat epidermis was unaffected as the hairs remaining on the epidermis helped to contain the liquid dose. However, this might be arguable, since skin was shaved previously to sample collection.

Summarizing, the results obtained in this study demonstrate that the rate of penetration of endosulfan, as the commercially available 350g/l EC formulation or its aqueous dilutions (1/2.06 and 1/333 v/v), through human epidermis *in vitro* is much lower than through pig and rat epidermis. The following amounts of Endosulfan are considere available for absorption after 8 and 24 hours exposure:

	8 hours			**24 hours**		
Species	**Human**	**Pig**	**Rat**	**Human**	**Pig**	**Rat**
350 g/l conc. form. (3.58 mg/cm^2)	0.82	1.30	28.37	0.96	2.06	24.60
1/2.06 v/v aq. dil. (1.71 mg/cm^2)	0.37	7.20	21.48	0.69	5.48	33.11
1/333 v/v aq. dil. (0.01 mg/cm^2)	3.69	28.73	73.70	2.69	44.81	81.70

Data requirement 4.6: Main data submitter to recalculate exposure scenarios

Background

In a previous document (C010955) the notifier had proposed the values described below for the calculations of the operator exposure level:

- A NOAEL to define the systemic AOEL based on subchronic oral rat study
- Skin penetration factor for concentrate of 0.5% and for diluted of 1.5%, based on *in vivo* rat and in *vitro* human/rat/pig (C021864) studies.
- Additional data to demonstrate that there was not need to correct for bioavailability when calculating the systemic AOEL.

The RMS, since the submission:

- Considered that the AOEL should be based on the NOAEL of 0.6 mg/kg bw from the 1-year dog study.
- Correction factor of 70% should be applied for systemic absorption, based on toxicokinetics studies done in rat
- Taking into account all data, an AOEL of 0.0042 mg/kg/day could be established
- Skin penetration factors, based also on *in vivo* rat and on *in vitro* study that compared dermal absorption between human/rat/pig (C021864), were determined to be:
    - For concentrate 0.6%
    - For diluted 2.3%

Discussion of the different opinion between the notifier and the RMS about C021864 study is included in the open point 4.5.

The re-evaluation of the operator exposure submitted by the notifier had been done in this addendum including three points:

- summary of relevant use scenarios for exposure calculations
- summary of the re-evaluation submitted by the notifier
- new re-calculation of the acceptable exposure scenarios for operator, taking into account dermal penetration factors proposed by the RMS.

Summary

Safety uses for operator using PPE are expected in three scenarios:

- Tractor mounted boom sprayer in field crops
- Tractor mounted airblast sprayer in high crops
- Hand held sprayer Greenhouse (high crops)

Hand-held spray lance in citrus is not considered acceptable when BBA-model was applied. The notifier included an exposure study with acceptable levels of exposure for operator, taking into account the next PPE: gloves, coverall and mask during mixing and loading; gloves, coverall and hood+visor during application. In the RMS opinion, this last scenario is considered acceptable, as exposure study was well characterized

Individual study evaluation

B.6.14 Exposure data

Re-evaluation of the operator exposure and risk assessment for Thiodan 35 EC with a new data on skin penetration. Urtizberea m., Reader S. (2002) C022980

Performing laboratory: Aventis CropScience.

Date of the report: 22 May 2002

1-. Summary of relevant use scenarios for exposure calculations

Table 6.14-1: Use scenarios

Use scenarios		Application technique	Crop	Max. rate (kga.s./ha)	Expected work rate (ha/day)
1	Field crops	Tractor mounted boom sprayer	Cotton Sugar beet Potato	0.84 0.63 0.53	20
2	High crops	Tractor mounted airblast sprayer	Pome fruit Stone fruit	1.05 0.80	8
3	High crops	Hand held sprayer	Citrus Vines Tomato Cucurbits	1.05 1.05 0.53 0.53	1
4	Greenhouse (high crops)	Hand held sprayer	Tomato	0.80	1

Representative crops with the highest dose rates were taking for ensuring exposure evaluation that will cover the other crop uses in each use scenario:

- cotton for tractor mounted boom applications in field crops
- pome fruit for tractor mounted airblast applications in high crops
- citrus for hand held applications in high crops
- tomato for hand held applications in greenhouses

As Endosulfan is classified T+, the following specific instructions for operator protection have therefore been established:

- Wear protective gloves when handling the undiluted product
- Wear protective garment and sturdy footwear (e.g. rubber boots) when handling the undiluted product
- Wear rubber apron when handling the undiluted product
- Wear tight fitting goggles when handling the undiluted product
- Wear particle filtering half-mask FF2-SL or half-mask with particle filter P2 when handling the undiluted product.

2-. Summary of the re-evaluation submitted by the notifier

Evaluation of the operator exposure was carried out with the generic data base of the BBA-model (that better reflects the agricultural use conditions in shouthern Europe) and in addition with recently performed modern operator exposure studies in the relevant use scenarios. Results are described in tables 6.14-2 and 6.14-3.

Table 6.14-2: Systemic exposures obtained with BBA-model (with and without PPE)

Exposure scenarios					
Appl. technique	**Crop**	**Dose rate (kg a.s./ha)**	**Work rate (ha/day)**	**BBA model**	
				Without PPE	With PPE
Tractor boom	Cotton	0.84	20	0.0106	0.000793
Tractor airblast	Pome fruit	1.05	8	0.02437	0.00111
Hand held orchand	Citrus	1.05	1	0.013779	0.004861
Hand held greenhouse	Tomato	0.80	1	0.0105	0.00370

Table 6.14-3: Systemic exposures obtained with modern operator exposure studies (with PPE)

Modern operator exposure studies								
Appl. technique	**PPE used**	**Crop**	**Dose rate (kg a.s./ha)**	**Work Rate (ha/day)**	**Generic study, potato**	**Thiodan study, orchard airblast**	**Thiodan study, orchard hand held**	**Generic study, green house**
Tractor boom	Gloves, coverall, mask, hood+visor during mixing/loading; coverall during application	Potato	0.25	19-41	0.0000962			
Tractor airblast	Gloves, mask during mixing/loading;gloves, coverall, mask, hood+visor during application	Orchard	1.05	8		0.00224		
Hand held orchard	Gloves, coverall, mask during mixing/loading; gloves, hood+visor, coverall, mask during application	Orchard	1.05	1			0.00101	
Hand held greenhouse	Gloves, coverall, mask during mixing/loading; gloves, coverall during application	Tomato	0.80	1				0.00175

The degree of exposure is described in the table 6.14-4

Table 6.14-4: Risk assessment for Thiodan 35 EC

Crop	**Application technique**	**Source of data**	**Systemic exposure (mg/kg bw/day)**	**%AOEL (0.0042 mg/kgbw/day)**	**% AOEL (0.0147 mg/kg bw/day)**
Cotton	Tractor boom	BBA-model	0.000793	18.8%	5.4%
Potato	Tractor boom	Exposure study	0.0000962	2.3%	5.4%
Pome fruit	Tractor airblast	BBA-model	0.00111	26.4%	7.6%
		Exposure Study	0.00224	53%	16.3%
Citrus	Hand-held spray lance	BBA-model	0.004861	116%	33.1%
		Exposure Study	0.00101	24%	6.9%
Tomato	Hand-held glasshouse	BBA-model	0.0037	88%	25%
		Exposure Study	0.001752	42%	11.9%

3-. Summary of the re-evaluation submitted by the RMS

Table 6.14-5: Systemic exposures obtained with BBA-model (with and without PPE)

Exposure scenarios					
Appl. technique	**Crop**	**Dose rate (kg a.s./ha)**	**Work Rate (ha/day)**	**BBA-model**	
				Without PPE	With PPE
Tractor boom	Cotton	0.84	20	0.015	0.001075
Tractor airblast	Pome fruit	1.05	8	0.0357	0.001638
Hand held orchard	Citrus	1.05	1	0.01866	0.005052
Hand held greenhouse	Tomato	0.80	1	0.01421	0.00385

Table 6.14-6: Systemic exposures obtained with modern operator exposure studies (with PPE)

Modern operator exposure studies								
Appl. technique	**PPE used**	**Crop**	**Dose rate (kg a.s./ha)**	**Work Rate (ha/day)**	**Generic study, potato**	**Thiodan study, orchard airblast**	**Thiodan study, orchard hand held**	**Generic study, green house**
Tractor boom	Gloves, coverall, mask, hood+visor during mixing/loading; coverall during application	Potato	0.25	19-41	0.0001315			
Tractor airblast	Gloves, mask during mixing/loading; gloves, coverall, mask, hood+visor during application	Orchard	1.05	8		0.0023		
Hand held orchard	Gloves, coverall, mask during mixing/loading; gloves, hood+visor, coverall, mask during application	Orchard	1.05	1			0.0013	
Hand held greenhouse	Gloves, coverall, mask during mixing/loading; gloves, coverall during application	Tomato	0.80	1				0.001978

The degree of exposure is described in the table 6.14-7**.**

Table 6.14-7: Risk assessment for Thiodan 35 EC using PPE

Crop	Application technique	Source of data	PPE (with or without)	Systemic exposure (mg/kg bw/day)	%AOEL (0.0042 mg/kgbw/day)
Cotton	Tractor boom	BBA-model	Without PPE	0.015	357.14
			With PPE	0.001075	25.59
Potato	Tractor boom	Exposure study	With PPE	0.0001315	3.13
Pome fruit	Tractor airblast	BBA-model	Without PPE	0.0357	850
			With PPE	0.001638	39
		Exposure Study	With PPE	0.0023	54.76
Citrus	Hand-held spray lance	BBA-model	Without PPE	0.01866	444.28
			With PPE	0.005052	120.28
		Exposure Study	With PPE	0.0013	30.95
Tomato	Hand-held glasshouse	BBA-model	Without PPE	0.01421	338.33
			With PPE	0.00385	91.66
		Exposure Study	With PPE	0.001978	47.09

Conclusion:

According the table below, safety uses for operator using PPE are expected in three scenarios:

- Tractor mounted boom sprayer in field crops
- Tractor mounted airblast sprayer in high crops
- Hand held sprayer Greenhouse (high crops)

Hand-held spray lance in citrus is not considered acceptable when BBA-model was applied. The notifier included an exposure study with acceptable levels of exposure for operator, taking into account the next PPE: gloves, coverall and mask during mixing and loading; gloves, coverall and hood+visor during application. In the RMS opinion, this last scenario is considered acceptable, as exposure study was well characterized

Data requirement 4.5: Main data submitter to address the toxicity of endosulfate-lactone

Background

As it is considered necessary to study the toxicity of endosulfan-lactone, the notifier had submitted news studies.

Previous acute oral toxicity studies of endosulfan-lactone in rats had been evaluated (see the addendum to Annex B of the Endosulfan Monograph of may 2001 and October 2001). In them, it was concluded that males were more sensitive to the test substance than females, and the LD_{50} for males should be calculated. As the LD_{50} for males was determined to be <200 mg/kg bw, endosulfan-lactone is considered as toxicologically significant metabolite. The notifier had submitted new study in order to re-calculate the LD_{50} for males (C024720)

Furthermore, Ames test, preliminary 28-day and a 90-day toxicity studies in rat with endosulfan lactone (C013516, C032189, C032788) had been reported.

In the other hand, endosulfan lactone is present in equilibrium with endosulfan hydroxycarboxylic acid (metabolite that is included in the water residue definition, and present in tomato and cucumber leaves). Therefore, toxicological data of this metabolite is required. The notifier had send an acute oral toxicity test (C024725), and another study about the conversion rate from endosulfan hydroxy carboxylic acid

to endosulfan lactone (C023002)

Summary

As a conclusion obtained from the evaluation of the study C024720, the LD_{50} of endosulfan-lactone for male rats was between 25 to 200 mg/kg, and T ("Toxic") symbol is required. This metabolite should be considered as a toxicologically significant metabolite, based on acute toxicity study.

From the Preliminary 28-day toxicity study in the rat by dietary administration of endosulfan lactone (C032189), it can be established a NOAEL of 500 ppm (corresponding to 36.6 and 39.6 mg/kg bw/day in males and females, respectively) based on body weight decreased and on liver alterations.

In the 90-day toxicity study by dietary administration (C032788), liver was established as the target organ. Main alteration was a centrilobular hypertrophy of the hepatocites. Also, hyaline droplets in proximal tubules of the kidneys due to the accumulation of α_{2u}-globulin was observed. This last finding was not considered toxicologically relevant for humans. Considering that the response observed in the liver and kidney of males at 10 ppm was slight, it can be established a NOAEL of 10 ppm (corresponding to 0.6 mg/kg bw/day in males and 0.7 mg/kg/day in females).

One genotoxicity study (Ames test, C013516) had been evaluated and concluded that endosulfan-lactone showed no mutagenic potential under the conditions of this study.

The notifier had submitted acute oral toxicity test in rats using Endosulfan Hydroxy Carboxilic Acid. The LD_{50} was comprised between 200 and 2000mg/kg bw in both males and females, and according to the classification criteria, this metabolite should be assigned the symbol Xn, the indication of danger "Harmful" and the risk phrase R22: "Harmful if swallowed". This metabolite is not considered as a toxicologically significant metabolite based on this acute oral study.

Individual studies evaluation

B.6.8 Further toxicological studies

B.6.8.2 Toxicity of endosulfan metabolites

ENDOSULFAN LACTONE

B.6.8.2.1 Endosulfan-lactone. Acute oral toxicity in rats. "Acute toxic class method". B. Griffon (2002) C024720

Performing laboratory: CIT. BP 563-27005 E VREUX-France.

Date of experimental work: 20 April 2002 to 17 May 2002

Objective: To determine the LD_{50} in males rats

Guidelines: OECD No. 423 (Dated: December 2001)

Deviations: Purity of the test substance was 99.6%, however, purity of the test substance in previous study was 96%

GLP: yes

Date of report: June 2002

The study is acceptable

Materials and methods:

Test substance used was Endosulfan lactone (batch number 8273X) and was determined to be 99.6% purity. The test item was prepared in 0.5 % methylcellulose and was administered by oral route (gavage) to groups of fasted Sprague-Dawley rats. Three animals of one sex were used for each step. Males were used in the initial step. The starting dose of 200 mg/kg was chosen.

Dose levels are described in the table below:

Table 6.8.2.1-1: Dose regimen

Dose (mg/kg)	Vehicle	Volume (mL/kg)	Male	Female
200	0.5% methylcellulose	10	3	-
25	0.5% methylcellulose	10	3	-
25	0.5% methylcellulose	10	-	3

Clinical signs, mortality and body weight gain were checked for a period of up to 14 days following the single administration of the test item. All animals were subjected to necropsy.

Findings:

After the first assay, 2/3 animals dosed with 200 mg/kg died. Another assay was carried out on three males at the next lower dose-level (25 mg/kg) After the second assay, as 0/3 animals died, the results were confirmed on three females.

Clinical examinations: At the 200 mg/kg dose-level, hypoactivity, piloerection and dyspnea were observed prior to death of these animals, as well as in the surviving animal from day 1 up to day 4. At the 25 mg/kg, no clinical signs were observed.

Body weight gain at both dose levels and both sexes was not affected by treatment with the test item. Pathology examination of the main organs of the animals revealed not apparent abnormalities.

Conclusion:

The oral LD_{50} is comprised between 25 and 200 mg/kg in male rats. According to the classification criteria, Endosulfan lactone should be assigned the symbol T, “Toxic” and the risk phrase R25: “Toxic for Swallowed”. Therefore, **endosulfan-lactone should be considered as a toxicologically significant metabolite.**

B.6.8.2.2 Endosulfan lactone: preliminary 28-day toxicity study in the rat by dietary administration (2003) Labay K. C032189

Performing laboratory: Bayer CropScience

Date of experimental work: August 28, 2002 to October 02, 2002

Objective: To determine the potential toxic effects of endosulfan lactone in rats following continuous dietary administration for 28 days and to provide information for selection of dose levels for future toxicity studies in this species.

GLP: yes

Date of report: January 31, 2003

The study is acceptable

Material and methods

Endosulfan lactone, a metabolite of endosulfan (batch number DJA 119, purity 95.1%) was administered continuously via diet to groups of Wistar rats (5/sex/group) for 28 days at concentrations of 10, 100, 500 and 2000 ppm, corresponding to 0.8 and 0.8, 7.3 and 8.3, 36.6 and 39.6, 149.6 and 161.7 mg/kg/day in males and females, respectively. A similarly constituted group received untreated diet and acted as a control.

All animals were checked for moribundity and mortality twice daily, clinical signs at least once daily, and detailed physical examinations at least weekly during treatment period. Body weight was recorded twice during the acclimatization period, on the first day of test substance administration, at weekly intervals throughout the treatment period and before the necropsy. Furthermore, food consumption was recorded weekly during the treatment period, and weekly mean achived dosage intake in mg/kg/day for each week and for weeks 1 to 4 was calculated.

On days 29 and 30, all animals were sacrificed by exsanguinations under deep anesthesia. All animals were necropsied and fresh organs were weighed.

Statistical analysis included the calculation of means and standards deviations achieved sex and dose groups. Bartlett´s test for homogeneity of variances was applied and then, ANOVA and Dunnet´s test or Kruskall-Wallis and Mann-Whitney test were chosen.

Findings

Homogeneity and concentration results of the substance in the diet were within target ranges. There were no mortalities during the study, and no clinical signs were observed in animals dosed at 500, 100 and 10 ppm. At 2000 ppm one female had convulsions on day 28. A relation with treatment was not excluded.

Body weight gains were statistically significantly lower at 2000 ppm when compared with control group, during all weekly intervals. Mean body weight gains and its percentages of body weight gains at 2000 ppm related to control values is included in table 6.8.2.2-1 and table 6.8.2.2-2.

Table 6.8.2.2-1: Absolute weight gains referenced to dose level (mean and standard deviation)

Dose Level										
	Control (0 ppm)		10 ppm		100 ppm		500 ppm		2000 ppm	
Sex	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Day 8	59±4	23±3	57±7	20±4	55±4	24±7	54±3	17±2	25.4±16	6.2±6
Day 15	108±9	37±5	104±15	30±5	103±5	37±6	102±3	29±7	62.2±23	20.4±8
Day 22	141±9	48±6	142±19	45±3	137±5	49±6	141±4	10±5	97.4±21	29.6±6
Day 28	162±9	57±5	166±25	51±4	163±9	61±8	164±7	49±7	126±26	38.6±9

Table 6.8.2.2-2: Body weight gains at 2000 ppm (% gain when compared to controls)

	Dose level:2000 ppm	
	Males	Females
Days 1-8	-58%	-74%
Days 1-15	-43%	-46%
Days 1-22	-31%	-38%
Days 1-28	-22%	-32%

At 2000 ppm, the mean body weights were lower in males and females than in controls during all weekly intervals and were statistically significant except in females on days 15 and 28. At 500 ppm, the mean absolute body weight gain was decreased (-26%) in females on day 8 compared to controls, but was not statistically significant. At lower doses, the body weight evolution was unaffected by the treatment. Data are included in table 6.8.2.2-3 and table 6.8.2.2-4.

Table 6.8.2.2-3: Body weight (mean and standard deviation)

Dose Level										
	Control (0 ppm)		10 ppm		100 ppm		500 ppm		2000 ppm	
Sex	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Day 1	254±7	183±8	253±8	186±7	251±5	182±2	255±8	184±6	252±7	183±5
Day 8	314±5	205±11	310±13	206±11	306±4	206±8	309±10	201±6	**277±16**	**189±10**
Day 15	362±5	220±12	358±21	216±9	**354±3**	219±6	357±10	213±12	**314±21**	203±10
Day 22	395±8	231±11	396±25	231±8	388±7	231±8	395±10	223±14	**349±21**	**213±9**
Day 28	417±10	240±12	419±31	237±11	414±10	243±9	419±10	234±11	**378±26**	222±11

Table 6.8.2.2-4: Body weight at 2000 ppm (% when compared to controls)

	Dose level:2000 ppm	
	Males	Females
Days 8	-12%	-8%
Days 15	-13%	-8%
Days 22	-12%	-8%
Days 28	-9%	-8%

Food consumption: At 2000 ppm food consumption was statistically significantly lower than controls on days 8 and 15 in males (-22% and –17%, respectively).

Terminal body weight and organs weight: At the sacrifice of the animals, mean terminal body weight was significantly lower in males treated at 2000 ppm (-10%), when compared to controls. In females at the top dose, a slightly lower mean terminal body weight was noticed, but was not statistically significant. Mean absolute relative liver weights were statistically significantly higher in males and females at 2000 ppm, and there was a tendency towards higher absolute and relative kidney weights in males at the same dose, that was statistically significant only for mean kidney to body weight ratio.

Gross pathology: Treatment-related macroscopic findings were noticed in liver of all males and 4/5 females treated at 2000 ppm, and comprised large and/or dark livers as well as a prominent liver lobulation.

The kidneys of 2/5 males at 2000 ppm were pale at the necropsy, but no histophatological changes were

noticed.

Conclusion:

At 2000 ppm, one female had convulsions on day 28. Mean body weight and body weight gain were consistently lower than controls for both males and females throughout the treatment period (-22% in males and –32% in females). This observation was essentially due to a marked decrease in body weight gain during the first week of treatment (-58% in males and –74% in females). Food consumption was statistically significantly lower than controls (-22% and –17%, respectively) on days 8 and 15 in males only. At sacrifice, mean absolute and relative liver weights were higher than controls in both sexes and were found to be large and/or dark with prominent lobulation in a majority of animals.

The NOAEL of endosulfan lactone was established at 500 ppm (corresponding to 36.6 and 39.6 mg/kg bw/day for males and females, respectively), based on body weights decreases and on liver alterations.

B.6.8.2.3 Endosulfan lactone. 90-day toxicity study in the rat by dietary administration (2003) Langrand-Lerche C. (C032788)

Performing laboratory: Bayer CropScience

Date of experimental work: October 22, 2002 to April 30, 2003

Objective: To determine the potential toxic effects of endosulfan lactone in rats following continuous dietary administration for at least 90 days

Guidelines: 408 OCDE guideline (1998)

Deviations: none

GLP: yes

Date of report: January 31, 2003

The study is acceptable

Material and methods

Endosulfan lactone (batch number DJA 119, purity 95.1%) was administered into the diet to groups of Wistar rats (10/group/sex) at three dose levels: 10 ppm, 250 ppm and 2000 ppm (corresponding to 0.6 and 0.7, 9.2 and 10.6, 129.2 and 141.5 mg/kg/day in males and females, respectively). A similarly constituted group received untreated diet and acted as a control.

All animals were checked for moribundity and mortality twice daily, observed clinical signs were recorded at least once daily, physical examinations were performed during the acclimatization phase and at least weekly during the treatment period, neurotoxicity assessment (consisted of the test of grasping, righting, corneal, papillary, auditory startle and head shaking reflexes) was done during the acclimatization period and during week 12, body weight was recorded twice during acclimatization period and on the first day of test substance administration and weekly during the treatment period and just before necropsy, food consumption was determined weekly, ophthalmological examination was done during acclimatization period and during week 12 in control and 2000 ppm group. Urine samples were collected overnight during week 13 from all animals and, before necropsy a blood sample was collected from the retro-orbital venous plexus of each animal for hematology and clinical chemistry determinations. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

Statistical analysis was applied to body and organ weight, food consumption, blood and urinary

parameters.

Findings

Mortality: At 2000 ppm, one female was found death on day 5, no abnormal clinical sign were recorded before death. Another female of the same group had convulsions on day 7 and 8 together with laboured respiration, reduced motor activity, general pallor and soiled fur and nose on day 7 and/or 8. In addition, this female showed a 31 g body weight loss after a week of treatment and was killed for humane reasons on day 8.

Clinical signs: no treatment-related clinical signs were noted in any dose groups in surviving animals. Neurotoxicity assessment in both sexes did not reveal any treatment related-changes.

Body weight: At 2000 ppm, males mean body weights were statistically significant lower (between 7 to 10%) than controls since day 8 to day 57 of the treatment period. No statistically significantly differences were found in females at the same dose during the treatment period, either in the rest of dose groups.

Mean body weight gain: Males group dosed at 2000 ppm, significantly statistically lower body weight gain was found in days 8 and 22 (44% and 16%, respectively). Females treated with 2000 ppm did not presented significant differences when compared to control group.

In females dosed at 10 ppm, and 150 ppm, statistically significantly higher values were found on days 8 and 15, respectively, when compared to control groups.

Food consumption: At 2000 ppm, significant reduced food consumption was found in both males and females on day 8. No one else difference was found in the rest of the dose group.

Ophthalmological examination: no ocular abnormalities were found in either sex at 2000 ppm.

Clinical pathology: At 2000 ppm, in males only, statistically significant differences were noted in mean erythrocyte count, hemoglobin concentration and hematocrit (-6%, -5% and –5%, respectively).

Clinical chemistry: higher mean total cholesterol concentrations were noted in females at 2000 ppm and 150 ppm (+34% and +31%, respectively) Statistically significant lower mean tryglicerides concentration was noted in males at 150 ppm (-58%). The same tendency was observed at 2000 ppm (-38%, not statistically significant)

A tendency towards lower aspartate and alanine aminotransferase activities (-15%, not statistically significant and –25%, respectively) was seen in females at 150 ppm. No significant changes were observed at 2000 ppm.

There were statistically significant differences in albumin/globulin ratio at 150 ppm in both sexes.

Urinalysis: no relevant changes were observed.

Post-mortem examinations: No significant differences were observed between treated and control group in the mean terminal body weight.

Mean liver weights were statistically significantly higher in males at 2000 and 150 ppm and in females at 150 ppm only. Thymus weights were found to be statistically significantly lower in females at 150 ppm, but this changes were not found in females treated at 2000 ppm.

Table 6.8.2.3-1: Liver weight changes at terminal sacrifice (%of change when compared to controls)

Sex	**Males**			**Females**		
Dose group (ppm)	**10**	**150**	**2000**	**10**	**150**	**2000**
Mean absolute liver weight	NC	+24 p<0.01	+13	NC	+29 p<0.01	NC
Mean liver to body weight ratio	NC	+27 p<0.01	+19 p<0.01	NC	+23 p<0.01	NC
Mean liver to brain weight ratio	+12 NS	+29 p<0.01	+18 p<0.05	NC	+26 p<0.01	NC

NC: no relevant change
NS: no statistically significant

Gross pathology: The necropsy done in female sacrificed on day 8 revealed black foci on the glandular mucosa of the stomach associated with dark content.

Treatment-related changes were found in the liver, like enlarged livers with dark color and prominent lobulation in animals treated at 2000 and 150 ppm.

Other changes were not treatment-related.

Microscopic pathology: Females that died on days 5 and 8 presented treatment-related changes in the liver and the brain. In the brain, a diffuse degeneration/necrosis of the cerebral cortex was noted in both females. Also, autolysis was found in female found dead on day 5.

In the liver, a moderate centrilobular necrosis was found in sacrificed female. Also slight centrilobular hepatocellular hypertrophy in both females, associated with a slight valuolation of centrilobular hepatocytes in one female was observed.

Microscopic pathology done in animals sacrificed at the end of the treatment period reveals treatment-related changes on the liver of both sexes and in the kidney and adrenal glandule in males.

In liver, slight to moderate centrilobular hepatocellular hypertrophy was noted in all animals treated at 150 and 2000 ppm, and in 6 males dosed at 10 ppm. Also, vacuolation of centrilobular hepatocytes was observed in males treated at 150 and 2000 ppm, and in one and two females dosed at 150 and 2000 ppm, respectively. Males appeared to be more sensitive to the effects of Endosulfan. Degeneration/necrosis of single hepatocytes was seen in 3/10 males treated at 150 and 2000 ppm.

Table 6.8.2.3-2: Incidence of treatment related lesions in the liver

Sex	Male				Female			
Dosage level (ppm)	0	10	150	2000	0	10	150	2000
Number examined	10	10	10	10	10	10	10	10
Hypertrophy, hepatocellular, centrilobular								
Slight	0	6	0	1	0	0	6	8
Mild	0	0	0	5	0	0	4	0
Moderate	0	0	10	4	0	0	0	0
Total	0	6	10	10	0	0	10	8
Vacuolation, hepatocellular, centrilobular								
Slight	0	0	10	5	0	0	1	2
Mild	0	0	0	4	0	0	0	0
Total	0	0	10	9	0	0	1	2
Degeneration/necrosis, hepatocellular, single cell								
Slight	0	0	3	3	0	0	0	0

In kidney, hyaline droplets in proximal tubules were seen in most males from all groups but the severity was greater than control in all treated groups. There were no evidence of a dose response at 150 and 2000ppm. Due to the sex-relationship and appearance of the droplets, this finding was considered to represent an accumulation of α_{2u}-globulin. This protein is naturally reabsorbed and accumulated in the renal tubular epithelium of the young male rat. As humans secreted the protein only in trace amounts, this mechanism is generally accepted as not relevant for humans. Therefore, although this accumulation was considered to be treatment-related, it was not considered to be toxicologically significant. Basophilic tubules were seen in males from all groups but the incidence and severity were slightly greater than control in all treated groups with no evidence of a dose response.

Table 6.8.2.3-3: Incidence of treatment related lesions in the kidneys

Sex	Male				Female			
Dosage level (ppm)	0	10	150	2000	0	10	150	2000
Number examined	10	10	10	10	10	10	10	10
Hyaline droplets, proximal tubules, prominent								
Slight	8	7	1	7	0	0	0	0
Mild	0	2	8	3	0	0	0	0
Moderate	0	0	1	0	0	0	0	0
Total	8	9	10	10	0	0	0	0
Basophilic tubules, focal								
Slight	3	8	8	5	1	2	1	0
Mild	0	0	1	1	0	0	0	0
Total	3	8	9	6	1	2	1	0

In the adrenal gland, slight to mild vacuolation of the zona fasciculate was seen in 5/10 males treated at 2000 ppm.

A variety of spontaneous changes was noted in control and treated animals with no indication of relationship to the treatment.

Conclusion:

At 2000 ppm one female was found death and another one was sacrificed due to laboured respiration, reduced motor activity, general pallor, soiled fur and weight loss.

Mean body weight from males dosed at 2000 ppm was statistically significant lower (between 7 to 10%) than controls since day 8 to day 57 of the treatment period and their body weight gain was found to be lower than control in days 8 and 22 (44% and 16%, respectively). Those effects were not observed in female neither in lower dosage. Clinical chemistry analysis revealed higher mean total cholesterol concentrations in females at 2000 ppm and 150 ppm (+34% and +31%, respectively) and statistically significant lower mean tryglicerides concentration in males at 150 ppm (-58%). Also, a tendency towards lower aspartate and alanine aminotransferase activities (-15%, not statistically significant and –25%, respectively) was seen in females at 150 ppm. There were statistically significant differences in albumin/globulin ratio at 150 ppm in both sexes.

Liver was identified as target organ of endosulfate lactone, due to the treatment related findings: macroscopic examination of the organ revealed enlarged livers in rats dosed at 150 and 2000 ppm., and presented dark colour and prominent lobulation . Slight to moderate centrilobular hepatocellular hypertrophy was noted in all animals treated at 150 and 2000 ppm, and in 6 males dosed at 10 ppm.

Also, vacuolation of centrilobular hepatocytes was observed in males treated at 150 and 2000 ppm, and in one and two females dosed at 150 and 2000 ppm, respectively. Males appeared to be more sensitive to the effects of Endosulfan. Degeneration/necrosis of single hepatocytes was seen in 3/10 males treated at 150 and 2000 ppm.

Kidneys from males only presented hyaline droplets in proximal tubules due to the accumulation of α_{2u}-globulin. This finding was not considered toxicologically relevant for humans.

Based on the results of the study, and considering the slight response observed in the liver and kidney of males at 10 ppm, it can be established a NOAEL of 10 ppm (corresponding to 0.6 mg/kg bw/day in males and 0.7 mg/kg/day in females).

B.6.8.2.4 Genotoxicity of Endosulfan-lactone

Ballantyne M., 2001 f (Aventis CropScience C013516)

Dates of experimental work: January 16, 2001 to February 23, 2001. Date of report: April 27, 2001.

The objective of this study was to evaluate the mutagenic activity of Endosulfan-lactone by examining its ability to revert four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 471 (1997), EEC Annex V Tests B13 and B14 (1993), UKEMS (1990), Japanese MHW (1989) and MAFF (1985), ICH Harmonised Tripartite (1997) and EPA-OPPTS 870.5100 (1998).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan-lactone, batch number 0161X with purity 96.7%. It was dissolved in DMSO. Appropriate positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline-1-oxide, benzo[a]pyrene and 2-amino-anthracene) were included. The study was conducted using four *Salmonella typhimurium* strains (TA1535, TA1537, TA98 and TA100) and two *Escherichia coli* strains (WP2 pKM101 and WP2 uvrA pKM101). The mammalian metabolic activation system was S9 (10%) from Aroclor 1254-induced male Sprague Dawley rats. The plate incorporation procedure was used. The m-statistic was calculated to check that the data were Poisson-distributed, and Dunnett's test was used to compare the counts of each dose level with the control. The presence or otherwise of a dose level response was checked by linear regression analysis. The test article is considered to be mutagenic if: 1) Dunnett's test give a significant response ($p \leq 0.01$) and the data set shows a significant dose level correlation; and 2) the positive responses described before are reproducible.

An initial toxicity range-finder experiment was carried out in TA100 strain only, using Endosulfan-lactone at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls, with and without S9. Evidence of toxicity in the form of a diminution of the background bacterial lawn was observed at the highest one or two test concentrations. There was not precipitation of the test article.

Endosulfan-lactone was assayed for mutation in two separate experiments. Negative and positive controls were included for all strains in each experiment.

In the first mutation experiment, Endosulfan-lactone was tested at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, in the presence and absence of S9, in all strains except TA100. Data from the range-finder experiment were considered acceptable for mutation assessment, and presented in the report as the experiment 1 mutation data for TA100 strain. Evidence of toxicity (diminution of the background bacterial lawn) was observed, in the absence of S9, at 1000 and 5000 µg/plate in TA98, TA100 and TA1537; and in the presence of S9, at 1000 and 5000 µg/plate in TA98, and at 5000 µg/plate in TA100 and TA1537. Further evidence of toxicity (marked reduction in revertant numbers) was observed in the two *E. coli* strains at 5000 µg/platel (± S9). No clear evidence of toxicity was observed in TA1535 strain. Negative mutagenicity results were obtained in all strains both in the presence and absence of S9.

In the second mutation experiment, Endosulfan-lactone was tested, in the absence of S9, at 39.0625, 78.125, 156.25, 312.5, 625 and 1250 µg/plate in TA98, TA100 and TA1537; and at 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate in TA1535, WP2 pKM101 and WP2 uvrA pKM101. Concentrations tested in the presence of S9 were 39.0625, 78.125, 156.25, 312.5, 625 and 1250 µg/plate in TA98; 78.125, 156.25, 312.5, 625, 1250 and 2500 µg/plate in TA100 and TA1537; and 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate in TA1535, WP2 pKM101 and WP2 uvrA pKM101. As mutagenicity results of the first experiment were negative, treatments in the presence of S9 in experiment 2 included a pre-incubation step. Evidence of toxicity was observed in all the tester strains except TA1535. In the absence of S9, a diminution of the background bacterial lawn was observed at 625 and 1250 µg/plate in TA100 and TA1537; at 1250 µg/plate in TA98; and at 2500 and 5000 µg/plate in WP2 uvrA pKM101. In addition, a reduction in revertant numbers was observed, in the absence of S9, in the two *E. coli* strains, starting at the lowest concentration tested in WP2 pKM101 and at 1250 µg/plate in WP2 uvrA pKM101, being marked at 5000 µg/plate in both strains. In the presence of S9, toxic effects were observed at the four highest test dose levels in *Salmonella* strains, and at 5000 µg/plate in *E. coli* strains. Only treatments of WP2 uvrA pKM101 strain in the presence of S9 resulted in an increase (not dose level related) in revertant numbers that was statistically significant at all concentrations tested except at 5000 µg/plate.

The maximum increase in revertant numbers above control (1.41) was obtained at 1250 µg/plate. A direct repeat of these strain treatments determined that this increase was not reproducible, as no comparable increase was observed. In this repeat experiment, a marked reduction in revertant numbers was observed at the maximum concentration tested (5000 µg/plate).

Positive controls gave a satisfactory response in all strains used in each experiment except 2-amino-anthracene that did not provided the expected increase in revertant numbers in WP2 pKM101 strain, when tested in the presence of S9. Nevertheless, metabolic activity of the *E. coli* S9 mix was established from the strain WP2 uvrA pKM101 data.

Conclusion

Endosulfan-lactone showed no mutagenic potential under the conditions of this study.

ENDOSULFAN HYDROXY CARBOXYLIC ACID

B.6.8.2.5 Endosulfan Hydroxy Carboxylic Acid. Acute oral toxicity in rats. "Acute toxic class method" B. Griffon (2002) **C024725**

Performing laboratory: CIT BP 563-27005 Evreux-France

Data of experimental work: 30 April 2002 to 28 May 2002

Objective: To evaluate the acute toxicity of Endosulfan Hydroxy Carboxylic Acid.

Guidelines: OECD 423

GLP: yes

Date of report: June 2002

The study is acceptable

Materials and methods:

The test material used was Endosulfan Hydroxy Carboxylicc Acid (batch number AEF114151) determined to be 96.6% purity. This test material was administered to two groups of three males and two groups of three females Sprague-Dawley rats in a single administration by oral route using a metal gavage tube. The dose-level used as starting dose was selected from one of three fixed levels, 25, 200 or 2000 mg/kg body weight. As no information of the toxic potential of the test item was available, for animal welfare reasons, the starting dose of 200 mg/kg was chosen. After the first assay, as 0/3 animals died, another assay was carried out on three females at the next higher dose-level (2000 mg/kg). After the second assay, as 3/3 animals died, the results were confirmed in three females at 200 mg/kg and three males at 2000mg/kg.

Dose regimen is described in the table below.

Table B.6.8.2.5 1- Dose regimen

Dose (mg/kg)	Vehicle	Volume (mL/kg)	Male	Female
200	0.5% methylcellulose	10	3	-
2000	0.5% methylcellulose	10	-	3
200	0.5% methylcellulose	10	-	3
2000	0.5% methylcellulose	10	3	-

Clinical signs, mortality and body weight gain were checked for a period of up to 14 days following the single administration of the test item. All animals were subjected to necropsy.

Findings:

Clinical examinations: At the 200 mg/kg dose-level, no clinical signs no mortality were observed in males. In females, no death occurred but piloerection and dyspnea were observed in all of them from day 1 up to day 5. At the 2000 mg/kg all animals (males and females) died on day 1 within 6 hours following treatment; hypoactivity or sedation, piloerection and tremors were observed prior to death.

Body weight gain of the animals given 200 mg/kg was not affected by treatment with the test item.

Macroscopic examination of the main organs of the animals revealed not apparent abnormalities.

Conclusion:

The oral LD_{50} of the test Endosulfan Hydroxy Carboxilic Acid is comprised between 200 and 2000 mg/kg in males and females rats. According to the classification criteria, Hydroxy Carboxylic Acid should be assigned the symbol Xn, the indication of danger "Harmful" and the risk phrase R22: "Harmful if swallowed".

Response to Annotation by Bayer CropScience

The notifier had submitted acute toxicity and genotoxicity data of endosulfan isomers and metabolites

- α-endosulfan: C013750, C016335
- β-endosulfan: C013756, C017228
- Endosulfan sulfate: C016518, C017165, C017169
- Endosulfan ether: C013508, C013514
- Endosulfan hydroxyether: C013510, C013518, C017622

Evaluation of genotoxicity potential of the metabolites

B.6.8 Further toxicological studies (IIA, 5.8)

B.6.8.2 Genotoxicity of Endosulfan-metabolites

After the ECCO-Peer Review Meeting, the notifier was requested to provide genotoxicity studies performed with Endosulfan-sulfate. The ECCO 106-Overview Meeting considered this data requirement essential for unconditional Annex I inclusion of Endosulfan, and, in addition, proposed that the main data submitter to address the toxicity of Endosulfan-lactone.

In this sense, the main notifier, Aventis, submitted, on January, eight new genotoxicity studies performed on the metabolites: Endosulfan-sulfate, Endosulfan-lactone, Endosulfan-ether, Endosulfan-hydroxyether, as well as on the isomers alpha and beta Endosulfan.

The genotoxicity studies conducted were all to the GLP and regulatory guidelines current at the time the studies were performed. These studies are summarised in table B.6.8.2-1 and B.6.8.2-2.

All tested isomers and metabolites were not genotoxic under the conditions of these studies. Further studies are not necessary.

Table B.6.8.2-1 Summary of genotoxicity studies on Endosulfan isomers

SUBSTANCE	TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
Alpha-Endosulfan	Bacterial gene mutation assay	*S. typhimurium*, TA1535, TA1537, TA100, TA98 *E. coli* WP2 pKM101 WP2 uvrA pKM101	1st exp. plate incorporation test (±S9) 1.6, 8, 40, 200, 1000, 5000 µg/plate 2nd exp. plate incorporation (-S9) and pre-incubation test (+S9) 51.2, 128, 320, 800, 2000, 5000 µg/plate	Negative The only significant increase in revertant number did not meet the criteria for a positive response (WP2 pKM101 +S9).	Precipitation at 800 µg/plate and above. Toxicity at 5000 µg/plate.	Ballantyne, 2001 a (Doc.C016335)
Beta-Endosulfan	Bacterial gene mutation assay	*S. typhimurium*, TA1535, TA1537, TA100, TA98 *E. coli* WP2-pKM101 WP2 uvrA pKM101	1st exp. plate incorporation test (±S9) 1.6, 8, 40, 200, 1000, 5000 µg/plate 2nd exp. plate incorporation (-S9) and pre-incubation test (+S9) 10.24, 25.64, 64, 160, 400, 1000 µg/plate	Negative	Precipitation at 1000 µg/plate (±S9) and also at 400 µg/plate (+S9). No clear evidence of toxicity.	Ballantyne, 2001 b (Doc.C017228)

Table B.6.8.2-2 Summary of genotoxicity studies on Endosulfan metabolites

SUBSTANCE	TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
Endosulfan-sulfate	Bacterial gene mutation assay	*S. typhimurium*, TA1535, TA1537, TA100, TA98 *E. coli* WP2 pKM101 WP2 uvrA pKM101	6 dose levels 1st exp. plate incorporation test (±S9) 1.6-5000 µg/plate 2nd exp. plate incorporation (-S9) and pre-incubation test (+S9) 51.2-5000 µg/plate	Negative Significant increases in revertant number did not meet the criteria for a positive response (TA1537 and WP2 uvrA pKM101, both +S9)	Precipitation at 800 µg/plate and above. Toxicity within the precipitate dose level range.	Ballantyne, 2001 c (Doc.C017165)
	In vitro clastogenic assay	Human lymphocytes	1st exp. trial 1 3h-treatment (+S9): 67.11, 131.1, 204.8 µg/mL 1st exp. trial 2 3h-treatment (-S9): 40, 80, 130 µg/mL 2nd exp. 3h-treatment (+S9): 109, 245.6, 400 µg/mL 20h-treatment (-S9): 18.14, 29.53, 56.57 µg/mL	Negative The cases where the aberrant cell frequency exceeded the historical negative control range (+S9) did not meet the criteria for a positive response.	Precipitation at the top dose level tested in all experiments except in 2nd exp. (-S9). A reduction in mitotic index of about 50 % was induced by the top dose level tested in all experiments except in the 2nd exp. (+S9).	Whitwell, 2001 (Doc.C017169)
Endosulfan-ether	Bacterial gene mutation assay	*S. typhimurium*, TA1535, TA1537, TA100, TA98 *E. coli* WP2 pKM101 WP2 uvrA pKM101	6 dose levels Range finder in TA100 and 1st exp. in all strains plate incorporation test (±S9): 1.6-5000 µg/plate 2nd exp: plate incorporation (-S9) and pre-incubation test (+S9) in all strains: 31.25-1000 µg/plate (-S9) 62.5-2000 µg/plate (+S9); and TA100 plate incorporation test (+S9): 62.5-2000 µg/plate.	Negative Significant increases in revertant number did not meet the criteria for a positive response (TA100 +S9, TA98 –S9)	Precipitation at the top dose level tested in all experiments No evidence of toxicity.	Ballantyne, 2001 d (Doc.C013514)

SUBSTANCE	TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
Endosulfan-hydroxyether	Bacterial gene mutation assay	*S. typhimurium*, TA1535, TA1537, TA100, TA98 *E. coli:* WP2 pKM101 WP2 uvrA pKM101	6 dose levels Range finder in TA100 and 1st exp in all strains plate incorporation test (±S9): 1.6-5000 µg/plate (range finder) 0.32-1000 µg/plate (1st exp.) 2nd exp. plate incorporation (-S9) and pre-incubation test (+S9): 31.25-1000 µg/plate in TA1535 and *E. coli* strains 6.25-200 µg/plate in the remaining strains. 2nd exp. plate incorporation test (+S9) in TA100 and TA98: 6.25-200 µg/plate 2nd exp repeat (TA100 pre-incubation test +S9) 6.25-200 µg/plate	Negative Significant increases in revertant number did not meet the criteria for a positive response (TA100 +S9, TA98 ±S9, WP2 uvrA pKM101±S9, TA1535 –S9)	Precipitation at 5000 µg/plate. Toxicity at the top dose level tested in all treated strains except in TA100 in the 2nd exp. (plate incorporation test + S9).	Ballantyne, 2001 e (Doc.C013518)
	In vivo UDS assay	Hepatocytes from male Han Wistar rats	560 and 1400 mg/kg 1st exp.: the exposure time was 12-14 h 2nd exp.: the exposure time was 2-4 h	Negative	Excessive toxicity at 2000 mg/kg in the range finder experiment. Piloerection at 1400 mg/kg, in 1st exp. from the main study.	Howe, 2001 (Doc.C017622)

SUBSTANCE	TEST	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
Endosulfan-lactone	Bacterial gene mutation assay	*S. typhimurium*, TA1535, TA1537, TA100, TA98 *E. coli* WP2 pKM101 WP2 uvrA pKM101	6 dose levels 1st exp. plate incorporation test (±S9) 1.6-5000 µg/plate 2nd exp. plate incorporation (-S9) and pre-incubation test (+S9) 39.0625-1250 µg/plate in TA98 (±S9). 39.0625-1250 µg/plate in TA1537 and TA100 (-S9). 78.125-2500 µg/plate in TA1537 and TA100 (+S9). 156.25-5000 µg/plate in remaining strains (±S9). 2nd exp repeat WP2 uvrA pKM101 pre-incubation test (+S9): 156.25-5000 µg/plate.	Negative Significant increases in revertant number did not meet the criteria for a positive response (WP2 uvrA pKM101+S9)	There was not precipitation. Toxicity in all strains at the top dose level tested except in TA1535.	Ballantyne, 2001 f (Doc.C013516)

B.6.8.2.1 Genotoxicity of alpha-Endosulfan

Ballantyne M., 2001 a (Aventis CropScience C016335)

Dates of experimental work: March 7, 2001 to May 4, 2001. Date of report: June 25, 2001.

The objective of this study was to evaluate the mutagenic activity of alpha-Endosulfan by examining its ability to revert four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 471 (1997), EEC Annex V Tests B13 and B14 (1993), UKEMS (1990), Japanese MHW (1989) and MAFF (1985), ICH Harmonised Tripartite (1997) and EPA-OPPTS 870.5100 (1998).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was alpha-Endosulfan, batch number 0118X with purity 99.9%. It was dissolved in DMSO. Appropriate positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline-1-oxide, benzo[a]pyrene and 2-amino-anthracene) were included. The study was conducted using four *Salmonella typhimurium* strains (TA1535, TA1537, TA98 and TA100) and two *Escherichia coli* strains (WP2 pKM101 and WP2 uvrA pKM101). The mammalian metabolic activation system was S9 (10%) from Aroclor 1254-induced male Sprague Dawley rats. The plate incorporation procedure was used. The m-statistic was calculated to check that the data were Poisson-distributed, and Dunnett's test was used to compare the counts of each dose level with the control. The presence or otherwise of a dose level response was checked by linear regression analysis. The test article is considered to be mutagenic if: 1) Dunnett's test give a significant response ($p \leq 0.01$) and the data set shows a significant dose level correlation; and 2) the positive responses described before are reproducible.

An initial toxicity range-finder experiment was carried out in TA100 strain only, using alpha-Endosulfan at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls, with and without S9. Following these treatments, no clear evidence of toxicity (diminution of the background bacterial lawn or reduction in revertant numbers) was observed. There was precipitation of test article at 1000 and 5000 µg/plate.

Alpha-Endosulfan was assayed for mutation in two separate experiments. Negative and positive controls were included for all strains in each experiment.

In the first mutation experiment, alpha-Endosulfan was tested at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, in the presence and absence of S9, in all strains except TA100. Data from the range-finder experiment were considered acceptable for mutation assessment, and presented in the report as the experiment 1 mutation data for TA100 strain. Evidence of toxicity (diminution of the background bacterial lawn) was observed at 5000 µg/plate in all strains except TA100 in the absence of S9, and also in TA1537, WP2 pKM101 and WP2 uvrA pKM101 in the presence of S9. Precipitation of test article was observed on all plates treated at 1000 µg/plate and above. When mutagenicity data were analysed at the

1% level using Dunnett's test, the only statistically significant increase in revertant numbers (1.29 above control) was obtained at 1.6 µg/plate in WP2 pKM101 strain in the presence of S9.

Due to the appearance of toxic effects within the precipitating dose level range with at least some strain treatments in experiment 1, it was considered that the lower limit of precipitation was not an appropriate dose level limiting factor to be employed for this study. Therefore, in the second mutation experiment, alpha-Endosulfan was tested at concentrations of 51.2, 128, 320, 800, 2000 and 5000 µg/plate in the presence and absence of S9, in all strains. In addition, treatments in the presence of S9 were modified by the inclusion of a pre-incubation step. Evidence of toxicity (diminution of the background bacterial lawn) was observed at 5000 µg/plate for all strains in the absence and presence of S9. Precipitation of test article was observed on all plates treated at 800 µg/plate and above. Negative mutagenicity results were obtained in all strains both in the presence and absence of S9.

Alpha-Endosulfan was considered non mutagenic because the only statistically significant increase in revertant numbers, obtained in WP2 pKM101 strain in the presence of S9 (experiment 1), was extremely small in magnitude (1.29 above control), and no concentration related (occurred only at the lowest dose level).

Positive controls gave a satisfactory response in all strains used in each experiment except 2-amino-anthracene that did not provided the expected increase in revertant numbers in WP2 pKM101 strain, when tested in the presence of S9. Nevertheless, metabolic activity of the *E. coli* S9 mix was established from the strain WP2 uvrA pKM101 data. It should be also noted that experiment 1 mean solvent control revertant colony counts with strain WP2 pKM101 in the presence of S9, and strain WP2 uvrA pKM101 in the absence and presence of S9, were just above the laboratory historical control range.

Conclusion

Alpha-Endosulfan showed no mutagenic potential under the conditions of this study.

B.6.8.2.2 Genotoxicity of beta-Endosulfan

Ballantyne M., 2001 b (Aventis CropScience C017228)

Dates of experimental work: March 23, 2001 to May 20, 2001. Date of report: July 13, 2001.

The objective of this study was to evaluate the mutagenic activity of beta-Endosulfan by examining its ability to revert four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 471 (1997), EEC Annex V Tests B13 and B14 (1993), UKEMS (1990), Japanese MHW (1989) and MAFF (1985), ICH Harmonised Tripartite (1997) and EPA-OPPTS 870.5100 (1998).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was beta-Endosulfan, batch number RU141/1 with purity 99.6%. It was dissolved in dimethyl formamide (DMF). Appropriate positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline-1-oxide, benzo[a]pyrene and 2-amino-anthracene) were included. The study was conducted using four *Salmonella typhimurium* strains (TA1535, TA1537, TA98 and TA100) and two *Escherichia coli* strains (WP2 pKM101 and WP2 uvrA pKM101). The mammalian metabolic activation system was S9 (10%) from Aroclor 1254-induced male Sprague Dawley rats. The plate incorporation procedure was used. The m-statistic was calculated to check that the data were Poisson-distributed, and Dunnett's test was used to compare the counts of each dose level with the control. The presence or otherwise of a dose level response was checked by linear regression analysis. The test article is considered to be mutagenic if: 1) Dunnett's test give a significant response ($p \leq 0.01$) and the data set shows a significant dose level correlation; and 2) the positive responses described before are reproducible.

An initial toxicity range-finder experiment was carried out in TA100 strain only, using beta-Endosulfan at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls, with and without S9. Following these treatments, no clear evidence of toxicity (diminution of the background bacterial lawn or reduction in revertant numbers) was observed. There was precipitation of test article at 1000 and 5000 µg/plate.

Beta-Endosulfan was assayed for mutation in two separate experiments. Negative and positive controls were included for all strains in each experiment.

In the first mutation experiment, beta-Endosulfan was tested at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, in the presence and absence of S9, in all strains except TA100. Data from the range-finder experiment were considered acceptable for mutation assessment, and presented in the report as the experiment 1 mutation data for TA100 strain. There was no clear evidence of toxicity, although some small reductions in revertant numbers were observed at the highest test dose levels with several strains. There was precipitation of test article on all plates treated at 1000 µg/plate and above. Negative mutagenicity results were obtained in all strains both in the presence and absence of S9.

In the second mutation experiment, beta-Endosulfan was tested at concentrations of 10.24, 25.6, 64, 160, 400 and 1000 µg/plate in the presence and absence of S9, in all strains. In addition, treatments in the presence of S9 were modified by the inclusion of a pre-incubation step. As previously, there was no clear evidence of toxicity, although some small reductions in revertant numbers were observed at the highest test dose levels with several strains. Precipitation of test article was observed on all plates treated at 1000 µg/plate, and also on plates treated at 400 µg/plate in the presence of S9. Negative mutagenicity results were obtained in all strains both in the presence and absence of S9.

Positive controls gave a satisfactory response in all strains used in each experiment except 2-amino-anthracene that did not provided the expected increase in revertant numbers in WP2 pKM101 strain,

when tested in the presence of S9. Nevertheless, metabolic activity of the *E. coli* S9 mix was established from the strain WP2 uvrA pKM101 data.

Conclusion

Beta-Endosulfan showed no mutagenic potential under the conditions of this study.

B.6.8.2.3 Genotoxicity of Endosulfan-sulfate

Ballantyne M., 2001 c (Aventis CropScience C017165)

Dates of experimental work: April 27, 2001 to May 25, 2001. Date of report: July 31, 2001.

The objective of this study was to evaluate the mutagenic activity of Endosulfan-sulfate by examining its ability to revert four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 471 (1997), EEC Annex V Tests B13 and B14 (1993), UKEMS (1990), Japanese MHW (1989) and MAFF (1985), ICH Harmonised Tripartite (1997) and EPA-OPPTS 870.5100 (1998).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan-sulfate, batch number CIW999 with purity 99.3%. It was dissolved in DMSO. Appropriate positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline-1-oxide, benzo[a]pyrene and 2-amino-anthracene) were included. The study was conducted using four *Salmonella typhimurium* strains (TA1535, TA1537, TA98 and TA100) and two *Escherichia coli* strains (WP2 pKM101 and WP2 uvrA pKM101). The mammalian metabolic activation system was S9 (10%) from Aroclor 1254-induced male Sprague Dawley rats. The plate incorporation procedure was used. The m-statistic was calculated to check that the data were Poisson-distributed, and Dunnett's test was used to compare the counts of each dose level with the control. The presence or otherwise of a dose level response was checked by linear regression analysis. The test article is considered to be mutagenic if: 1) Dunnett's test give a significant response ($p \leq 0.01$) and the data set shows a significant dose level correlation; and 2) the positive responses described before are reproducible.

An initial toxicity range-finder experiment was carried out in TA100 strain only, using Endosulfan-sulfate at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls, with and without S9. Following these treatments, there was no clear evidence of toxicity (diminution of the background bacterial lawn or reduction in revertant numbers). Precipitation of test article was observed on all plates treated at the two highest concentrations during the treatment, but only at 5000 µg/plate following 3 days incubation.

Endosulfan-sulfate was assayed for mutation in two separate experiments. Negative and positive controls were included for all strains in each experiment.

In the first mutation experiment, Endosulfan-sulfate was tested at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, in the presence and absence of S9, in all strains except TA100. Data from the range-finder experiment were considered acceptable for mutation assessment, and presented in the report as the experiment 1 mutation data for TA100 strain. A diminution of the background bacterial lawn was observed, in the absence of S9, at 5000 µg/plate in TA1537, and, in the presence of S9, at 1000 and 5000 µg/plate in TA98, and at 200 µg/plate and above in TA1537. Some small reductions in revertant numbers were also observed at the highest dose levels in several strains, in the absence and/or in the presence of S9. There was precipitation of test article on all plates treated at 1000 µg/plate and above. Only treatments of WP2 uvrA pKM101 strain in the presence of S9 resulted in an increase (not dose level related) in revertant numbers that was statistically significant at 1.6 and 200 µg/plate. The maximum increase in revertant numbers above control (1.21) was obtained at 1.6 µg/plate.

Due to the appearance of toxic effects within the precipitating dose level range with at least some strain treatments in experiment 1, it was considered that the lower limit of precipitation was not an appropriate dose level-limiting factor to be employed for this study. Therefore, in the second mutation experiment, Endosulfan-sulfate was tested at concentrations of 51.2, 128, 320, 800, 2000 and 5000 µg/plate in the presence and absence of S9, in all strains. In addition, treatments in the presence of S9 were modified by the inclusion of a pre-incubation step. A diminution of the background bacterial lawn was observed, only in the absence of S9, from 320 µg/plate in TA100 and TA1537, and from 800 µg/plate in TA98. Some small reductions in revertant numbers were also observed at the highest dose levels in several strains, in the absence and/or in the presence of S9. Precipitation of test article was observed on all plates treated at 800 µg/plate and above. When mutagenicity data were analysed at the 1% level using Dunnett's test, the only statistically significant increase in revertant numbers (1.77 above control) was obtained at 320 µg/plate in TA1537 strain in the presence of S9.

Endosulfan-sulfate was considered non mutagenic because the only statistically significant increases in revertant numbers, obtained in WP2 uvrA pKM101 strain (experiment 1) and in TA1537 (experiment 2), both in the presence of S9, were extremely small in magnitude (< 2 above control), and no concentration related.

Positive controls gave a satisfactory response in all strains used in each experiment except 2-amino-anthracene that did not provided the expected increase in revertant numbers in WP2 pKM101 strain, when tested in the presence of S9. Nevertheless, metabolic activity of the *E. coli* S9 mix was established from the strain WP2 uvrA pKM101 data.

Conclusion

Endosulfan-sulfate showed no mutagenic potential under the conditions of this study.

Whitwell J., 2001 (Aventis CropScience C017169)

Dates of experimental work: May 17, 2001 to July 23, 2001. Date of report: August 22, 2001.

The objective of this study was to evaluate the clastogenic potential of Endosulfan-sulfate by examining its effects on the chromosomes of the lymphocytes of human donors, cultured *in vitro* and treated in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 473 (1997), ICH Harmonised Tripartite (1995) and EPA-OPPTS 870.5375 (1998).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan-sulfate, batch number CIW999, with purity 99.3%, and LOT 1059X, with purity 97.4%. It was dissolved in DMSO. The test article solutions were used within 2.75 hours of initial formulation. Appropriate positive controls, 4-nitroquinoline 1-oxide and cyclophosphamide, were included. Blood from three healthy, non-smoking male volunteers was used for each experiment of this study. The mammalian metabolic activation system was S9 (2%) from Aroclor 1254-induced male Sprague Dawley rats.

For each experiment, an appropriate volume of whole blood was drawn from the peripheral circulation within one day of culture initiation. Blood was stored refrigerated prior use. Cultures were set up placing heparinised blood in tubes containing culture medium, foetal calf serum, gentamycin and PHA. Blood cultures were incubated for 48 hours before treatment. All cultures received colchicine 2 hours before harvesting. Slides were examined for mitotic index (at least 200 cells were counted per concentration tested, i. e. 100 cells per replicate culture). The highest concentration used for chromosome analysis was in general that produced a suppression of the mitotic activity of about 50%. Slides from the highest selected concentration and two lower dose levels were taken for microscope analysis. Where possible, 200 metaphases were examined at each level of treatment for chromosome aberrations. The classification system used in scoring aberrations was based on the scheme described by ISCN (1995) and is detailed in Appendix 2 of this report. The aberrant cells in each culture were categorised as follows: 1) cells with structural aberrations including gaps; 2) cells with structural aberrations excluding gaps; and 3) polyploid, endoreduplicated or hyperdiploid cells. A test article is considered as positive if: a) the proportions of cells with structural aberrations at one or more concentrations exceeded the historical negative control range in both replicates, and b) a statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) occurs at these concentrations. The statistical method used was the Fisher's exact test ($p \leq 0.05$).

Preliminary solubility data indicated that Endosulfan-sulfate dissolved in DMSO precipitated into culture medium at concentrations down to approximately 544 µg/mL. Therefore, a concentration of 400 µg/mL was chosen as a suitable maximum for the chromosome aberration study. Osmolality measurements on post-treatment media were not performed because the highest concentration tested (400 µg/mL) was less than 10 mM (molecular weight of Endosulfan-sulfate = 422.9). The test article had no observed effect on

the pH of culture medium. Two mutation experiments were carried out with Endosulfan-sulfate along with concurrent negative (solvent) and positive controls.

In experiment 1, trial 1, treatment of blood cultures from donor 1 in the absence and presence of S9 was for 3 hours followed by a 17-hour recovery period prior to harvest. Endosulfan-sulfate was tested at concentrations of 9.007, 11.26, 14.07, 17.59, 21.99, 27.49, 34.36, 42.95, 53.69, 67.11, 83.89, 104.9, 131.1, 163.8, 204.8, 256, 320 and 400 µg/mL. Precipitation of test article was observed at treatment from 256 µg/mL (without and with S9), and at harvest from 104.9 µg/mL (without S9) or from 131.1 µg/mL (with S9). The mitotic index was determined at concentrations from 42.95 µg/mL to 400 µg/mL (to see Table B.6.8.2.3-1)

Table B.6.8.2.3-1 Mitototic index determination in experiment 1 trial 1 (donor 1)

Treatment (µg/mL) for 3 h	MI[a] (-S9)	MI[a] (+ S9)
42.95	5	0
53.69	15	6
67.11	22	6
83.89	36	21
104.9	69 E	22
131.1	69 E	40 E
163.8	70 E	38 E
204.8	86 E	55 E
256.0	73 PE	62 PE
320.0	69 PE	58 PE
400.0	79 PE	72 PE

[a]MI: Mitotic inhibition (%) = [1-(mean MI_T/mean MI_C)] x 100%
where treatment T= treatment and C= negative control)
P: indicates precipitation observed at treatment
E: indicates precipitation observed at harvest.

Due to the observed toxicity curve of the test article, it was not possible to identify a suitable top concentration for chromosome analysis in the absence of S9. It was therefore necessary to repeat this treatment in a separate trial (Experiment 1, trial 2), using blood cultures from donor 2 and Endosulfan-sulfate concentrations of 30, 40, 50, 60, 70, 80, 85, 90, 95, 100, 105, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200 and 210 µg/mL. Precipitation of test article was observed at treatment from 190 µg/mL, and at harvest from 90 µg/mL. The mitotic index was determined at concentrations from 40 µg/mL to 210 µg/mL (to see Table B.6.8.2.3-2). The mitotic inhibition was 54%, in the absence of S9, at 130 µg/mL (trial 2) and 55%, in the presence of S9, at 204.8 µg/mL (trial 1). Thus, the concentrations selected for chromosome analysis were 40, 80 and 130 µg/mL (- S9), and 67.11, 131.1 and 204.8 µg/mL (+ S9).

Table B.6.8.2.3-2: Mitototic index determination in experiment 1 trial 2 (donor 2)

Treatment (µg/mL) for 3 h	MI[a] (- S9)
40	0
50	9
60	5
70	7
80	27
85	36
90	41 E
95	34 E
100	40 E
105	39 E
110	43 E
120	49 E
130	54 E
140	61 E
150	54 E
160	68 E
170	48 E
180	56 E
190	53 PE
200	62 PE
210	51 PE

[a]MI: Mitotic inhibition (%) = [1-(mean MI_T/mean MI_C)] x 100%
where treatment T= treatment and C= negative control)
P: indicates precipitation observed at treatment
E: indicates precipitation observed at harvest.

In experiment 2, blood cultures from donor 3 were used. The treatment in the absence of S9 was continuous for 20 hours. Treatment in the presence of S9 was for 3 hours only followed by a 17-hour recovery period prior to harvest. Endosulfan-sulfate was tested at concentrations of: 8.047, 9.467, 11.14, 13.10, 15.42, 18.14, 21.34, 25.10, 29.53, 34.74, 40.87, 48.09, 56.57, 66.56, 78.30, 92.12, 108.4, 127.5 and 150 µg/mL (without S9), and 25.25, 29.70, 34.94, 41.11, 48.36, 56.90, 66.94, 78.75, 92.65, 109, 128.2, 150.9, 177.5, 208.8, 245.6, 289, 340 and 400 µg/mL (with S9). Precipitation of test article was observed at harvest from 108.4 µg/mL (without S9) and from 177.5 µg/mL (with S9), and at treatment, only at 400 µg/mL (with S9). The mitotic index was determined at concentrations from 18.14 µg/mL to 150 µg/mL, without S9, and from 92.65 µg/mL to 400 µg/mL, with S9 (to see Table B.6.8.2.3-3). In the absence of S9, the mitotic inhibition was 47% at 56.57 µg/mL. In the presence of S9, the maximum mitotic inhibition (44%) was observed at 245.6 µg/mL, while at the highest concentration tested (400 µg/mL) the mitotic inhibition was only 29%. The concentrations selected for chromosome analysis were 18.14, 29.53 and 56.57 µg/mL (without S9) and 109, 245.6 and 400 µg/mL (with S9).

Table B.6.8.2.3-3: Mitototic index determination in experiment 2 (donor 3)

Treatment (µg/mL) for 20 h (-S9)	MIH[a] (-S9)	Treatment (µg/mL) for 3 h (+ S9)	MI[a] (+ S9)
18.14	0		
21.34	20		
25.10	17		
29.53	25		
34.74	35	92.65	0
40.87	27	109	8
48.09	26	128.2	22
56.57	47	150.9	36
66.56	65	177.5	30 E
78.30	64	208.8	26 E
92.12	62	245.6	44 E
108.4	66 E	289	38 E
127.5	59 E	340	26 E
150.0	61 E	400	29 PE

[a]MI: Mitotic inhibition (%) = [1-(mean MI_T/mean MI_C)] x 100% where treatment T= treatment and C= negative control)
P: indicates precipitation observed at treatment; E: indicates precipitation observed at harvest.

According to the report a difference in toxicity was observed between experiment 1 and 2 for 3 h treatment in the presence of S9. We consider that this difference was also observed between trials 1 and 2 (experiment 1) for 3 h treatment in the absence of S9. The report indicates that reason for this disparity was not clear but that may have had something to do with blood donor variability and/or the presence of precipitate at the beginning and end of the treatment phases at a number of concentration levels. In addition, it should be taken into account the test substance assayed because there were two batches of technical material with different purity but it was not reported which batch was used in each experiment.

Treatment of cultures with Endosulfan-sulfate in the absence and presence of S9 (both experiments) resulted in frequencies of cells with structural chromosome aberrations that were within the historical solvent control range for the majority of concentrations analysed (to see Tables B.6.8.2.3-4, B.6.8.2.3-5, and B.6.8.2.3-6). The only exception was observed at the intermediate concentration (245.6 µg/mL) in the presence of S9 in experiment 2. However, although the aberrant cell frequency was seen to exceed the historical negative control range, this increase was small and present in just one of the two replicate cultures and was not dose related.

Positive controls gave a satisfactory response in each experiment.

Table B.6.8.2.3-4: Cells with structural chromosome aberrations excluding gaps in experiment 1

Without S9 (3 h treatment)				With S9 (3 h treatment)			
Treatment (µg/mL	Replicate	Cells scored	Cells with structural aberrations (%)	Treatment (µg/mL	Replicate	Cells scored	Cells with Structural aberrations (%)
Solvent	A	100	1 (1)	Solvent	A	100	0 (0)
	B	100	3 (3)		B	100	2 (2)
	Total	200	4 (2)		Total	200	2 (1)
40	A	100	0 (0)	67.11	A	100	2 (2)
	B	100	2 (2)		B	100	3 (3)
	Total	200	2 (1)		Total	200	5 (2.5)
80	A	100	0 (0)	131.1	A	100	1 (1)
	B	100	2 (2)		B	100	4 (4)
	Total	200	2 (1)		Total	200	5 (2.5)
130	A	87	2 (2)	204.8	A	100	3 (3)
	B	97	2 (2)		B	100	3 (3)
	Total	184	4 (2)		Total	200	6 (3)
NQO	A	100	16 (16)	CPA	A	100	57 (57)
	B	100	23 (23)		B	100	72 (72)
	Total	200	39 (19.5) *		Total	200	129 (64.5) *

*Statistical significance p≤ 0.01
NQO: 4-nitroquinoline 1-oxide
CPA: cyclophosphamide

Table B.6.8.2.3-5: Cells with structural chromosome aberrations excluding gaps in experiment 2

Without S9 (20 h treatment)				With S9 (3 h treatment)			
Treatment (µg/mL	Replicate	Cells scored	Cells with structural aberrations (%)	Treatment (µg/mL	Replicate	Cells scored	Cells with structural aberrations (%)
Solvent	A	100	1 (1)	Solvent	A	100	0 (0)
	B	100	1 (1)		B	100	2 (2)
	Total	200	2 (1)		Total	200	2 (1)
18.14	A	100	0 (0)	109	A	100	2 (2)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	200	2 (1)
29.53	A	100	2 (2)	245.6	A	100	5 (5)
	B	100	2 (2)		B	100	1 (1)
	Total	200	4 (2)		Total	200	6 (3)
56.57	A	100	3 (3)	400	A	100	2 (2)
	B	100	2 (2)		B	100	1 (1)
	Total	200	5 (2.5)		Total	200	3 (1.5)
NQO	A	100	26 (26)	CPA	A	100	38 (38)
	B	100	23 (23)		B	100	38 (38)
	Total	200	49 (24.5) *		Total	200	76 (38) *

*Statistical significance p≤ 0.01
NQO: 4-nitroquinoline 1-oxide
CPA: cyclophosphamide

Table B.6.8.2.3-6: Historical ranges for solvent controls (cells with structural chromosome aberrations excluding gaps)

Sex and S9 treatment	Total number of cells scored	Mean per 100 cells	Calculated range per 100 cells
Male (-S9)	20600	0.87	0-4
Male (+S9)	16200	0.69	0-4

On the other hand, in the majority of Endosulfan-sulfate treated cultures, the frequencies of cells with numerical chromosome aberrations were within the historical solvent control range (to see Tables B.6.8.2.3-7, B.6.8.2.3-8 and B.6.8.2.3-9). Exceptions to this were observed for treatment in the presence of S9 in experiment 1. Single cultures at the lowest and intermediate concentrations analysed (67.11 and 131.1 µg/mL) had aberrant cell frequencies that exceeded the historical negative control range. However, these increases were marginal, present in just one of the two replicate cultures in each case, and were not dose-related.

Table B.6.8.2.3-7: Cells with numerical chromosome aberrations in experiment 1

Without S9 (3 h treatment)				With S9 (3 h treatment)			
Treatment (µg/mL	Replicate	Cells scored	Cells with numerical aberrations (%)	Treatment (µg/mL	Replicate	Cells scored	Cells with numerical aberrations (%)
Solvent	A	100	0 (0)	Solvent	A	101	1 (1)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	201	1 (0.5)
40	A	100	0 (0)	67.11	A	100	0 (0)
	B	100	0 (0)		B	103	3 (2.9)
	Total	200	0 (0)		Total	203	3 (1.5)
80	A	100	0 (0)	131.1	A	102	2 (2)
	B	101	1 (1)		B	103	3 (2.9)
	Total	201	1 (0.5)		Total	205	5 (2.4)
130	A	88	1 (1.1)	204.8	A	100	0 (0)
	B	98	1 (1)		B	101	1 (1)
	Total	186	2 (1.1)		Total	201	1 (0.5)
NQO	A	100	0 (0)	CPA	A	101	1 (1)
	B	100	0 (0)		B	101	1 (1)
	Total	200	0 (0)		Total	202	2 (1)

NQO: 4-nitroquinoline 1-oxide
CPA: cyclophosphamide

Table B.6.8.2.3-8: Cells with numerical chromosome aberrations in experiment 2

Without S9 (20 h treatment)				With S9 (3 h treatment)			
Treatment (µg/mL	Replicate	Cells scored	Cells with numerical aberrations (%)	Treatment (µg/mL	Replicate	Cells scored	Cells with numerical aberrations (%)
Solvent	A	100	0 (0)	Solvent	A	100	0 (0)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	200	0 (0)
18.14	A	100	0 (0)	109	A	100	0 (0)
	B	101	1 (1)		B	100	0 (0)
	Total	201	1 (0.5)		Total	200	0 (0)
29.53	A	100	0 (0)	245.6	A	100	0 (0)
	B	101	1 (1)		B	100	0 (0)
	Total	201	1 (0.5)		Total	200	0 (0)
56.57	A	100	0 (0)	400	A	100	0 (0)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	200	0 (0)
NQO	A	100	0 (0)	CPA	A	101	1 (1)
	B	100	0 (0)		B	100	0 (0)
	Total	200	0 (0)		Total	201	1 (0.5)

NQO: 4-nitroquinoline 1-oxide
CPA: cyclophosphamide

Table B.6.8.2.3-9: Historical ranges for solvent controls of cells with numerical chromosome aberrations

Sex and S9 treatment	Total number of cells scored	Mean per 100 cells	Calculated range per 100 cells
Male (-S9)	20686	0.41	0-3
Male (+S9)	16246	0.28	0-2

Conclusion

Endosulfan-sulfate did not exhibit clastogenic activity in cultured human lymphocytes, under the conditions of this study.

B.6.8.2.4 Genotoxicity of Endosulfan-ether

Ballantyne M., 2001 d (Aventis CropScience C013514)

Dates of experimental work: January 16, 2001 to February 20, 2001. Date of report: April 27, 2001.

The objective of this study was to evaluate the mutagenic activity of Endosulfan-ether by examining its ability to revert four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 471 (1997), EEC Annex V Tests B13 and B14 (1993), UKEMS (1990), Japanese MHW (1989) and MAFF (1985), ICH Harmonised Tripartite (1997) and EPA-OPPTS 870.5100 (1998).

Deviations: Due to solubility limitations of the test article, it was considered inadvisable to reduce the volume of the test article solution added for the pre-incubation treatments. In order to prevent any possible toxic effects of the solvent (DMSO), an additional 0.5 mL volume of sodium phosphate buffer was added to each pre-incubation tube. To counteract this increased volume on the test plates, the volume of molten agar to which the pre-incubation mixes were added was reduced to 2 mL, but the agar concentration increased from 0.9% to 1.125%.

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan-ether, lot number 0155X with purity 99.8%. It was dissolved in DMSO. Appropriate positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline-1-oxide, benzo[a]pyrene and 2-amino-anthracene) were included. The study was conducted using four *Salmonella typhimurium* strains (TA1535, TA1537, TA98 and TA100) and two *Escherichia coli* strains (WP2 pKM101 and WP2 uvrA pKM101). The mammalian metabolic activation system was S9 (10%) from Aroclor 1254-induced male Sprague Dawley rats. The plate incorporation procedure was used. The m-statistic was calculated to check that the data were Poisson-distributed, and Dunnett's test was used to compare the counts of each dose level with the control. The presence or otherwise of a dose level response was checked by linear regression analysis. The test article is considered to be mutagenic if: 1) Dunnett's test give a significant response ($p \leq 0.01$) and the data set shows a significant dose level correlation; and 2) the positive responses described before are reproducible.

An initial toxicity range-finder experiment was carried out in TA100 strain only, using Endosulfan-ether at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls, with and without S9. Following these treatments, there was no clear evidence of toxicity (diminution of the background bacterial lawn or reduction in revertant numbers). Precipitation of test article was observed on all plates treated at 1000 and 5000 µg/plate. Only treatments in the presence of S9 resulted in increases in revertant numbers that were statistically significant at 200, 1000 and 5000 µg/plate. The maximum increase in revertant numbers above control (1.43) was obtained at the highest dose level.

Endosulfan-ether was assayed for mutation in two separate experiments. Negative and positive controls were included for all strains in each experiment.

In the first mutation experiment, Endosulfan-ether was tested at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, in the presence and absence of S9, in all strains. There was no clear evidence of toxicity, whilst precipitation of test article was observed on all plates treated at 5000 µg/plate, and also on all plates treated at 1000 µg/plate in the absence of S9, and in TA1537 strain in the presence of S9. Negative mutagenicity results were obtained in all strains both in the presence and absence of S9.

In the second mutation experiment, Endosulfan-ether was tested at 31.25, 62.5, 125, 250, 500 and 1000 µg/plate in the absence of S9, and at 62.5, 125, 250, 500, 1000 and 2000 µg/plate in the presence of S9, in all strains. In addition, a pre-incubation methodology was employed for all strain treatments in the presence of S9. In order to investigate small increases in revertant numbers in the presence of S9 in TA100 strain (range-finder experiment), treatments of this strain in the presence of S9 were also performed using the plate incorporation methodology. There was again no evidence of toxicity, whilst precipitation of test article was observed on all plates treated at the highest two test dose levels in the absence and presence of S9 (including the plate incorporation treatments of TA100 strain). When mutagenicity data were analysed at the 1% level using Dunnett's test, a statistically significant increase in revertant numbers (1.65 above control) was obtained only at 125 µg/plate in TA98 strain in the absence of S9. In addition, treatments of TA100 in the presence of S9 (only when the plate incorporation methodology was used) resulted in an increase (not dose level related) in revertant numbers that was statistically significant at all concentrations tested except at 62.5 µg/plate. The maximum increase in revertant numbers above control (1.47) was obtained at 125 µg/plate.

Endosulfan-ether was considered no mutagenic for all strains, including TA98 and TA100. In TA98, the statistically significant increase in revertant numbers in the absence of S9 (experiment 2) was extremely small in magnitude (1.65 above control), and no concentration related (occurred only at a single intermediate dose level). In TA100, statistical significant increases in revertant numbers in the presence of S9 (when the plate incorporation methodology was used) were also extremely small in magnitude (<2 above control). Besides, no clear concentration related was observed (maximum increase at 5000 µg/plate in the range finder experiment and at 125 µg/plate in experiment 2), and no clear reproducible (only on two of the three treatment occasions).

Positive controls gave a satisfactory response in all strains used in each experiment except 2-amino-anthracene that did not provided the expected increase in revertant numbers in WP2 pKM101 strain, when tested in the presence of S9. Nevertheless, metabolic activity of the *E. coli* S9 mix was established from the strain WP2 uvrA pKM101 data. It should be also noted that the mean solvent control revertant colony counts with strain WP2 pKM101 were slightly above the laboratory historical control range, except in experiment 2 in the presence of S9.

Conclusion

Endosulfan-ether showed no mutagenic potential under the conditions of this study.

B.6.8.2.5 Genotoxicity of Endosulfan-hydroxyether

Ballantyne M., 2001 e (Aventis CropScience C013518)

Dates of experimental work: January 16, 2001 to February 26, 2001. Date of report: May 9, 2001.

The objective of this study was to evaluate the mutagenic activity of Endosulfan-hydroxyether by examining its ability to revert four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 471 (1997), EEC Annex V Tests B13 and B14 (1993), UKEMS (1990), Japanese MHW (1989) and MAFF (1985), ICH Harmonised Tripartite (1997) and EPA-OPPTS 870.5100 (1998).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan-hydroxyether, bath number 6R-1370 with purity 95.7%. It was dissolved in DMSO. Appropriate positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline-1-oxide, benzo[a]pyrene and 2-amino-anthracene) were included. The study was conducted using four *Salmonella typhimurium* strains (TA1535, TA1537, TA98 and TA100) and two *Escherichia coli* strains (WP2 pKM101 and WP2 uvrA pKM101). The mammalian metabolic activation system was S9 (10%) from Aroclor 1254-induced male Sprague Dawley rats. The plate incorporation procedure was used. The m-statistic was calculated to check that the data were Poisson-distributed, and Dunnett's test was used to compare the counts of each dose level with the control. The presence or otherwise of a dose level response was checked by linear regression analysis. The test article is considered to be mutagenic if: 1) Dunnett's test give a significant response ($p \leq 0.01$) and the data set shows a significant dose level correlation; and 2) the positive responses described before are reproducible.

An initial toxicity range-finder experiment was carried out in TA100 strain only, using Endosulfan-hydroxyether at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls, with and without S9. Following these treatments, complete killing of the bacterial cells

occurred at 1000 µg/plate and above in the absence of S9, and at 5000 µg/plate in the presence of S9. Further evidence of toxicity (diminution of the background bacterial lawn) was observed at 1000 µg/plate in the presence of S9. Precipitation of test article was also observed on all plates treated at 5000 µg/plate. Only one statistically significant increase in revertant numbers (1.74 above control) was obtained at 200 µg/plate in the presence of S9.

Endosulfan-hydroxyether was assayed for mutation in two separate experiments. Negative and positive controls were included for all strains in each experiment.

In the first mutation experiment, Endosulfan-hydroxyether was tested at concentrations of 0.32, 1.6, 8, 40, 200 and 1000 µg/plate, in the presence and absence of S9, in all strains. Following these treatments, a complete killing of test bacteria (TA98, TA100 and TA1537) occurred at 1000 µg/plate in the absence and presence of S9. In addition, a diminution of the background bacterial lawn was observed at 200 µg/plate in TA98, TA100 and TA1537, and at 1000 µg/plate in the remaining test strains. There was no precipitation of test article. In TA98, statistically significant increases in revertant numbers were observed at 8 µg/plate (1.63 above control) in the absence of S9, and at 200 µg/plate (1.51 above control) in the presence of S9. In TA100, one statistically significant increase in revertant numbers was observed at 200 µg/plate (1.52 above control) in the presence of S9. In WP2 uvrA pKM101, one statistically significant increase in revertant numbers was observed at 0.32 µg/plate (1.26 above control) in the absence of S9. Negative mutagenicity results were obtained in the remaining strains both in the presence and absence of S9.

In the second mutation experiment, Endosulfan-hydroxy ether was tested, in the absence and presence of S9, at 31.25, 62.5, 125, 250, 500 and 1000 µg/plate in TA1535, WP2 pKM101 and WP2 uvrA pKM101, and at 6.25, 12.5, 25, 50, 100 and 200 µg/plate in TA98, TA100 and TA1537. Treatments of each strain in the presence of S9 were performed using a pre-incubation step and due to previously observed increases in revertant numbers, treatments with S9 of strains TA98 and TA100 were also performed using the plate incorporation methodology. Besides, a repeat pre-incubation test was performed with TA100 strain. A diminution of the background bacterial lawn was observed at the highest test dose level in all treated strains (except in TA100 in the plate incorporation treatment with S9). Further diminution of the background bacterial lawn was observed following pre-incubation treatments of all strains (except in TA100 in the repeat pre-incubation test) at the next highest test dose level, and also in *E. coli* strain treatments at the next highest test dose level in the absence of S9. There was no precipitation of test article. In TA100, statistically significant increases in revertant numbers were only observed in the plate incorporation treatments with S9 at 100 µg/plate (1.44 above control) and 200 µg/plate (1.33 above control). In TA1535, statistically significant increases in revertant numbers were observed at 62.5, 125 and 250 µg/plate, in the absence of S9, being the maximum (2.05 above control) obtained at 250 µg/plate. In WP2 uvrA pKM101, one statistically significant increase in revertant numbers was observed at 31.25

µg/plate (1.60 above control) in the presence of S9. Negative mutagenicity results were obtained in the remaining strains both in the presence and absence of S9.

Endosulfan-hydroxyether was considered no mutagenic for all strains. The statistically significant increases in revertant numbers observed in TA98 and WP2 uvrA pKM101 strains (experiment 1) were extremely small in magnitude (< 2 above control), occurred only at a single dose level and were no concentration related. In TA1535, the statistical significant increases in revertant numbers in the absence of S9 (experiment 2) were no clear dose level related (the increase at the highest no toxic dose level was not statistically significant) and no reproducible although one increase was sufficient in magnitude (2.05 above control at 250 µg/plate). In TA100, statistically significant increases in revertant numbers in the presence of S9 were reproducible over all three experimental occasions for plate incorporation treatments, although the increase was not observed when pre-incubation methodology was used. These increases provided some evidence of a dose level relationship, however they were all very small in magnitude (<2 above control), and occurred at only one, or at most two dose levels.

Positive controls gave a satisfactory response in all strains used in each experiment except 2-amino-anthracene that did not provided the expected increase in revertant numbers in WP2 pKM101 strain, when tested in the presence of S9. Nevertheless, metabolic activity of the *E. coli* S9 mix was established from the strain WP2 uvrA pKM101 data. It should be also noted that the mean solvent control revertant colony counts with strain WP2 pKM101 in the absence and presence of S9 (experiment 1), and strain WP2 uvrA pKM101 in the absence of S9 (experiment 2) were slightly above the laboratory historical control range.

Conclusion

Endosulfan-hydroxyether showed no mutagenic potential under the conditions of this study.

Howe J., 2001 (Aventis CropScience C017622)

Dates of experimental work: June 25, 2001 to September 4, 2001. Date of report: October 31, 2001.

The objective of this study was to evaluate the ability of Endosulfan-hydroxyether to induce unscheduled DNA synthesis (UDS) in hepatocytes of treated rats.

Guidelines: OECD 486 (1997), UKEMS (1993) and EPA-OPPTS 870.5550 (1998)

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan-hydroxyether, bath number 6R-1370A with purity 99.4%. The vehicle used in the initial toxicity range-finder study was 0.5% w/v methylcellulose (0.5% MC). However, when tested in a homogeneity trial this did not produce an acceptable homogeneous formulation, therefore, a further trial was performed using 1% w/v methylcellulose + 0.1% w/v Tween 80 (1% MC + 0.1% Tween

80). This second trial produced an acceptable homogeneous formulation and then, 1% MC + 0.1% Tween 80 was used as the vehicle in the main study experiments. Positive controls were 2-acetamidofluorene (2-AAF), for the 12-14 hour experiment, and dimethylnitrosamine (DMN) for the 2-4 hour experiment. The study was conducted using Han Wistar rats.

Information provided by the sponsor indicated that the LD_{50} value for Endosulfan-hydroxyether in male and female rats was greater than 2000 mg/kg (the recommended maximum dose for the UDS assay). Accordingly, a range-finder experiment was performed using 3 male and 3 female rats dosed once with 2000 mg/kg Endosulfan-hidroxyether via oral gavage. During a 2-day post-dose observation period clinical signs, including protruding eyes, piloerection, tremors, hunching, abnormal breathing, staining of the nose, convulsions, lethargy and weight loss, were observed in males. Additionally, one male was also killed *in extremis*. Females exhibited signs including protruding eyes, piloerection, hunching, abnormal breathing and weight loss. Due to this excessive toxicity observed, especially in males, a further 3 male and 3 female rats were dosed once with 1400 mg/kg Endosulfan-hydroxyether. During a 2-day post-dose observation period acceptable clinical signs, including protruding eyes, piloerection and weight loss were observed in both males and females, without differences between sexes. Therefore, 1400 mg/kg was considered representative of a maximum tolerated dose and chosen as the highest dose for the main experiments performed with males.

In the main study, two experiments were carried with male rats treated once by oral gavage. The exposure period was 12-14 hour in experiment 1, and 2-4 hour in experiment 2. Each experiment consisted of two treatment groups (rats received Endosulfan-hydroxyether at 560 and 1400 mg/kg), and concurrent negative (vehicle) and positive control groups. Four animals were assigned to each test group. After the treatment periods, animals were sacrificed and their livers perfused with collagenase to provide a primary culture of hepatocytes. Cultures were made from three animals in each dose group. Viability of the hepatocytes was assessed by the trypan blue exclusion method. In addition, the number of the isolated cells was determined. Three mL of hepatocyte suspension (1.5×10^5 viable cells/mL) were added to each of a six-well multiplate and incubated for at least 90 minutes to allow cells to attach. After the attachment period, hepatocytes were exposed for 4 hours to ^{3}H-thymidine. Six slides from each animal were prepared with fixed hepatocytes and of these, three were dipped in photographic emulsion to prepare autoradiograms. Slides were examined microscopically after development of the emulsion and staining. Nuclear and mean cytoplasm grain counts were recorded, and the net nuclear grain count (NNG) determined. Where possible, 100 cells were analysed per animal using two slides in each case. The following data were calculated for each slide, animal and dose point: 1) the population average NNG and standard deviation; 2) the percent of cells responding or in repair (i.e. $\geq$ 5 NNG); 3) the population average cytoplasmic and nuclear grain count. A test article is considered as positive in this assay if, at any dose and at either time point: a) it produces group mean NNG values greater than 0 NNG and 20% or more cells responding; b) an increase above solvent control levels is seen in both NNG and the percentage of cells in repair.

Piloerection was observed in the main study (1400 mg/kg dose group, experiment 1). No other clinical signs were observed in any other animal dosed. Viability of liver cells from all group animals was ≥50%. Negative (vehicle) control animals gave a group mean NNG value of zero or less with only 1.3 to 4.7% cells in repair. Group mean NNG values were increased by 2-AAF and DMN treatment to more than 5 (8.6 and 15.1 respectively) and more than 50% cells found to be in repair. Treatment with 560 or 1400 mg/kg Endosulfan-hydroxyether did not produce a group mean value greater than –0.8 nor were any more than 1.3% cells found in repair at either dose.

It may be noted that in experiment 1, it was only possible to score 9 cells from one of the three maximum dose group animals (1400 mg/kg). This was probably due to delayed toxicity in this single animal. However, it was possible to score 100 cells for each of the remaining two animals in this group, and as there was no evidence of UDS induction in any animal tested this reduced cell number is not considered to affect the validity of the study in any way. Additionally, the vehicle control NNG value for experiment 2 was slightly raised (0.0). This was due to slide variability and in particular a single slide which had a raised mean NNG value. However, as the overall mean NNG value for the group is within the negative historical control range, this value is considered to be acceptable.

Conclusion

Endosulfan-hydroxyether did not induce UDS in hepatocytes of treated rats, under the conditions of this study.

B.6.8.2.6 Genotoxicity of Endosulfan-lactone

Ballantyne M., 2001 f (Aventis CropScience C013516)

Dates of experimental work: January 16, 2001 to February 23, 2001. Date of report: April 27, 2001.

The objective of this study was to evaluate the mutagenic activity of Endosulfan-lactone by examining its ability to revert four histidine-requiring strains of *Salmonella typhimurium* and two tryptophan-requiring strains of *Escherichia coli* in the absence and presence of a rat liver metabolising system (S9).

Guidelines: OECD 471 (1997), EEC Annex V Tests B13 and B14 (1993), UKEMS (1990), Japanese MHW (1989) and MAFF (1985), ICH Harmonised Tripartite (1997) and EPA-OPPTS 870.5100 (1998).

GLP: Yes.

The study is considered acceptable.

Material and methods together with findings

The test substance was Endosulfan-lactone, batch number 0161X with purity 96.7%. It was dissolved in DMSO. Appropriate positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline-1-oxide, benzo[a]pyrene and 2-amino-anthracene) were included. The study was conducted using four *Salmonella typhimurium* strains (TA1535, TA1537, TA98 and TA100) and two *Escherichia coli* strains (WP2 pKM101 and WP2 uvrA pKM101). The mammalian metabolic activation system was S9 (10%) from Aroclor 1254-induced male Sprague Dawley rats. The plate incorporation procedure was used. The m-statistic was calculated to check that the data were Poisson-distributed, and Dunnett's test was used to compare the counts of each dose level with the control. The presence or otherwise of a dose level

response was checked by linear regression analysis. The test article is considered to be mutagenic if: 1) Dunnett's test give a significant response ($p \leq 0.01$) and the data set shows a significant dose level correlation; and 2) the positive responses described before are reproducible.

An initial toxicity range-finder experiment was carried out in TA100 strain only, using Endosulfan-lactone at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls, with and without S9. Evidence of toxicity in the form of a diminution of the background bacterial lawn was observed at the highest one or two test concentrations. There was not precipitation of the test article.

Endosulfan-lactone was assayed for mutation in two separate experiments. Negative and positive controls were included for all strains in each experiment.

In the first mutation experiment, Endosulfan-lactone was tested at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, in the presence and absence of S9, in all strains except TA100. Data from the range-finder experiment were considered acceptable for mutation assessment, and presented in the report as the experiment 1 mutation data for TA100 strain. Evidence of toxicity (diminution of the background bacterial lawn) was observed, in the absence of S9, at 1000 and 5000 µg/plate in TA98, TA100 and TA1537; and in the presence of S9, at 1000 and 5000 µg/plate in TA98, and at 5000 µg/plate in TA100 and TA1537. Further evidence of toxicity (marked reduction in revertant numbers) was observed in the two *E. coli* strains at 5000 µg/platel (± S9). No clear evidence of toxicity was observed in TA1535 strain. Negative mutagenicity results were obtained in all strains both in the presence and absence of S9.

In the second mutation experiment, Endosulfan-lactone was tested, in the absence of S9, at 39.0625, 78.125, 156.25, 312.5, 625 and 1250 µg/plate in TA98, TA100 and TA1537; and at 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate in TA1535, WP2 pKM101 and WP2 uvrA pKM101. Concentrations tested in the presence of S9 were 39.0625, 78.125, 156.25, 312.5, 625 and 1250 µg/plate in TA98; 78.125, 156.25, 312.5, 625, 1250 and 2500 µg/plate in TA100 and TA1537; and 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate in TA1535, WP2 pKM101 and WP2 uvrA pKM101. As mutagenicity results of the first experiment were negative, treatments in the presence of S9 in experiment 2 included a pre-incubation step. Evidence of toxicity was observed in all the tester strains except TA1535. In the absence of S9, a diminution of the background bacterial lawn was observed at 625 and 1250 µg/plate in TA100 and TA1537; at 1250 µg/plate in TA98; and at 2500 and 5000 µg/plate in WP2 uvrA pKM101. In addition, a reduction in revertant numbers was observed, in the absence of S9, in the two *E. coli* strains, starting at the lowest concentration tested in WP2 pKM101 and at 1250 µg/plate in WP2 uvrA pKM101, being marked at 5000 µg/plate in both strains. In the presence of S9, toxic effects were observed at the four highest test dose levels in *Salmonella* strains, and at 5000 µg/plate in *E. coli* strains. Only treatments of WP2 uvrA pKM101 strain in the presence of S9 resulted in an increase (not dose level related) in revertant numbers that was statistically significant at all concentrations tested except at 5000 µg/plate.

The maximum increase in revertant numbers above control (1.41) was obtained at 1250 µg/plate. A direct repeat of these strain treatments determined that this increase was not reproducible, as no comparable increase was observed. In this repeat experiment, a marked reduction in revertant numbers was observed at the maximum concentration tested (5000 µg/plate).

Positive controls gave a satisfactory response in all strains used in each experiment except 2-amino-anthracene that did not provided the expected increase in revertant numbers in WP2 pKM101 strain, when tested in the presence of S9. Nevertheless, metabolic activity of the *E. coli* S9 mix was established from the strain WP2 uvrA pKM101 data.

Conclusion

Endosulfan-lactone showed no mutagenic potential under the conditions of this study.

B 6.9 Referents on.

Annex II or annex III point	Author (s) Year Title Reference	GLP GEP Y/N	Published	Owner	Data protection
IIA/5.4	Honarver N 2003 In vivo chromosome aberration assay in mouse spermatogonial cells C032454	Y	N	Aventis	NO
IIIA	Craine, E.M. 1986 Dermal absorption in vivo rats A35730	Y	N	AgrAv	NO
IIIA	Craine, E.M. 1988 Dermal absorption in vivo rats A39677	Y	N	AgrAv	NO
IIIA	Lachmann, G 1987 Dermal absorption in vivo monkeys A36685	Y	N	AgrAv	NO
IIIA	Noctor JC, John SA 1995 Dermal absorption in vitro human and rats skin A54103	Y	N	AgrAv	NO
IIIA	Singer GM 1997 Biomonitoring study AA950305	Y	N	AgrAv	NO
IIIA	Davies DJ 2002 Dermal absorption in vitro human, rat and pig skin C021864	Y	N	Aventis	YES
IIIA	Urtizberea M, Reader S 2002 Re.evaluation of the operator exposure and risk assessment for Thiodan 35 EC with a new data on skin penetration C022980	Y	N	Aventis	NO
IIA/5.8	Griffon 2002 Endosulfan lactone. Acute oral toxicity in rats. Acute classic method C024720	Y	N	Bayer	NO
IIA/5.8	Labay K 2003 Endosulfan lactone: preliminary 28-day toxicity study in the rat by dietary administration C032189	Y	N	Bayer	NO
IIA/5.8	Langrand-Lerche C. 2003 Endosulfan lactone. 90-day toxicity study in the rat by dietary administration C032788	Y	N	Bayer	NO
IIA/5.8	Griffon B 2002 Endosulfan hydroxy carboxylic acid. Acute oral toxicity in rats. Acute toxic class method	Y	N	Bayer	NO

Annex II or annex III point	Author (s) Year Title Reference	GLP GEP Y/N	Published	Owner	Data protection
	C024725				
IIA/5.8	Ballantyne, M 2001 Alpha-endosulfan: reverse mutation in four histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requering strains of *Escherichia coli* C016335	Y	N	Aventis	NO
IIA/5.8	Ballantyne, M 2001 Beta-endosulfan: reverse mutation in four histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requering strains of *Escherichia coli* C017228	Y	N	Aventis	NO
IIA/5.8	Ballantyne, M 2001 Endosulfan-sulfate: reverse mutation in four histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requering strains of *Escherichia coli* C017165	Y	N	Aventis	NO
IIA/5.8	Ballantyne, M 2001 Endosulfan ether: reverse mutation in four histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requering strains of *Escherichia coli* C013514	Y	N	Aventis	NO
IIA/5.8	Ballantyne, M 2001 Endosulfan hydroxyether: reverse mutation in four histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requering strains of *Escherichia coli* C013518	Y	N	Aventis	NO
IIA/5.8	Ballantyne, M 2001 Endosulfan-lactone: reverse mutation in four histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requering strains of *Escherichia coli* C013516	Y	N	Aventis	NO
IIA/5.8	Howe J. 2001 Endosulfan hydroxyether: measurement of unscheduled DNA sintesis in rat liver using *in vivo/in vitro* procedure C017622	Y	N	Aventis	NO
IIA/5.8	Whitwell J 2001 Endosulfan sulfate: induction of chromosome aberrations in cultured human peripheral blood lymphocytes C017169	Y	N	Aventis	NO

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA/5.8	Ballantyne, M. 2001 a Alpha-Endosulfan: Reverse Mutation in four Histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requiring strains of *Escherichia coli*. Document No.C016335 Report No. 605335 [redacted] 1905/33-D6171	YES	NO	Aventis	NO
IIA/5.8	Ballantyne, M. 2001 b Beta-Endosulfan: Reverse Mutation in four Histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requiring strains of *Escherichia coli*. Document No.C017228 Report No. 605334 ([redacted] 1905/53-D6171	YES	NO	Aventis	NO
IIA/5.8	Ballantyne, M. 2001 c Endosulfan-sulfate: Reverse Mutation in four Histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requiring strains of *Escherichia coli*. Document No.C017165 Report No. 605322 ([redacted] 1905/29-D6171	YES	NO	Aventis	NO
IIA/5.8	Ballantyne, M. 2001 d Endosulfan-ether: Reverse Mutation in four Histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requiring strains of *Escherichia coli*. Document No.C013514 Report No. 605229 ([redacted] 1905/30-D6171	YES	NO	Aventis	NO
IIA/5.8	Ballantyne, M. 2001 e Endosulfan-hydroxyether: Reverse Mutation in four Histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requiring	YES	NO	Aventis	NO

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA/5.8	Ballantyne, M. 2001 f Endosulfan-lactone: Reverse Mutation in four Histidine-requering strains of *Salmonella typhimurium* and two Tryptophan-requiring strains of *Escherichia coli*. Document No.C013516 Report No. 605230 [redacted] 1905/31-D6171	YES	NO	Aventis	NO
IIA/5.8	Howe, J. 2001 Endosulfan hydroxyether: Measurement of unscheduled DNA síntesis in rat liver using an *in vivo/in vitro* procedure Document No.C017622 Report No. 2014/22-D6173	YES	NO	Aventis	NO
IIA/5.8	Whitwell, J. 2001 Endosulfan-sulfate: induction of chromosome aberrations in cultured human peripheral blood lymphocytes Document No.C017169 Report No. 605365 [redacted] 2014/18-D6172	YES	NO	Aventis	NO