Section A6.6.1 **6.6.1 Genotoxicity in vitro (gene mutation in bacteria)**

Annex Point IIA6.6.1

Key Study

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*29/11/05*
Materials and Methods	***3.1.2*** *To what* ***exactly*** *does 'specification' refer?*
	Applicants version is acceptable.
Results and discussion	*Adopt applicant's version.*
Conclusion	*Other conclusions:*
	(Adopt applicant's version or include revised version)
Reliability	*2*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.1 6.6.1Genotoxicity in vitro (gene mutation in bacteria)

Annex Point IIA6.6.1

Table A6_6_1(2)-1. Table for Gene Mutation Assay

SALMONELLA MUTAGENESIS ASSAY
AVERAGED PLATE COUNTS AND REVERSION INDEX*
PLATE INCORPORATION

Burroughs Wellcome Company (Client)
BW 0021673 Batch #816912
Permethrin tech (MRI #150A) (Test Compound Identity)

B. Richard 5/17/79 (Investigator)
DMSO (Solvent)

0.5 µl - 50.0 µl (Dose Range)
None (Metabolic Activation)

STRAIN	DATE	SOLVENT CONTROL AVG. REVERT.	SOLVENT CONTROL REVERSION INDEX	1st CONCENTRATION 2.5 AVG. REVERT.	1st CONCENTRATION 2.5 REVERSION INDEX	2nd CONCENTRATION 5.0 AVG. REVERT.	2nd CONCENTRATION 5.0 REVERSION INDEX	3rd CONCENTRATION 10.0 AVG. REVERT.	3rd CONCENTRATION 10.0 REVERSION INDEX	4th CONCENTRATION 25.0 AVG. REVERT.	4th CONCENTRATION 25.0 REVERSION INDEX	5th CONCENTRATION 50.0 AVG. REVERT.	5th CONCENTRATION 50.0 REVERSION INDEX
TA 98	5-10-79	15	12.6	13	11.5	13	11.8	18	16.4	16	14.5	9	8.21
TA 100	5-10-79	11.7	106.4	131	119.1	128	116.4	119	108.2	136	123.6	86	78.7
TA 1535	5-10-79	16	12.3	14	11.7	14	11.7	10	8.3	10	8.3	7	5.8
TA 1537	5-10-79	6	2.9	3	1.4	7	3.3	3	1.4	3	1.4	4	1.9
TA 1538	5-10-79	10	10.2	9	9.0	11	11.0	7	7.0	6	6.0	5	5.0

POSITIVE CONTROLS

TA98 2AA @ 0.5ug/plate with activation Avg. Revert.	TA98 2AA @ 0.5ug/plate with activation Revers. Index	TA100 2AA @ 0.5ug/plate with activation Avg. Revert.	TA100 2AA @ 0.5ug/plate with activation Revers. Index	TA1535 2AA @ 0.5ug/plate with activation Avg. Revert.	TA1535 2AA @ 0.5ug/plate with activation Revers. Index	TA1535 FS @ 0.02ul/plate without activation Avg. Revert.	TA1535 FS @ 0.02ul/plate without activation Revers. Index	TA1537 9AAD @ 75ug/plate without activation Avg. Revert.	TA1537 9AAD @ 75ug/plate without activation Revers. Index
807	77.6	1231	119.1	384	384.0	607	505.8	255	191.4

EST'M. CELLS PLATED PER PLATE

TA98	1.1×10^8
TA100	1.1×10^8
TA1535	1.2×10^8
TA1537	2.1×10^8
TA1538	1.5×10^8

* Revertants observed per 10^8 cells plated.

Form No. MU-99

Section A6.6.1 6.6.1Genotoxicity in vitro (gene mutation in bacteria)

Annex Point IIA6.6.1

Table A6_6_1(2)-2. Table for Gene Mutation Assay

SALMONELLA MUTAGENESIS ASSAY
AVERAGED PLATE COUNTS AND REVERSION INDEX*
PLATE INCORPORATION

Burroughs Wellcome Company (Client) — L Reichard 5/7/79 (Investigator) — 0.5 µl - 50.0 µl (Dose Range)

BW 0021873 Batch 4818012 Permethrin tech (MRI #150A) (Test Compound Identity) — DMSO (Solvent) — Rat Liver Microsomes (Metabolic Activation)

STRAIN	DATE	SOLVENT CONTROL AVG. REVERT.	SOLVENT CONTROL REVERSION INDEX	1st CONCENTRATION 0.5 AVG. REVERT.	1st CONCENTRATION 0.5 REVERSION INDEX	2nd CONCENTRATION 2.6 AVG. REVERT.	2nd CONCENTRATION 2.6 REVERSION INDEX	3rd CONCENTRATION 12.0 AVG. REVERT.	3rd CONCENTRATION 12.0 REVERSION INDEX	4th CONCENTRATION 25.0 AVG. REVERT.	4th CONCENTRATION 25.0 REVERSION INDEX	5th CONCENTRATION 50.0 AVG. REVERT.	5th CONCENTRATION 50.0 REVERSION INDEX
TA98	5.10.79	23	20.0	23	20.9	20	18.2	24	21.8	20	18.2	121	109
TA100	5.10.79	113	102.7	106	96.4	111	100.9	156	141.8	165	150.0	115	104.5
TA1535	5.10.79	10	8.3	9	7.5	7	5.8	8	6.7	6	5.0	4	3.3
TA1537	5.10.79	6	2.9	6	2.9	8	3.8	4	1.9	4	1.9	4	1.9
TA1538	5.10.79	11	11.0	12	12.0	14	14.0	13	13.0	11	11.0	5	5.0

POSITIVE CONTROLS

TA98 2AA @ 0.4ug/plate with activation Avg. Revert.	TA98 2AA @ 0.4ug/plate with activation Revers. Index	TA100 2AA @ 0.4ug/plate with activation Avg. Revert.	TA100 2AA @ 0.4ug/plate with activation Revers. Index	TA1535 2AA @ 0.4ug/plate with activation Avg. Revert.	TA1535 2AA @ 0.4ug/plate with activation Revers. Index	TA1535 FS @ 0.05µl/plate without activation Avg. Revert.	TA1535 FS @ 0.05µl/plate without activation Revers. Index	TA1537 9AAD @ 75ug/plate without activation Avg. Revert.	TA1537 9AAD @ 75ug/plate without activation Revers. Index
507	733.6	1281	1191	354	294.0	607	505.8	255	121.4

TOTAL CELLS PLATED PER PLATE

TA98	1.1 X 10^8
TA100	1.1 X 10^8
TA1535	1.2 X 10^8
TA1537	2.1 X 10^8
TA1538	1.0 X 10^8

* Revertants observed per 10^8 cells plated.

Form No. ML-59

Section A6.6.1 6.6.1Genotoxicity in vitro (gene mutation in bacteria)

Annex Point IIA6.6.1

Table A6_6_1(2)-3. Table for Gene Mutation Assay

SALMONELLA MUTAGENESIS ASSAY

AVERAGED PLATE COUNTS AND REVERSION INDEX*

PRE-INCUBATION

Burroughs Wellcome Company — Client
BW 0021273 Batch #818012 Permethrin tech (NRI #150A) — Test Compound Accuracy
H Reichard 6/1/79 — Investigator
DMSO — Solvent
0.5 µl - 50.0 µl/plate; 0.8 µl - 81.3 µl/ml — Dose Range
None — Metabolic Activation

STRAIN	DATE	SOLVENT CONTROL AVG. REVERT.	SOLVENT CONTROL REVERSION INDEX	1st CONCENTRATION 0.5 µl/plate 0.8 µl/ml AVG. REVERT.	1st CONCENTRATION REVERSION INDEX	2nd CONCENTRATION 2.6 µl/plate 4.3 µl/ml AVG. REVERT.	2nd CONCENTRATION REVERSION INDEX	3rd CONCENTRATION 13.0 µl/plate 21.7 µl/ml AVG. REVERT.	3rd CONCENTRATION REVERSION INDEX	4th CONCENTRATION 25.0 µl/plate 41.7 µl/ml AVG. REVERT.	4th CONCENTRATION REVERSION INDEX	5th CONCENTRATION 500 µl/plate PS 8 µl/ml AVG. REVERT.	5th CONCENTRATION REVERSION INDEX
TA98	5.11.79	15	16.7	21	23.3	15	20.0	18	20.0	24	26.7	18	20.0
TA100	5.11.79	101	112.2	105	116.7	108	120.0	110	122.2	114	126.7	112	124.4
TA1535	5.11.79	36	40.0	36	45.0	27	33.8	21	26.3	14	17.5	13	16.3
TA1537	5.11.79	8	11.4	8	11.4	15	21.4	9	12.9	6	8.6	13	18.6
TA1538	5.11.79	8	6.2	11	8.5	10	7.7	18	13.8	13	10.0	13	10.0

POSITIVE CONTROLS

TA98 2AA # 0.4ug/plate with activation Avg. Revert.	TA98 Revers. Index	TA100 2AA # 0.4ug/plate with activation Avg. Revert.	TA100 Revers. Index	TA1538 2AA # 0.4ug/plate with activation Avg. Revert.	TA1538 Revers. Index	TA1535 PS # 0.02µl/plate without activation Avg. Revert.	TA1535 Revers. Index	TA1537 9AAD # 75ug/plate without activation Avg. Revert.	TA1537 Revers. Index
856	951.1	1258	1377.7	839	645.4	730	975.0	244	348.6

TOTAL CELLS PLATED PER PLATE

TA98	0.9×10^8
TA100	0.9×10^8
TA1535	0.8×10^8
TA1537	0.7×10^8
TA1538	1.2×10^8

* - Revertants observed per 10^8 cells plated.

Form No. GL-29

Table A6_6_1(2)-4. Table for Gene Mutation Assay

SALMONELLA MUTAGENESIS ASSAY
AVERAGED PLATE COUNTS AND REVERSION INDEX*
PRE-INCUBATION

Burroughs Wellcome Company (client)
BW 0021273 Batch #81B012 Permethrin tech (IMRI #1509A) (Test Compound Identity)
E. Eickhoff 5/17/79 (Investigator)
DMSO (Solvent)
0.5 µl - 50.0 µl/plate; 0.8 µl - 83.3 µl/ml (Dose Range)
Rat Liver Microsomes (Metabolic Activation)

STRAIN	DATE	SOLVENT CONTROL AVG. REVERT.	SOLVENT CONTROL REVERSION INDEX	1st CONCENTRATION 0.5 µl/plate 0.8 µl/ml AVG. REVERT.	1st CONCENTRATION REVERSION INDEX	2nd CONCENTRATION 2.0 µl/plate 3.3 µl/ml AVG. REVERT.	2nd CONCENTRATION REVERSION INDEX	3rd CONCENTRATION 12.0 µl/plate 20 µl/ml AVG. REVERT.	3rd CONCENTRATION REVERSION INDEX	4th CONCENTRATION 25.0 µl/plate 41.7 µl/ml AVG. REVERT.	4th CONCENTRATION REVERSION INDEX	5th CONCENTRATION 50.0 µl/plate 83.3 µl/ml AVG. REVERT.	5th CONCENTRATION REVERSION INDEX
TA 98	5-8-79	27	30.0	24	26.7	23	25.6	36	40.0	25	27.8	26	28.9
TA 100	5-11-79	108	120.0	115	127.8	106	117.7	148	164.4	128	142.2	131	145.6
TA 1535	5-11-79	16	22.9	11	15.7	9	12.9	8	11.4	8	11.4	7	10.0
TA 1537	5-11-79	11	13.8	10	12.5	14	17.5	6	7.5	5	6.3	4	5.0
TA 1538	5-11-79	23	17.7	18	13.8	25	19.2	17	13.1	14	10.8	13	10.0

POSITIVE CONTROLS

TA98 2AA @ 0.4ug/plate with activation		TA100 2AA @ 0.4ug/plate with activation		TA1535 2AA @ 0.1ug/plate with activation		TA1535 SA @ 0.02ug/plate without activation		TA1537 9AAD @ 50ug/plate without activation	
Avg. Revert.	Revers. Index	Avg. Revert.	Revers. Index	Avg. Revert.	Revers. Index	Avg. Revert.	Revers. Index	Avg. Revert.	Revers. Index
856	951.1	1258	1397.8	839	645.4	780	975.0	244	348.6

TOTAL CELLS PLATED PER PLATE

TA98	0.9×10^8
TA100	0.9×10^8
TA1535	0.8×10^8
TA1537	0.7×10^8
TA1538	1.3×10^8

* Revertants observed per 10^8 cells plated.

Form No. 92-59

Section A6.6.2 **6.6.2 Genotoxicity in vitro (chromosome aberrations)**

Annex Point IIA6.6.2

Key Study

		Official use only
	1 REFERENCE	
1.1 Reference	Barrueco C[1], Herrera A[2], Caballo C[2] & de la Peña E[3]; 1994; Induction of Structural Chromosome Aberrations in Human Lymphocyte Cultures and CHO Cells by permethrin; Centro Nacional de Sanidad Ambiental, Instituto de Salud Carlos III[1], Seguridad Química, Ministerio de Sanidad y Consumo[2], Centro de Ciencias Medioambientales, Consejo Superior de Investigaciones Científicas[3], Madrid, Spain; Teratogenesis, Carcinogenesis, and Mutagenesis 14:31-38; 1994.	
1.2 Data protection	No	
1.2.1 Data owner	Public domain	
1.2.2 Companies with letter of access	Not applicable	
1.2.3 Criteria for data protection	No data protection claimed	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes; UKEMS Recommended Procedures.	
2.2 GLP	No; not designed as a regulatory study.	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Permethrin (3-phenoxybenzyl [1RS,3RS;1RS,3SR]-3-(2,2-dichloro-vinyl)-2,2-dimethylcyclopropane carboxylate) (CAS Registry 52645-53-1), with a purity of 99.5%, was provided by Chem. Service, Inc. (West Chester, PA)	
3.1.1 Lot/Batch number	Not available	
3.1.2 Specification	Deviating from specification given in section 2 as follows	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	99.5%	
3.1.2.3 Stability	Not applicable (short-term administration)	
3.2 Study Type	In Vitro mammalian chromosome aberration test	
3.2.1 Organism/cell type	mammalian cell lines: Chinese Hamster Ovary (CHO)	X
	primary cultures: lymphocytes	
3.2.2 Deficiencies / Proficiencies	Not applicable	X
3.2.3 METABOLIC ACTIVATION SYSTEM	S9 mix, rat, liver, induced, Aroclor 1254	
3.2.4 Positive control	Not applicable	X

Section A6.6.2 **6.6.2 Genotoxicity in vitro (chromosome aberrations)**

Annex Point IIA6.6.2

		Key Study
3.3 Administration / Exposure; Application of test substance		
3.3.1 Concentrations	Human lymphocytes:	0, 50, 75, 100, 150, 200 µg/mL -S9
		0, 50, 75, 100, 150 µg/mL +S9
	CHO cells:	0, 20, 50, 100 µg/mL ±S9
3.3.2 WAY OF APPLICATION	added to cultures	
3.3.3 Pre-incubation time	Human lymphocytes:	48 hours
	CHO cells:	20 hours
3.3.4 Other modifications	Human lymphocytes: -S9, cultures were incubated in the presence of permethrin for periods of either 21 or 2 hours; +S9, cultures were incubated in the presence of permethrin for a period of 2 hours. CHO cells: -S9, cultures were incubated in the presence of permethrin for periods of either 20 or 3 hours; +S9, cultures were incubated in the presence of permethrin for a period of 3 hours.	
3.4 Examinations	see tables in appendix for examinations and results	
3.4.1 Number of cells evaluated	Human lymphocytes: a total of 50 or 100 well-spread metaphases containing 46 ± 2 chromosomes were examined per treatment for each donor. CHO cells: a total of 100 well-spread metaphases containing 20 ± 2 chromosomes were examined per treatment for each experiment.	

Section A6.6.2 **6.6.2 Genotoxicity in vitro (chromosome aberrations)**

Annex Point IIA6.6.2

Key Study

4 RESULTS AND DISCUSSION

4.1 Genotoxicity

4.1.1 without metabolic activation

Human lymphocytes

When permethrin was present in the culture medium all along (21 hours) and cultures were harvested, increases observed in the frequency of chromosome aberrations were statistically significant in the range of 75-100 µg/mL for the first donor and in the range of 100-150 µg/mL for the second donor. The effect of permethrin seemed to be dose-dependent. Permethrin induced chromosome- and chromatid-type aberrations. The highest frequency of chromosome aberrations, mainly chromosome-type, was induced by 100 µg/mL of permethrin in both donors.

When permethrin was only present 2 hours in the culture medium of lymphocytes, the increases observed in the chromosome aberrations' frequency were statistically significant in the range of 150-200 µg/mL. Permethrin induced chromatid-type aberrations.

CHO cells

When permethrin was present in the culture medium all along (20 hours) and cultures were harvested, increases observed in the frequency of chromosome aberrations were statistically significant in the range of 50-100 µg/mL. The effect of permethrin seemed to be dose-dependent. Permethrin induced chromosome- and chromatid-type aberrations. The highest frequency of chromosome aberrations, mainly chromosome-type, was induced by 100 µg/mL of permethrin in both cultures.

In CHO cells exposed to permethrin during 3 hours, increases observed in the chromosome aberrations' frequency were not statistically significant.

4.1.2 WITH METABOLIC ACTIVATION

Human lymphocytes

Increases observed in the chromosome aberrations' frequency were not statistically significant.

CHO cells

In cells exposed to permethrin during 3 hours, increases observed in the chromosome aberrations' frequency were not statistically significant.

4.2 Cytotoxicity

The chromosome aberration data from the human lymphocyte cultures have already been published. The permethrin dose levels were selected on the basis of two preliminary cytotoxicity tests: the proliferative rate index (PRI) and the percentage of binucleated cells with regard to the total cells. Thus, the highest dose chosen caused a cell-cycle delay and a decrease in the percentage of binucleated cells.

Section A6.6.2 **6.6.2 Genotoxicity in vitro (chromosome aberrations)**

Annex Point IIA6.6.2

	Key Study	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 MATERIALS AND METHODS	Permethrin was tested for its ability to induce structural chromosome aberrations in human lymphocyte cultures and CHO cells. Permethrin was tested in the range of 50-200 µg/mL in human lymphocyte cultures and in the range of 20-100 µg/mL in CHO cells. In both lymphocyte and CHO cultures, assays were performed in the absence and in the presence of a rat liver activation system (S9 mix). In the absence of S9 mix, two experiments with different duration of the treatment were carried out.	
5.2 Results and discussion	Permethrin induced chromosomal aberrations in both human lymphocyte and CHO cell cultures when it was evaluated in the absence of a metabolic activation system. The activity of a given concentration of permethrin seemed to be decreased more by the reduction of the time of exposure than by the presence of S9 mix. Aberrations induced by permethrin were mainly chromosome-type aberrations in both human lymphocyte and CHO cell cultures.	
5.3 Conclusion	Permethrin can be characterised as an S-phase independent clastogenic agent *in vitro*.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Not GLP	X

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*29/11/05*
Materials and Methods	***3.2.1*** *Applicant should state here that* ***human*** *lympocytes were used.* ***3.2.2*** *Not apparent, rather than not applicable.* ***3.2.4*** *OECD Guidline 473 require concurrent positive and negative controls to be included in each experiment.* ***5.3.2*** *No controls were included either.* *Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version.*
Conclusion	*Adopt applicant's version.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ...

Section A6.6.2 **6.6.2 Genotoxicity in vitro (chromosome aberrations)**

Annex Point IIA6.6.2

	Key Study
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_6_1(3)-1. Table for Chromosomal Aberrations

Induction of Chromosome Aberrations in Cultured Human Lymphocytes Treated With Permethrin

		Treatment	No. of cells		Chromatid type[a]			Chromosome type[a]			Aberrations/cell		% Abnormal cells	
μg/ml	% S9	h	scored	Gaps	B	E	T	B	E	T	(− gaps)	(+ gaps)	(− gaps)	(+ gaps)
Donor 1														
0	0	21	100	4	1	0	1	1	0	1	0.02	0.06	2	5
50	0	21	100	4	1	0	1	2	1	3	0.04	0.08	4	8
75	0	21	100	8	0	0	0	14	0	14‡	0.14†	0.22‡	13‡	17*
100	0	21	100	21	6	1	7*	11	2	13‡	0.20‡	0.41‡	16‡	29‡
Donor 2														
0	0	21	50	5	0	0	0	2	0	2	0.04	0.10	4	10
50	0	21	50	5	2	0	2	3	0	3	0.10	0.16	10	14
75	0	21	50	2	1	2	3	0	2	2	0.10	0.14	8	12
100	0	21	50	6	0	1	1	6	4	10*	0.22‡	0.34‡	20‡	28‡
150	0	21	50	9	3	1	4*	2	3	5	0.18†	0.36‡	14*	28‡
200	0	21	0											
Donor 2														
0	0	2	50	4	0	0	0	2	0	2	0.04	0.12	4	10
50	0	2	50	5	1	0	1	0	0	0	0.02	0.08	2	8
75	0	2	44	1	1	0	1	0	0	0	0.02	0.04	2	4
100	0	2	50	1	0	0	0	2	0	2	0.04	0.06	4	6
150	0	2	50	1	3	2	5*	0	1	1	0.12*	0.14	12*	12
200	0	2	50	4	2	4	6*	1	4	5	0.22‡	0.30†	18*	22*
Donor 3														
0	10	2	50	3	2	1	3	1	0	1	0.08	0.14	8	14
50	10	2	50	4	1	3	1	1	0	1	0.04	0.12	4	12
75	10	2	50	1	1	3	1	2	0	2	0.06	0.08	6	8
100	10	2	50	5	0	1	1	1	0	1	0.04	0.14	4	12
150	10	2	50	8	2	3	7	3	2	5	0.14	0.30*	14	24

[a]Chromosome aberrations: B, breaks; E, exchanges; T, total.
*$P < .05$.
†$P < .01$.
‡$P < .001$ (χ^2 test)

Induction of Chromosome Aberrations in CHO Cells Treated With Permethrin

		Treatment	No. of cells			Chromatid type[a]			Chromosome type[a]			Aberrations/cell		% Abnormal cells	
μg/ml	% S9	h	scored	MI	Gaps	B	E	T	B	E	T	(− gaps)	(+ gaps)	(− gaps)	(+ gaps)
0	0	20	100	5.3	4	1	0	1	3	0	3	0.04	0.08	4	6
20	0	20	100	4.2	5	2	0	2	5	0	5	0.07	0.12	7	12
50	0	20	100	3.5*	4	2	0	2	6	0	6	0.08	0.12	8	11
100	0	20	65	2.7†	5	3	0	3	8	0	8*	0.16‡	0.24‡	15‡	21‡
0	0	20	100	5.5	2	0	0	0	2	0	2	0.02	0.04	2	4
20	0	20	100	4.8	1	1	0	1	4	0	4	0.05	0.06	5	6
50	0	20	100	3.7*	4	1	1	2	7	0	7	0.09*	0.13*	9*	12*
100	0	20	74	3.0†	7	4	0	4*	7	1	8*	0.16‡	0.25‡	18‡	26‡
0	0	3	100	5.6	3	0	0	0	2	0	2	0.02	0.05	2	4
20	0	3	100	5.1	3	0	0	0	4	0	4	0.04	0.09	4	7
50	0	3	100	4.2	5	1	0	1	6	1	7	0.08	0.13	8	11
100	0	3	100	3.9*	4	2	0	2	6	0	6	0.08	0.12	8	11
0	0	3	100	5.2	3	1	0	1	2	0	2	0.03	0.05	3	5
20	0	3	100	5.0	3	1	0	1	4	0	4	0.05	0.09	5	8
50	0	3	100	4.5	6	3	0	3	6	0	6	0.09	0.13	9	13
100	0	3	100	4.0	8	5	0	5	5	0	5	0.10	0.13	9	12
0	10	3	100	4.9	3	1	0	1	3	0	3	0.04	0.07	4	6
20	10	3	100	4.1	6	3	0	3	4	0	4	0.07	0.11	7	10
50	10	3	100	3.8	6	3	0	3	6	0	6	0.07	0.13	7	12
100	10	3	100	4.0	8	5	0	5	5	0	5	0.08	0.14	7	12
0	10	3	100	5.5	3	2	0	2	4	0	4	0.06	0.09	6	9
20	10	3	100	4.7	7	4	0	4	5	0	5	0.09	0.13	5	11
50	10	3	100	4.2	6	3	0	3	6	0	6	0.09	0.15	9	14
100	10	3	100	3.6*	6	2	0	2	6	1	7	0.09	0.15	9	13

[a]Chromosome aberrations: B, breaks; E, exchanges; T, total.
MI: mitotic index.
*$P < .5$.
†$P < .01$.
‡$P < .001$ (χ^2 test).

Section A6.6.3 **6.6.3 Genotoxicity in vitro (mouse lymphoma test)**

Annex Point IIA 6.6.3

	Key Study	Official use only
	1 REFERENCE	
1.1 Reference	Clive D; 1977; Mutagenicity of BW 21Z73 in L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells with and without Metabolic Activation; Burroughs Wellcome Co., Research Triangle Park, North Carolina, USA; unpublished Report (Doc.) No. TTEP / 77 / 0001; 05.01.1977.	
1.2 Data protection	Yes	
1.2.1 Data owner	Sumitomo Chemical (UK) PLC	
1.2.2 Companies with letter of access	Bayer Environmental Science	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	No; no guidelines available.	
2.2 GLP	No; GLP was not compulsory at the time the study was performed.	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2 (name used in study report: BW 21Z)	
3.1.1 Lot/Batch number	Not available	
3.1.2 Specification	As given in section 2	X
3.1.2.1 Description	Pale yellow liquid changing gradually to white waxy solid. Liquid may contain small amount of precipitate early in transition to solid.	
3.1.2.2 Purity	As given in section 2	
3.1.2.3 Stability	Not applicable (short-term administration)	
3.2 Study Type	In vitro mammalian chromosome aberration test In vitro mammalian cell gene mutation test	
3.2.1 Organism/cell type	mammalian cell lines: Mouse lymphoma L5178Y cells	
3.2.2 Deficiencies / Proficiencies	Thymidine kinase proficient/deficient	
3.2.3 METABOLIC ACTIVATION SYSTEM	S9 mix, rat, liver, induced, Aroclor 1254, 500 mg/kg i.p.	
3.2.4 Positive control	Ethyl methane sulphonate (EMS), -S9 2-Acetylaminofluorene (2-AAF), +S9	

Section A6.6.3 **6.6.3 Genotoxicity in vitro (mouse lymphoma test)**

Annex Point IIA 6.6.3

		Key Study
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	**Concentrations**	-S9 Experiment 1: 0, 31, 47, 62, 94, 125 µg/mL Experiment 2: 0, 45 µg/mL Experiment 3: 0, 30, 40, 50 µg/mL +S9 Experiment 1: 0, 16, 31, 47, 62, 94 µg/mL Experiment 2: 0, 20, 30, 40, 50 µg/mL
3.3.2	**Way of application**	Dissolved in medium
3.3.3	**Pre-incubation time**	4 hours
3.3.4	**Other modifications**	+S9 Two separate dose-response experiments were run using different S9 preparations. In the first, in-house S9 was used while S9 purchased from Litton Bionetics served in the second experiment.
3.4	**Examinations**	*see tables in appendix for examinations and results*
3.4.1	**Number of cells evaluated**	Results expressed as number of induced mutants per 10^6 survivors.
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	**without metabolic activation**	No
4.1.2	**with metabolic activation**	No

Section A6.6.3 **6.6.3 Genotoxicity in vitro (mouse lymphoma test)**

Annex Point IIA 6.6.3

Key Study

4.2 Cytotoxicity

Yes.

-S9

Greater than two-fold variation in equi-toxic doses was observed among the 3 experiments (for example, ID_{90}s varied from slightly greater than 94 µg/mL for experiment 1 down to approximately 40 µg/mL for experiments 2 and 3). This is distinctly greater than normal variation associated with this system; it might be related to whether the permethrin had been heated to dissolve visible precipitate (experiments 2 and 3) or not (experiment 1) prior to preparing stock solution. Such an explanation is consistent with the difference in melting points between the *cis* and *trans* isomers (*cis* = 65-69°C; *trans* = 42-45°C) and their relative toxicities (*cis* is more toxic than *trans*) (O. Murch, personal communication). Despite these high melting points, once melted the mixture appears liquid at room temperature for several days. If some of the more readily solidified *cis* isomer had been unknowingly lost from solution at the running of the first experiment, the remaining liquid solution which was used to treat the cells would have been enriched in the less toxic *trans* isomer and the observed higher inhibitory concentration would have been required.

+S9

Both in-house and bought-in S9 preparations were more cytotoxic than usually observed with the 2-AAF control (usually 5-20% survival under these conditions). Because of the increased ratio of toxicity to mutagenicity in the case of 2-AAF, data were accrued for similarly lowered survivals of cultures treated with permethrin as well (6.1 % and 2.9% for the two experiments).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Permethrin technical was tested for mutagenic potential in cultured L5178Y/TK$^{+/-}$ mouse lymphoma cells both with and without supplementary rat liver homogenate microsomal metabolic activation (S9) in 5 experiments (3 with S9, 2 without). For each of these metabolic conditions a solvent control and an appropriate positive control were run.

X

Section A6.6.3 **6.6.3 Genotoxicity in vitro (mouse lymphoma test)**

Annex Point IIA 6.6.3

Key Study

5.2 Results and discussion

-S9

In 3 separate dose-response determinations no significant (at least double that seen in the negative control culture) mutagenicity was observed at survivals as low as (or lower than) 10% of the untreated (solvent) control, a toxic range determined as optimal in distinguishing between carcinogens and non-carcinogens by a mutagenic end-point in this system.

In contrast to the uniformity of these negative mutagenicity results, greater than two-fold variation in equi-toxic doses was observed among the 3 experiments (for example, ID_{90}s varied from slightly greater than 94 µg/mL for experiment 1 down to approximately 40 µg/mL for experiments 2 and 3). This is distinctly greater than normal variation associated with this system; it might be related to whether the permethrin had been heated to dissolve visible precipitate (experiments 2 and 3) or not (experiment 1) prior to preparing stock solution. Such an explanation is consistent with the difference in melting points between the *cis* and *trans* isomers (*cis* = 65-69°C; *trans* = 42-45°C) and their relative toxicities (*cis* is more toxic than *trans*) (O. Murch, personal communication). Despite these high melting points, once melted the mixture appears liquid at room temperature for several days. If some of the more readily solidified *cis* isomer had been unknowingly lost from solution at the running of the first experiment, the remaining liquid solution which was used to treat the cells would have been enriched in the less toxic *trans* isomer and the observed higher inhibitory concentration would have been required.

+S9

Both in-house and bought-in S9 preparations were more cytotoxic than usually observed with the 2-AAF positive control (usually 5-20% survival under these conditions); however, the mutagenicity of 2-AAF was within the acceptable range (100-200 x 10^{-6}) for this dose in both experiments. Therefore, the S9s, though not ideal preparations, were metabolically active in converting 2-AAF to a mutagen, and must be considered adequate for permethrin as well (assuming that permethrin can be activated by rat liver microsomal enzymes). Because of the increased ratio of toxicity to mutagenicity in the case of 2-AAF, data were accrued for similarly lowered survivals of cultures treated with permethrin as well (6.1 % and 2.9% for the two experiments). Even under this additional challenge no significant (at least double that seen in the negative control culture) mutagenicity was observed for permethrin.

5.3 Conclusion

In 5 experiments (3 with S9, 2 without) no evidence of mutagenicity of permethrin was found under conditions which are nearly 90% reliable for the quantitative as well as the qualitative prediction of oncogenic potency for a small series of chemicals. X

Section A6.6.3 **6.6.3 Genotoxicity in vitro (mouse lymphoma test)**

Annex Point IIA 6.6.3

Key Study

5.3.1 Reliability	2
5.3.2 Deficiencies	Yes; greater than two-fold variation in equi-toxic doses was observed among the 3 experiments without metabolic activation; S9 preparations were more cytotoxic than usually observed with the 2-AAF positive control. The variation in toxicity among the 3 experiments without metabolic activation may have been due to heating of the permethrin to dissolve visible precipitate. However, heating only occurred in 2 of the 3 experiments, and all of the experiments returned negative results for mutagenicity. Although the S9 preparations were more cytotoxic than usually observed with the 2-AAF positive control, the mutagenicity of 2-AAF was within the acceptable range (100-200 x 10^{-6}) for this dose in both experiments with metabolic activation. Therefore, the S9s, though not ideal preparations, were metabolically active in converting 2-AAF to a mutagen, and must be considered adequate for permethrin as well (assuming that permethrin can be activated by rat liver microsomal enzymes).

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*29/11/05*
Materials and Methods	***3.1.2*** *To what* ***exactly*** *does specification refer?* *Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version.*
Conclusion	***5.1*** *Permethrin technical was tested in 5 experiments (3* ***without*** *S9, 2* ***with).*** ***5.3*** *Should read - In 5 experiments (3* ***without*** *S9, 2* ***with).***
Reliability	*2*
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*

Section A6.6.3	6.6.3 Genotoxicity in vitro (mouse lymphoma test)
Annex Point IIA 6.6.3	
	Key Study
Remarks	

Table A6_6_3-1. Table for Mouse Lymphoma Assay

MUTAGENICITY AND CYTOTOXICITY OF BW 21Z IN L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS WITHOUT METABOLIC ACTIVATION

Experiment	Chemical	Conc. (µg/ml)	% Survival[1]	No. Induced Mutants per 10^6 Survivors[2]
1	BW 21Z[3]	0[6]	100	0 (background = 50)
		31	80	-12
		47	47	-1
		62	31	12
		94	12.8	31
		125	4.2	47
	EMS[4]	620	25	843
2	BW 21Z[5]	0[6]	100	0 (background = 46)
		45	3.7	35
	EMS[4]	620	23	745
3	BW 21Z[5]	0[6]	100	0 (background = 46)
		30	28	39
		40	17	32
		50	7.2	46
	EMS[4]	620	19	1420

[1] Relative to untreated solvent control

[2] After substracting background mutant frequency

[3] Not heated prior to preparing stock solution

[4] Ethyl methanesulfonate, positive control

[5] Heated (approx. 80°C) prior to preparing stock solution

[6] Solvent control: contains 1% DMSO during exposure

NUTAGENICITY AND CYTOTOXICITY OF BW 21Z
IN L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
SUPPLEMENTED WITH RAT LIVER METABOLIC ACTIVATION (S-9*)

Experiment[1]	Chemical	Conc. (ug/ml)	% Survival[2]	No. Induced Mutants per 10^6 Survivors[3]
1	BW 21Z[4]	0[6]	100	0 (background = 38)
		16	40	19
		31	62	2
		47	25	17
		62	15	4
		94	6.1	6
	2-AAF[5]	50	2.9	137
2	BW 21Z[6]	0[7]	100	0 (background = 48)
		20	45	-12
		30	25	6
		40	10.1	5
		50	2.9	30
	2-AAF[5]	50	1.0	106

[1] Experiment numbers correspond to those in Table 1

[2] Relative to untreated solvent control

[3] After substracting background mutant frequency

[4] Not heated prior to preparing stock solution

[5] 2-Acetylaminofluorene, positive control requiring metabolic activation

[6] Heated (approx. 80°C) prior to preparing stock solution

[7] Solvent control: contains 1% DMSO during exposure

Section A6.6.4 **6.6.4 Genotoxicity in vivo (mouse micronucleus test)**

Annex Point IIA6.6.4

	Key Study	Official use only
	1 REFERENCE	
1.1 Reference	[redacted]; 1997a; Micronucleus Test of Permethrin Technical in Mice; [redacted]; unpublished Report No. 1270/JRF/TOX/97; 27.09.1997.	
1.2 Data protection	Yes	
1.2.1 Data owner	Bayer CropScience AG	
1.2.2 Companies with letter of access	Sumitomo Chemical (UK) PLC	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes; OECD 474, EC B. 12 &US EPA PAG 84-2.	
2.2 GLP	Yes	
2.3 Deviations	Yes; there was a slight deviation in the temperature during the experimental period when compared to the protocol.	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	002 / 96, 38	
3.1.2 Specification	As given in section 2	X
3.1.2.1 Description	Yellow brown	
3.1.2.2 Purity	94%	
3.1.2.3 Stability	Not applicable (single administration)	
3.1.2.4 Maximum tolerable dose	430 mg/kg	
3.2 Test Animals		
3.2.1 Species	mouse	
3.2.2 Strain	Swiss albino	
3.2.3 Source	[redacted]	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Six to seven weeks old/24-30 g	
3.2.6 Number of animals per group	5 males + 5 females	
3.2.7 Control animals	Yes	

Section A6.6.4 **6.6.4 Genotoxicity in vivo (mouse micronucleus test)**

Annex Point IIA6.6.4

	Key Study
3.3 Administration/ Exposure	Oral
3.3.1 Number of applications	1
3.3.2 Interval between applications	Not applicable
3.3.3 Postexposure period	24 h
	Oral
3.3.4 Type	gavage
3.3.5 Concentration	0, 107, 215, 430 mg/kg bw
3.3.6 Vehicle	Peanut oil
3.3.7 Concentration in vehicle	Not reported
3.3.8 Total volume applied	10 mL/kg bodyweight
3.3.9 Controls	Vehicle and positive (mitomycin-C 4 mg/kg i.p.)
3.4 Examinations	
3.4.1 Clinical signs	Yes
3.4.2 Tissue	Bone marrow
	Number of animals: all animals
	Number of cells: 1000
	Time points: 24 h after treatment
	Type of cells: erythrocytes in bone marrow
	Parameters: polychromatic/normochromatic erythrocytes ratio (P/N)
	percentage frequency of micronucleated erythrocytes
3.5 Further remarks	The data on body weight, per cent micronucleated erythrocytes and P/N ratio were statistically analysed by Student's t-test.

Section A6.6.4 **6.6.4 Genotoxicity in vivo (mouse micronucleus test)**

Annex Point IIA6.6.4

Key Study

4 RESULTS AND DISCUSSION

4.1 Clinical signs

No mortality or toxicity symptoms were observed in mice treated up to the single oral dose level of 430 mg/kg bw. No significant change in body weight of mice treated with permethrin following the single oral dose of up to 430 mg/kg bw was observed.

4.2 Haematology / Tissue examination

The frequency of per cent micronucleated erythrocytes in permethrin treated groups did not significantly vary from that of control group up to the dose level of 430 mg/kg body weight. The P/N ratio did not significantly vary from that of control.

4.3 Genotoxicity

No

4.4 Other

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Fifty healthy Swiss albino mice were divided into 5 groups, each group comprising 10 animals (5 males and 5 females per group). Permethrin was suspended in peanut oil and orally administered to mice for one day only at the test dosages of 107, 215, 430 mg/kg body weight. The control group received peanut oil orally only. Positive control mice received single intraperitoneal injection of mitomycin-C at the dose level of 4 mg/kg body weight to assess the sensitivity of the test system.

All mice were sacrificed 24 hours after treatment. The femora of each animal was dissected out and bone marrow cells were flushed by using 3 mL of foetal calf serum. The cell pellet in 0.5 mL of foetal calf serum was obtained after centrifugation and removal of supernatant. Bone marrow smears were made on clean slides and stained with 5% Giemsa in phosphate buffer. The smears were examined using a Nikon Optiphot-2 microscope for the presence of micronucleated erythrocytes. A minimum of 1 000 polychromatic erythrocytes were checked for each mouse to detect the presence of micronucleus. The percentage frequency of micronucleated erythrocytes and the ratio between polychromatic and normochromatic erythrocytes (P/N) per animal was calculated. The values were statistically compared with control group.

5.2 Results and discussion

The experimental results showed that the frequency of per cent micronucleated erythrocytes in permethrin treated groups did not significantly vary from that of control group up to the dose level of 430 mg/kg bw. The P/N ratio did not significantly vary from that of control. No significant change in body weight of mice treated with permethrin following the single oral dose of up to 430 mg/kg bw was observed.

5.3 Conclusion

It is concluded that permethrin does not have micronucleus induction potential in mice following a single oral dose level of up to 430 mg/kg bw.

5.3.1 Reliability

2

Section A6.6.4 **6.6.4 Genotoxicity in vivo (mouse micronucleus test)**

Annex Point IIA6.6.4

Key Study

5.3.2 Deficiencies Yes; there was a slight deviation in the temperature during the experimental period when compared to the protocol, however, this is not thought to have influenced the quality or integrity of the data.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*29/11/05*
Materials and Methods	***3.1.2*** *To what* ***exactly*** *does 'specification' refer?* *Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version.*
Conclusion	Other conclusions:
Reliability	*2*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.4 6.6.4Genotoxicity in vivo (mouse micronucleus test)

Annex Point IIA6.6.4

Table A6_6_4(1)-1. Table for Micronucleus Test In Vivo
Summary Of Micronucleated Erythrocytes In Bone Marrow Cells – Male

DOSE AND GROUP	NO. OF OBSERVATIONS	TOTAL ERYTHROCYTES		MEAN % MNE	MEAN P/N RATIO
		SCORED	MNE		
Control I	5	7271	5	0.073 (0.077)	2.995 (1.615)
Permethrin technical 107 mg/kg bw II	5	6112	9	0.150 (0.111)	4.958 (1.598)
Permethrin technical 215 mg/kg bw III	5	6830	5	0.081 (0.113)	3.792 (1.502)
Permethrin technical 430 mg/kg bw IV	5	7034	11	0.170 (0.173)	3.079 (1.507)
Mitomycin-C 4.0 mg/kg bw V	5	8121	108	1.332* (0.351)	2.246 (0.787)

Summary Of Micronucleated Erythrocytes In Bone Marrow Cells – Female

DOSE AND GROUP	NO. OF OBSERVATIONS	TOTAL ERYTHROCYTES		MEAN % MNE	MEAN P/N RATIO
		SCORED	MNE		
Control I	5	8423	5	0.060 (0.040)	2.095 (1.488)
Permethrin technical 107 mg/kg bw II	5	6485	5	0.080 (0.057)	4.062 (1.323)
Permethrin technical 215 mg/kg bw III	5	7097	6	0.088 (0.099)	3.977 (1.962)
Permethrin technical 430 mg/kg bw IV	5	6977	7	0.091 (0.065)	3.112 (0.938)
Mitomycin-C 4.0 mg/kg bw V	5	9188	89	0.947* (0.184)	1.271 (0.407)

Values are mean and standard deviation
* = Significant at 5% level ($P \leq 0.05$)

Section A6.6.5
Annex Point IIA6.6.4

6.6.5 Genotoxicity in vivo (mouse cytogenetic test [chromosomal analysis])

Key Study

			Official use only
		1 REFERENCE	
1.1	**Reference**	*Prabakaran P*; 1997b; Chromosomal Aberration Study of Permethrin Technical in Mice; [redacted]; unpublished Report No. 1269/JRF/TOX/97; 14.10.1997.	
1.2	**Data protection**	Yes	
1.2.1	**Data owner**	Bayer CropScience AG	
1.2.2	**Companies with letter of access**	Sumitomo Chemical (UK) PLC	
1.2.3	**Criteria for data protection**	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; OECD 475, EC B. 11 &US EPA PAG 84-2.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes; there was a slight deviation in the relative humidity during the experimental period when compared to the protocol.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	**Lot/Batch number**	002 / 96	
3.1.2	**Specification**	As given in section 2	X
3.1.2.1	**Description**	Yellow brown	
3.1.2.2	**Purity**	94%	
3.1.2.3	**Stability**	Not applicable (single administration)	
3.1.2.4	**Maximum tolerable dose**	430 mg/kg	
3.2	**Test Animals**		
3.2.1	**Species**	mouse	
3.2.2	**Strain**	Swiss albino	
3.2.3	**Source**	[redacted]	
3.2.4	**Sex**	Male and female	
3.2.5	**Age/weight at study initiation**	Seven weeks old/21-28 g	
3.2.6	**Number of animals per group**	5 males + 5 females	

Section A6.6.5 Annex Point IIA6.6.4	6.6.5 Genotoxicity in vivo (mouse cytogenetic test [chromosomal analysis])

Key Study

3.2.7	Control animals	Yes
3.3	Administration/ Exposure	Oral
3.3.1	Number of applications	1
3.3.2	Interval between applications	Not applicable
3.3.3	Postexposure period	24 h
		Oral
3.3.4	Type	gavage
3.3.5	Concentration	0, 107, 215, 430 mg/kg bw
3.3.6	Vehicle	Peanut oil
3.3.7	Concentration in vehicle	Not reported
3.3.8	Total volume applied	10 mL/kg bodyweight
3.3.9	Controls	Vehicle and positive (mitomycin-C 4 mg/kg i.p.)
3.4	Examinations	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	Bone marrow
		Number of animals: all animals
		Number of cells: 500
		Time points: 24 h after treatment
		Type of cells: bone marrow
		Parameters: numbers and types of structural aberrations mitotic index
3.5	Further remarks	Data on body weight, mitotic index and percent aberrant cells were statistically analysed by student's t-test.

		4 RESULTS AND DISCUSSION
4.1	Clinical signs	Toxic symptoms were observed in the form of mild tremors and tail erection after dosing in a few mice treated with 215 and 430 mg/kg bw permethrin. These symptoms totally disappeared the following day prior to sacrifice. A single administration of permethrin did not affect the body weight in any dose groups.

Section A6.6.5
Annex Point IIA6.6.4

6.6.5 Genotoxicity in vivo (mouse cytogenetic test [chromosomal analysis])

Key Study

4.2	Haematology / Tissue examination	The mitotic index of Group V female mice (4.0 mg/kg bw Mitomycin-C) significantly increased from that of control, and was considered to be an incidental effect. The percent aberrated cells in permethrin treated mice up to the dose level of 430 mg/kg bw did not statistically differ from that of control. The encountered structural aberrations were mainly of chromatid breaks and ploidy were the recorded numerical anomalies. The results of the positive control group showed chromatid breaks, chromosomal breaks, fragments, higher incidence of numerical aberrations and pulverisation. The mean percent aberrant cell was 10.80 in male and 12.40 in female mice treated with Mitomycin-C and this proves the sensitivity of the test method.
4.3	Genotoxicity	No
4.4	Other	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	Materials and methods	Fifty (25 males and 25 females) healthy, Swiss albino mice were divided into 5 groups, each group comprising 5 males and 5 females. Permethrin was suspended in peanut oil and orally administered to mice for one day at the dose levels of 0, 107, 215 and 430 mg/kg body weight. The control group received only peanut oil orally. Mice from the positive control received single intraperitoneal injection of Mitomycin-C at the dose level of 4 mg/kg body weight to assess the sensitivity of the test system. All mice were sacrificed after colchicines injection and bone marrow cells were aspirated from femora. The cells were processed, hypotonically treated, fixed in Carnoy's fluid and slides were prepared. The cells on slides were stained with 5% Giemsa and examined under 100 X objective of a Nikon Optiphot-2 microscope. The mitotic index was calculated by counting metaphases in a minimum of 500 cells per animal. The structural and numerical changes in chromosomes were recorded by examining 50 metaphases for each animal. The percentage of aberrant cells was determined and the results were statistically analysed.

Section A6.6.5 Annex Point IIA6.6.4	6.6.5 Genotoxicity in vivo (mouse cytogenetic test [chromosomal analysis])
	Key Study
5.2 Results and discussion	The mitotic index of Group V female mice (4.0 mg/kg bw Mitomycin-C) significantly increased from that of control, and was considered to be an incidental effect. The percent aberrated cells in permethrin treated mice up to the dose level of 430 mg/kg bw did not statistically differ from that of control. The encountered structural aberrations were mainly of chromatid breaks and ploidy were the recorded numerical anomalies. The results of the positive control group showed chromatid breaks, chromosomal breaks, fragments, higher incidence of numerical aberrations and pulverisation. The mean percent aberrant cell was 10.80 in male and 12.40 in female mice treated with Mitomycin-C and this proves the sensitivity of the test method.
5.3 Conclusion	It is concluded that permethrin does not induce chromosomal aberration in mice treated up to the dose level of 430 mg/kg bw.
5.3.1 Reliability	2
5.3.2 Deficiencies	Yes; there was a slight deviation in the relative humidity during the experimental period when compared to the protocol, however, this is not thought to have influenced the quality or integrity of the data.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*29/11/05*
Materials and Methods	***3.1.2*** *To what does 'specification' refer?* *Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version.*
Conclusion	Other conclusions:
Reliability	*2*
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*

Section A6.6.5 Annex Point IIA6.6.4	6.6.5 Genotoxicity in vivo (mouse cytogenetic test [chromosomal analysis])
	Key Study
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.5 **6.6.5 Genotoxicity in vivo (mouse cytogenetic test [chromosomal analysis])**
Annex Point IIA6.6.4

Table A6_6_4(2)-1. Table for Cytogenetic In Vivo Test [chromosomal analysis]

SUMMARY OF CHROMOSOMAL ABERRATION IN BONE MARROW CELLS – MALE

Number of Observations = 5

DOSE AND GROUP	MITOTIC INDEX	% ABERRANT CELLS
CONTROL I	1.64 (0.877)	0.80 (1.095)
PERMETHRIN TECHNICAL 107 mg/kg bw II	1.41 (0.294)	0.80 (1.095)
PERMETHRIN TECHNICAL 215 mg/kg bw III	1.75 (0.720)	0.80 (1.095)
PERMETHRIN TECHNICAL 430 mg/kg bw IV	1.59 (0.576)	0.40 (0.894)
POSITIVE CONTROL MITOMYCIN-C V	1.57 (0.760)	10.80* (1.095)

SUMMARY OF CHROMOSOMAL ABERRATION IN BONE MARROW CELLS – FEMALE

Number of Observations = 5

DOSE AND GROUP	MITOTIC INDEX	% ABERRANT CELLS
CONTROL I	1.34 (0.437)	0.40 (0.894)
PERMETHRIN TECHNICAL 107 mg/kg bw II	1.97 (1.254)	0.80 (1.095)
PERMETHRIN TECHNICAL 215 mg/kg bw III	1.30 (0.511)	0.80 (1.095)
PERMETHRIN TECHNICAL 430 mg/kg bw IV	1.19 (0.169)	0.80 (1.095)
POSITIVE CONTROL MITOMYCIN-C V	2.59* (0.420)	12.40* (2.966)

Values are mean and standard deviation

* Significant at 5% level (P = 0.05)

Section A6.6.6 **6.6.6 Genotoxicity in vivo (mouse dominant lethal test)**

Annex Point IIA6.6.6

	Key Study	Official use only
	1 REFERENCE	
1.1 Reference	[redacted]; 1975; 21Z73, Dominant Lethal Study in Male Mice; [redacted]; unpublished Report (Doc. Code) No. HEFG 75-10; 27.11.1975.	
1.2 Data protection	Yes	
1.2.1 Data owner	Sumitomo Chemical (UK) PLC	
1.2.2 Companies with letter of access	Bayer Environmental Science	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	No; no guidelines available.	
2.2 GLP	No; GLP was not compulsory at the time the study was performed.	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2 (name used in study report: 21Z73)	
3.1.1 Lot/Batch number	Batch number ZB	
3.1.2 Specification	As given in section 2	X
3.1.2.1 Description	As given in section 2	
3.1.2.2 Purity	As given in section 2	
3.1.2.3 Stability	Not applicable (short-term administration)	
3.1.2.4 Maximum tolerable dose	452 mg/kg	
3.2 Test Animals		
3.2.1 Species	mouse	
3.2.2 Strain	CD 1	
3.2.3 Source	[redacted].	
3.2.4 Sex	Male (treated) and female (untreated) *m and f (see 0)*	
3.2.5 AGE/WEIGHT AT STUDY INITIATION	Sexually mature	
3.2.6 Number of animals per group	10 males + 180 females	
3.2.7 Control animals	Yes	

Section A6.6.6 **6.6.6 Genotoxicity in vivo (mouse dominant lethal test)**

Annex Point IIA6.6.6

	Key Study	
3.3 Administration/ Exposure	Oral	
3.3.1 Number of applications	5, as allowed by test guideline EC B. 22.	
3.3.2 Interval between applications	24 h	
3.3.3 Postexposure period	6 weeks	
	Oral	
3.3.4 Type	gavage	
3.3.5 Concentration	452 mg/kg bw	
3.3.6 Vehicle	Corn oil	
3.3.7 Concentration in vehicle	40% w/v	
3.3.8 Total volume applied	0.2 mL	
3.3.9 Controls	Vehicle (corn oil) and positive (trimethylphosphate (TP)) controls	
3.4 Examinations		
3.4.1 Clinical signs	Yes	
3.4.2 Tissue	implantations	X
	Number of animals: all animals	
	Time points: 1, 2, 3, 4, 5, 6 weeks after initial treatment	
	Parameters: number of live and dead implants	
3.5 Further remarks	Pregnancy rate recorded.	

4 RESULTS AND DISCUSSION

4.1 Clinical signs	The group of male mice dosed with permethrin showed signs of slight hypersensitivity after the 4th and 5th doses, the effects persisting for approximately 3 hours. No other signs of toxicity were seen.

Section A6.6.6 **6.6.6 Genotoxicity in vivo (mouse dominant lethal test)**

Annex Point IIA6.6.6

Key Study

4.2 Haematology / Tissue examination

Analysis of proportions of dead implants

The test statistics (chi square or F-ratio) for among group variation were significant for weeks 1, 2 and 5.

Comparison of group proportions

1. Comparison of Group 1 (corn oil) v. Group 2 (TP)

The proportion of dead implants was significantly greater ($p<0.05$) in Group 2 (TP) than in Group 1 (corn oil) for weeks 1 and 2. At Week 5 there was a significant difference ($p<0.05$) but the difference was in the opposite direction.

2. Comparison of Group 1 (corn oil) v. Group 3 (permethrin)

Comparison between Group 1 (corn oil) with Group 3 (permethrin) showed that at Week 5 there was a significant difference ($p<0.05$), there being a higher proportion of dead implants in Group 1 (corn oil).

Analysis of average implants

1. Comparison of Group 1 (corn oil) v. Group 2 (TP)

There were significantly fewer ($p<0.05$) implants in Group 2 (TP) than in Group 1 (corn oil) during weeks 1, 2 and 5.

2. Comparison of Group 1 (corn oil) v. Group 3 (permethrin)

These comparisons revealed that the average implants were significantly less ($p<0.05$) for Group 3 (permethrin) than for Group 1 (corn oil) in Week 5. This difference is not considered toxicologically important since in Week 5 there was a higher percentage of pregnancies and a significantly smaller proportion ($p<0.05$) of dead implants in Group 3 (permethrin) than in Group 1 (corn oil).

4.3 Genotoxicity

No

4.4 Other

Data on pregnant females

Statistical analysis of the pregnancy rates indicates that there were significant differences between Group 1 (corn oil) and Group 2 (TP positive control) in Week 1 and 2: a larger proportion of females was pregnant in Group 1 than in Group 2. In Week 1 the pregnancy rate was less for Group 1 (corn oil) than for Group 3 (permethrin) and this difference was statistically significant.

Section A6.6.6 **6.6.6 Genotoxicity in vivo (mouse dominant lethal test)**

Annex Point IIA6.6.6

Key Study

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Permethrin was administered by the oral route, once daily for 5 consecutive days, to groups of 10 sexually mature male mice. The dose given (452 mg/kg) was 1/5th of the acute LD_{50} value (2 262 mg/kg). A further group of 10 male mice was given the vehicle (corn oil) alone and this group served as negative controls. A further group of 10 male mice was given trimethylphosphate and this group served as positive controls. Immediately after the 5th dose each male was exposed to three untreated virgin female mice for one week. At the end of one week the females were replaced by three new virgin females. The procedure was repeated for a period of 6 weeks in order to cover the complete spermatogenic cycle.

The female mice were dissected 14 days following the midweek of their exposure to the males, and all implantations were recorded as living or dead.

During the study all mice were observed for either permethrin-induced effects or changes in health that might affect reproductive performance.

5.2 Results and discussion

Clinical signs

The group of male mice dosed with permethrin showed signs of slight hypersensitivity after the 4th and 5th doses, the effects persisting for approximately 3 hours. No other signs of toxicity were seen.

Data on pregnant females

Statistical analysis of the pregnancy rates indicates that there were significant differences between Group 1 (corn oil) and Group 2 (TP positive control) in Week 1 and 2: a larger proportion of females was pregnant in Group 1 than in Group 2. In Week 1 the pregnancy rate was less for Group 1 (corn oil) than for Group 3 (permethrin) and this difference was statistically significant.

Analysis of proportions of dead implants

The test statistics (chi square or F-ratio) for among group variation were significant for weeks 1, 2 and 5.

Comparison of group proportions

1. Comparison of Group 1 (corn oil) v. Group 2 (TP)

The proportion of dead implants was significantly greater ($p<0.05$) in Group 2 (TP) than in Group 1 (corn oil) for weeks 1 and 2. At Week 5 there was a significant difference ($p<0.05$) but the difference was in the opposite direction.

Section A6.6.6 **6.6.6 Genotoxicity in vivo (mouse dominant lethal test)**

Annex Point IIA6.6.6

Key Study

2. Comparison of Group 1 (corn oil) v. Group 3 (permethrin)

Comparison between Group 1 (corn oil) with Group 3 (permethrin) showed that at Week 5 there was a significant difference ($p<0.05$), there being a higher proportion of dead implants in Group 1 (corn oil).

Analysis of average implants

1. Comparison of Group 1 (corn oil) v. Group 2 (TP)

There were significantly fewer ($p<0.05$) implants in Group 2 (TP) than in Group 1 (corn oil) during weeks 1, 2 and 5.

2. Comparison of Group 1 (corn oil) v. Group 3 (permethrin)

These comparisons revealed that the average implants were significantly less ($p<0.05$) for Group 3 (permethrin) than for Group 1 (corn oil) in Week 5. This difference is not considered toxicologically important since in Week 5 there was a higher percentage of pregnancies and a significantly smaller proportion ($p<0.05$) of dead implants in Group 3 (permethrin) than in Group 1 (corn oil).

5.3 Conclusion Permethrin did not produce any dominant lethal mutations.

5.3.1 Reliability 2

5.3.2 Deficiencies Yes; not GLP

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*29/11/05*
Materials and Methods	***3.1.2*** *To what does 'specification' refer?* ***3.4.2*** *Both dead and live implants are recorded.* *Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version.*
Conclusion	Other conclusions:
Reliability	*2*
Acceptability	*Acceptable*
Remarks	

Section A6.6.6 Annex Point IIA6.6.6	6.6.6 Genotoxicity in vivo (mouse dominant lethal test)
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.6 6.6.6 Genotoxicity in vivo (mouse dominant lethal test)

Annex Point IIA6.6.6

Table A6.6.6-1. Table for In-Vivo-Test: Rodent Dominant Lethal Test

Week	Group 1 Corn oil		Group 2 Trimethylphosphate		Group 3 Permethrin	
	Implants		Implants		Implants	
	Living	Dead	Living	Dead	Living	Dead
1	208	10	0	2	309	18
2	305	10	81	55	326	23
3	300	13	216	17	332	21
4	300	12	255	9	273	14
5	243	29	212	11	245	14
6	312	24	263	5	356	13

Section A6.6.7	A6.6.7 Further testing on metabolites of concern	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [ ]	
Detailed justification:	The in vitro genotoxicity data generated on permethrin in the presence of metabolic activation, combined with the in vivo genotoxicity data generated on permethrin, provide adequate reassurance of the non-genotoxic nature of permethrin metabolites (a negative microbial reverse mutation assay on the permethrin metabolite DCVA ethyl ester further supports this position)	X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29/11/05*	
Evaluation of applicant's justification	*Reference is made to a negative microbial reverse mutation assay on the permethrin metabolite DCVA ethyl ester, but as this study is unidentified it should not be used as supporting evidence. The data which has been presented for permethrin itself is sufficient justification for no further studies to be required.*	
Conclusion	*Applicant's justification is acceptable.*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Appendix 1 to Doc III-A6

Bayer Environmental Science is a an affiliated company of Bayer CropScience, therefore the studies submitted by Bayer Environmental Science are owned by Bayer CropScience AG.

Reference List Doc. III-A6. sorted by reference no.

Section No/ Reference No	AUTHOR (S)	Year	Title. Source, Report No. GLP /(Un) Published	Data Protection Claimed (Yes/No)	Owner
6,1,1(1)	[redacted]	1975	Acute Oral Toxicity in Rats with Compound FMC 33297. [redacted] Report No. 2739-75 (Unpublished)	Yes	Sumitomo Chemical
6,1,1(2)	[redacted]	1974	Comparative Acute Oral Toxicity in Mice with FMC 33297, FMC 37400, FMC 35171 and FMC 30960. [redacted] Report No. HEFG 79-C76 (Unpublished)	Yes	Sumitomo Chemical
6,1,2	[redacted]	1975	Acute Dermal Toxicity in Rabbits. Compound FMC 33297. [redacted] Report No. 2908-75 (Unpublished)	Yes	Sumitomo Chemical
6,1,3	[redacted]	1976	Acute Inhalation. Compound No. FMC 33297. [redacted]. Report No. 2911-75 (Unpublished)	Yes	Sumitomo Chemical
6,1,4(1)	[redacted]	1975	Rabbit Eye Irritation. Compound No. FMC 33297. [redacted]. Report No. 2910-75 (Unpublished)	Yes	Sumitomo Chemical
6,1,4(2)	[redacted]	1975	Rabbit Primary Dermal Irritation. Compound No. FMC 33297. [redacted]. Report No. 2909-75 (Unpublished)	Yes	Sumitomo Chemical
6,1,5	[redacted]	1991	Skin Sensitisation in the Guinea Pig of a Permethrin 25/75 cis/trans Isomer RatioThe [redacted]. Report No. 91626D/WLC 159/SS	Yes	Sumitomo Chemical
6,2 (1)	Gaughan LC, Unai T & Casida JE	1977	Permethrin Metabolism in Rats; Department of Entomological Sciences, University of California, Berkeley, California 94720, USA; J. Agric. Food Chem., Vol. 25, No. 1, pp 9-17; 1977.	No	

6,2	Bartelt, N. & Hubbell, J.	1987	Percutaneous Absorption of Topically Applied 14C-Permethrin in Volunteers. Final Medical ReportBurroughs Wellcome Co. Report No. THRD/86/0047	Yes	Sumitomo Chemical
6,3,3	[redacted]	1980	Permethrin Technical. Inhalation Study in Rats – 16 x 6 Hour Exposures Over a 3 Week Period. [redacted]. Report No. WLC34/80323.	Yes	Sumitomo Chemical
6,4,1 (1)	[redacted]	1975	21z73, Rat Oral 90 Day Study. [redacted]. Report No. HEFG 76-1 (Unpublished)	Yes	Sumitomo Chemical
6,4,1 (2)	[redacted]	1978	Permethrin Oral Administration to Dogs for 6 Months. [redacted]. Report No. HEFG 78-14	Yes	Sumitomo Chemical
6,5 (1)	[redacted]	1980	21z: Potential Toxicity and Oncogenicity in Dietary Administration to Rats for a Period of 104 weeks. [redacted]. Report No. 80/WRL003/283 (Unpublished)	Yes	Sumitomo Chemical
6,5 (2)	Ishmael, J. & Litchfield, M.H.	1988	Chronic Toxicity and Carcinogenic Evaluation of Permethrin in Rats and Mice. Fundamental and Applied Toxicology. Vol. 11. pp308-322	No	N/A
6,6,1	Haworth SR	1979	Salmonella/Mammalian-Microsome Plate Incorporation and Pre-Incubation Mutagenesis Assays of Burroughs Wellcome Compound Permethrin Tech BW 0021Z73 #8E8026 and 8I8012; EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland 20852, USA; unpublished Report (Study) No. 015-560-150A-1 and 015-560-150A-2; 16.10.1979.	Yes	Sumitomo Chemical
6,6,2	Barrueco, C. et al	1994	Induction of structural chromosomal aberrations in human lymphocyte cultures and CHO cells by permethrin. Teratogenesis, Carcinogenesis, and Mutagenesis 14:31-38.	No	N/A
6,6,3	Clive, D.	1977	Mutagenicity of BW 21z73 in L5178Y/TK+/- Mouse Lymphoma Cells With and Without Exogenous Metabolic ActivationThe Wellcome Foundation Ltd. Report No. TTEP/77/0001	Yes	Sumitomo Chemical
6,6,4	[redacted]	1997	Micronucleus Test of Permethrin Technical in Mice. [redacted] Report No. 1270/JRF/TOX/97. (Unpublished)	Yes	Bayer CropScience AG

6,6,5	[redacted]	1997	Chromosomal Aberration Study of Permethrin Technical in Mice	Yes	Bayer CropScience AG
6,6,6	[redacted]	1975	21z73 Dominant Lethal Study in Male Mice. [redacted]. Report No. HEFG 75-10 (Unpublished)	Yes	Sumitomo Chemical
6,7 (2)	Ishmael, J. & Litchfield, M.H.	1988	Chronic Toxicity and Carcinogenic Evaluation of Permethrin in Rats and Mice. Fundamental and Applied Toxicology. Vol. 11. pp308-322	No	N/A
6,7 (1)	[redacted]	1980	21z: Potential Toxicity and Oncogenicity in Dietary Administration to Rats for a Period of 104 weeks. [redacted]. Report No. 80/WRL003/283 (Unpublished)	Yes	Sumitomo Chemical
6,8,1 (1)	[redacted]	1974	Foetal Toxicity of 21z73 (NRDC 143) in the Rat. [redacted] Report No. BPAT 74/10 (Unpublished)	Yes	Sumitomo Chemical
6,8,1 (2)	[redacted]	1979	21z: Effects of Oral Administration upon Pregnancy in the Rabbit. [redacted] Report No. HEFG 80-4.	Yes	Sumitomo Chemical
6,8,2	[redacted]	1979	A Multigeneration Reproduction Study of 21z73 (Permethrin) in the Rat. [redacted] No. BPAT 79/3.	Yes	Sumitomo Chemical
6,9	[redacted]	1997	Motor activity measurements in male and female mice postnatally exposed to Permethrin by inhalation; [redacted] unpublished Report No. 26418; 03.07.1997.	Yes	Sumitomo Chemical
6,13	[redacted]	1978	Permethrin Oral Administration to Dogs for 6 Months. [redacted] Report No. HEFG 78-14	Yes	Sumitomo Chemical

Competent Authority Report

Programme for Inclusion of Active Substances in Annex I to Council Directive 98/8/EC

Permethrin (PT 8)

CAS-No. 52645-53-1

DOCUMENT IIIA (A6)

Evaluation Report

Bayer Environmental Science

Sumitomo Chemical (UK) Plc.

Rapporteur: Ireland

August 2009

Permethrin PT8

Document IIIA (A6)

CONTENTS

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

	Key Study	Official use only
	1 REFERENCE	
1.1 Reference	[redacted] 1980; 21Z: Potential Toxicity and Oncogenicity in Dietary Administration to Rats for a Period of 104 Weeks; [redacted]; unpublished Report No. 80/WRL003/283; 10.1980.	
1.2 Data protection	Yes	
1.2.1 Data owner	Sumitomo Chemical (UK) PLC	
1.2.2 Companies with letter of access	Bayer Environmental Science	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	No; no guidelines available.	
2.2 GLP	No; GLP was not compulsory at the time the study was performed.	
2.3 Deviations	No	X
	3 MATERIALS AND METHODS	
3.1 TEST MATERIAL	As given in section 2 (name used in study report: 21Z)	X
3.1.1 Lot/Batch number	Not available	
3.1.2 Specification	As given in section 2	X
3.1.2.1 Description	As given in section 2	X
3.1.2.2 Purity	As given in section 2	X
3.1.2.3 Stability	Fresh batches of pre-mix of permethrin and powdered rodent diet were provided regularly (1-4 times a month) for use in the study.	
3.2 TEST ANIMALS		
3.2.1 Species	Rat	
3.2.2 Strain	Wistar	
3.2.3 Source	[redacted]	
3.2.4 Sex	♂ and ♀	
3.2.5 Age/weight at study initiation	4 weeks/60-80 g	
3.2.6 Number of animals per group	60 animals/group/sex main study 15 animals/group/sex satellite study of blood and urine	

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

	Key Study	
3.2.6.1 at interim sacrifice	0 animals/group/sex	
3.2.6.2 at terminal sacrifice	60 animals/group/sex	
3.2.7 Control animals	Yes	
3.3 Administration/ Exposure	*Oral*	
3.3.1 Duration of treatment	104 weeks	
3.3.2 Interim sacrifice(s)	not applicable	
3.3.3 Final sacrifice	♂ after 103 weeks, ♀ after 104 weeks	
3.3.4 Freqency of exposure	daily	
3.3.5 Postexposure period	not applicable	
	Oral	
3.3.6 Type	in food	
3.3.7 Concentration	food 0, 10, 50 and 250 mg/kg bw food consumption per day .....ad libitum	
3.3.8 Vehicle	not applicable	
3.3.9 Concentration in vehicle	not applicable	
3.3.10 Total volume applied	not applicable	
3.3.11 Controls	plain diet	X
3.4 Examinations		
3.4.1 Body weight	Yes	
3.4.2 Food consumption	Yes	
3.4.3 Water consumption	Yes	
3.4.4 Clinical signs	Yes	
3.4.5 Macroscopic investigations	Superficial or palpable masses	
3.4.6 Ophthalmoscopic examination	Yes	
3.4.7 Haematology	Yes	
	Number of animals: 10 animals/sex/group	
	Time points: After 6, 8, 26, 27, 29, 51, 53, 54, 78, 103, 104 weeks of treatment	

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

	Parameters:	Haemoglobin concentration (Hb), erythrocyte count (RBC), total and differential leucocyte count (WBC), prothrombin time; when intergroup differences were suggested by the results for prothrombin time or the erythrocytic characteristics, the above tests were supplemented by platelet count. Other: packed cell volume (PCV); when intergroup differences were suggested by the results for prothrombin time or the erythrocytic characteristics, the above tests were supplemented by reticulocyte count (Retics); values were derived for mean corpuscular volume, mean cell haemoglobin and mean cell haemoglobin concentration.
3.4.8 Clinical Chemistry		Yes
	Number of animals:	10 animals/sex/group
	Time points:	After 6, 26, 27, 29, 51, 78, 103 weeks of treatment
	Parameters:	Urea, glucose, total protein, alkaline phosphatase (AP), alanine aminotransferase (ALT; reported as glutamate pyruvate transaminase (SGPT) until Week 77), aspartate aminotransferase (AST; reported as glutamate oxalacetic transaminase (SGOT) until Week 77), sodium (Na; monitored from Week 26), potassium (K; monitored from Week 26).
	Other	Electrophoretic protein fractions
3.4.9 Urinalysis	Yes	
	Number of animals:	10 animals/sex/group
	Time points:	After 6, 26, 52, 78, 103 weeks of treatment
	Parameters:	Volume, pH, specific gravity (SG), glucose, protein; after centrifugation at 3 400 rpm for 5 minutes, the deposit was examined microscopically in respect of the following: blood (polymorph (P) and mononuclear (M) leucocytes, red blood cells (R)).
	Other	Reducing substances, ketones, bile pigments, urobilin; after centrifugation at 3 400 rpm for 5 minutes, the deposit was examined microscopically in respect of the following: epithelial cells (E), casts (C), other abnormalities (A).

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

		Key Study
3.4.10Pathology	Yes	
3.4.10.1Organ Weights	Yes	
	from:	all surviving animals, at terminal sacrifice
	Organs:	Adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, uterus
		Other: lungs, pituitary gland, thyroid
3.4.10.2Histopathology	Yes	
	from:	
		high dose group and controls; liver and thyroid only from low and intermediate dose groups
		other dose groups, if any effect
	from:	all surviving animals, all decedents
		at terminal sacrifice
	Organs:	Adrenals, aorta, bone marrow, brain, lymph node (cervical, mesenteric), small (duodenum, ileum) and large (colon) intestines, eyes (and optic nerves), heart, kidneys, liver, lungs, mammary gland, oesophagus, gonads (ovaries, testes), pancreas, pituitary, prostate, salivary glands, peripheral (sciatic) nerve, skin, spleen, stomach, thymus, thyroid, trachea, urinary bladder, uterus
		Other: presumptive neoplasms (with any adherent or invaded adnexa), seminal vesicles, skeletal muscle, tongue
Other examinations	Not applicable	
3.5 Statistics	The significance of any inter-group differences in blood composition or absolute or bodyweight-relative organ weights was assessed by a series of Student's 't' tests using a pooled within-treatment error variance. Mortality in female rats receiving permethrin was similar to that of the rats comprising the female control group; the data were therefore not subjected to statistical analysis. With the exception of the data on number of tissues examined as a positive group incidence presented for decedents, Weeks 53-Term, where inter-group differences in mortality distribution of male rats were assessed by using the chi-squared test, with Yates correction where appropriate, or by using Fisher's Exact Probability Test, analysis of male mortality data was *via* a computer programme designed to perform trend and homogeneity analyses of proportions and life-table data according to Thomas *et al*, 1977 (Computers and Biomedical Research, 10, 373). Animals killed at the termination of the study were entered as censored observation. This approach utilised the portion of the programme	

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

dealing with the life-table after Kaplan and Meier, 1958 (J. Am. Stat. Assoc., 53, 457).

The two-tailed probability associated with the observed difference between the proportion of animals surviving in the control group and that in the highest dosage group arising by chance, at each week of interest throughout the study, was determined by first calculating the normal standard deviate, Z, from values of $S(t_1)$ and $S(t_4)$, and SE_1 and SE_4, for the control and highest dosage groups respectively, as follows:

$$Z = \frac{S(t_1) - S(t_4)}{(SE_1^2 + SE_4^2)^{\frac{1}{2}}}$$

The exact probability associated with Z was determined by reference to a table of normal probability integrals.

The same computer programme was employed to examine for effects on latency of mammary gland benign fibro-epithelial tumours in female rats. No other type of tumour was treated in this way, since there were insufficient data to provide a meaningful analysis.

The significance of any inter-group differences in the distribution of non-neoplastic or neoplastic pathology was assessed by using Fisher's Exact Probability Test, applied as a two-tailed test.

3.5 Further remarks

Intake of test compound: achieved dosages, expressed as mg/kg/day, were calculated weekly for the first 26 weeks, bi-weekly for Weeks 27-28, and weekly for Weeks 79-103.

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

4 RESULTS AND DISCUSSION

4.1 BODY WEIGHT

In respect of rate of bodyweight gain, the treated and control groups were, throughout, essentially identical.

4.2 Food consumption

The amounts of food consumed by the treated animals were, throughout, essentially identical to those consumed by controls.

4.3 Water consumption

The amounts of water consumed by the treated animals were, throughout, essentially identical to those consumed by controls.

4.4 Clinical signs

From Week 90 onwards, body tremors were seen in ten male and five female rats receiving permethrin at the highest dosage. With one exception, a female, this was manifest either daily or intermittently for a minimum of two weeks. Prior to Week 90, body tremors had been seen in two male rats receiving 250 mg/kg/day and in one control female. It was considered that this phenomenon was, during the later stages of the study, related to treatment with permethrin.

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

Convulsive episodes, consisting of bouts of violent involuntary contractions of the skeletal muscles, were seen, at various times during the study, in 12 male rats (three in Group 1; two in Group 2; three in Group 3; four in Group 4) and in 15 female rats (four in Group 1; three in Group 2; one in Group 3; seven in Group 4); such episodes are recognised as spontaneous events in this strain of rat and were considered not to be related to treatment with permethrin.All other signs recorded were those generally associated with this strain of rat, and were considered to be unrelated to treatment.A total of 250 rats, distributed among the groups, died or were killed in extremis during the first 104 weeks of treatment. Any animal killed after completing 103 weeks of treatment was considered to be part of the terminal sacrifice.Mortality in female rats receiving permethrin at any dosage was similar to that of their respective control group throughout the treatment period. Between Weeks 40 and 66, more deaths occurred among males of Group 4 than in any other male subgroup, resulting in a statistically significant elevation in the cumulative mortality, which persisted from Weeks 48 to 83.From Weeks 66 to 83 the inter-group difference was gradually eroded by higher mortality rates in Groups 1, 2 and 3, and by Week 88 no statistically significant difference remained. Over Weeks 98 and 99, there were more deaths among treated males than among controls, and then the resultant relationship persisted to termination. It was concluded that permethrin administered at 250 mg/kg/day exerted significant adverse effect upon survival in male rats, but not in females. At 10 or 50 mg/kg/day, permethrin was without effect on survival in either sex.

Three female rats, from the satellite groups, died during blood sampling at Weeks 6 or 27. A further 69 animals from the satellite groups died or were killed in extremis.

4.5 MACROSCOPIC INVESTIGATIONS

During the course of the study, palpable swellings were recorded in a total of 275 rats. The anatomical position, times of appearance and group distribution (37 in Group 1♂; 22 in Group2♂; 30 in Group 3♂; 28 in Group 4♂; 37 in Group 1♀; 44 in Group2♀; 41 in Group 3♀; 36 in Group 4♀) were not suggestive of any relation to treatment. In females, treatment had no effect on the latency period of those palpable swellings which were subsequently diagnosed as mammary gland benign fibro-epithelial tumours.

4.6 OPHTHALMOSCOPIC EXAMINATION

Abnormalities and anomalies detected by ophthalmoscopic examination were typical of those commonly found in rats of this strain, and their distribution clearly did not associate with treatment with permethrin.

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

4.7 Haematology

The occasional statistically significant differences between control and treated rats were considered to represent chance variation unrelated to treatment with permethrin; the values noted were within the ranges normally found in rats of this strain (Table A6_7-1(1)a).

Estimation of the prothrombin time in female rats, after five or 26 weeks of treatment, was inconclusive because of an unusually high incidence of clotted samples; subsequent analyses performed during Weeks 8 and 29 confirmed that the prothrombin time was not altered by treatment.

In the light of results obtained after 26 weeks of treatment, the examination was extended to include male rats from the lowest and the intermediate dosage group. The parameters analysed were microhaematocrit, haemoglobin and erythrocyte count; no treatment-related effects were in evidence.

A significant lengthening of prothrombin time was observed after 50 weeks of treatment in male rats receiving the highest dosage; examination of male rats from the lowest and intermediate dosage groups revealed no treatment-related effects.

4.8 Clinical Chemistry

The statistically significant differences between control and treated rats were thought to be part of normal biological variation and were considered not to be related to treatment (Table A6_7-1(1)b). The values noted were within the ranges normally found in rats of this strain.

In the light of results obtained in the 27th week of treatment, the examination was extended two weeks later to include ten male and ten female rats from each of the remaining satellite groups. Although statistically significant differences in the group mean values were reported for many parameters, individual values were recorded largely within the ranges normally found in Wistar rats of this age in the laboratories of Life Science Research. There were no trends and it was considered that the results did not reflect a response to treatment with permethrin.

4.9 Urinalysis

Although, after five weeks of treatment, the protein concentrations in urine samples from male rats receiving permethrin at 250 mg/kg/day were higher than those recorded for the control groups, the values were within the range normally found in rats of this age and strain; the higher levels were not considered to be related to treatment. There were no other disturbances in the cellular or chemical constituents of the urine samples after 5, 25, 51, 77 and 102 weeks of treatment.

4.10 Pathology

Macropathological entities recorded at necropsy of decedents and of those animals surviving to the end of the treatment period were those commonly found in rats of this strain; they did not associate with treatment and gave no indication of a reason for the higher mortality recorded in male rats receiving permethrin at the highest dosage.

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

4.11 Organ Weights

The statistically significant differences (analysis of variance) from the control values of various absolute (A) and bodyweight-relative (R) organ weights are summarised below.

Dosage	Sex	Organ	Deviation	p value
250	♂	Liver (A)	+	<0.001
250	♂	Liver (R)	+	<0.05
250	♂	Adrenal glands (A)	+	<0.05
250	♂	Adrenal glands (R)	+	<0.01
50	♀	Pituitary gland (A)	+	<0.05
50	♀	Pituitary gland (R)	+	<0.05
10	♀	Heart (A)	+	<0.05
250	♀	Lungs (A)	-	<0.05
250	♀	Lungs (R)	-	<0.05
250	♀	Kidneys (A)	-	<0.05
250	♀	Kidneys (R)	-	<0.01

Terminal group mean bodyweights for each sex fell within a narrow range, so that the results of comparison of absolute organ weights were closely similar to those of comparing organ weights after relation to bodyweight.

Outlying group mean values were in some cases recognised as being due to a skewed distribution of massive tumours in small organs, the most marked example being adrenal weight in males given the highest dosage of permethrin: after exclusion of animal Nos. 201 and 218 (bearing an adenocarcinoma and phaeochromocytoma, respectively) the group mean adrenal weight was restored to the same range as that occupied by the other group mean values. Disturbance of group mean pituitary weight, by similar causes, was evident in Groups 1♂, 2♀, 3♀ and 4♀; group mean pituitary weights for all other subgroups fell within the range normally encountered in this laboratory. The incidence of cystic ovaries was also unevenly distributed, and the group mean ovary weight peaked in Group 3. After making allowance for such factors, it was evident that no dosage-related trends were present among the data for brain, pituitary, heart, spleen uterus, thyroids, ovaries and testes.

Adrenal weight in females was related to dosage, but the inter-group differences did not attain statistical significance at the 5% level and the trend was considered fortuitous. Kidney weight in females of the highest dosage group was significantly lower than that in control females, but an opposite relation in males indicated that treatment with permethrin was not implicated. In males given the highest dosage, liver weight was significantly higher than in controls.

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

Smaller elevations of liver weight occurred in females of the same group and in males given the intermediate dosage, but these did not attain statistical significance at the 5% level.

Finally, lung weight was not significantly altered by treatment in males, but a dosage-related downward trend was perceptible in females, statistical significance attaching only to the depression at the highest dosage. It is noteworthy that massive lung abscesses were responsible for the elevated lung weight in animals Nos. 258 and 279 in the female control group; after exclusion of these, there was no significant inter-group difference in lung weight.

It was concluded that there was evidence to suggest a treatment-related increase in liver weight in males. All other organs were evidently unaffected.

4.12 Histopathology

Animals dying or killed *in extremis* during Weeks 0-52 of treatment

Non-neoplastic findings

There was a range of banal degenerative and inflammatory changes similar in type and incidence to those commonly found in studies of Wistar rats at Life Science Research, and not considered to be related to treatment.

Two out of eight males in the highest dosage group were the only rats to have periacinar hepatocytic hypertrophy. This was considered to be biologically, although not statistically, significant, when considered in conjunction with other temporal groupings of rats.

Neoplastic findings

There were few neoplasms present; their random group-distribution indicated that they were not related to treatment.

Animals dying or killed *in extremis* between Week 53 and termination of study

Non-neoplastic findings

There was a wide range of banal degenerative and inflammatory changes similar in extent and type to those commonly found in studies of Wistar rats at Life Science Research, and not related to treatment with permethrin.

There were several changes present that, although marginally statistically significant, were not considered to be of biological significance. In these cases the apparent significance was as a result of an unusual control value or an isolated unusual incidence which bore no association with dosage relationship or possible pathogenesis.

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

Lesions which were apparently associated with treatment occurred in the kidneys, liver and thyroid glands. In the male rats there was an increase in moderate geriatric nephropathy in the intermediate dosage group, associated with a decrease in marked geriatric nephropathy in the intermediate and highest dosage groups. Hence, treatment with permethrin appeared to decrease the severity and incidence of geriatric nephropathy. There was a dosage-related increase in periacinar hepatocytic hypertrophy, statistically significant only in the female rats. In the thyroids there was a dosage-related increase in the incidence of focal disturbance in growth pattern of follicular cells, statistically significant only in the male rats.

Neoplastic findings

There was a range of neoplasms present, none of which was related to treatment. These included mammary gland benign fibro-epithelial tumours; pituitary adenomas; thyroid follicular cell adenomas; benign phaeochromocytomas; testicular interstitial cell tumours and benign and malignant mesemchymal skin and subcutis tumours.

Analysis of total benign and malignant tumours of all categories revealed a significant increase in the frequency of both benign and malignant neoplasms in the intermediate dosage group in the male rats. In the case of the benign tumours, this is largely due to the non-significant increased incidences of pituitary adenomas and thyroid follicular adenomas. In the case of the malignant tumours, many isolated incidences have caused the high total incidence. Neither result would appear to be attributable to an inherent oncogenic property of permethrin.

Animals killed at termination: Groups 1 and 4

Non-neoplastic findings

There was a wide range of banal degenerative and inflammatory changes similar in type and extent to those commonly found in Wistar rats of this age at Life Science Research, which were not considered to be related to treatment with permethrin.

Neoplastic findings

There was a wide range of neoplasms present, none of which was considered to be related to treatment. The most frequent neoplasms were mammary gland benign fibro-epithelial tumours and pituitary adenomas.

Analysis of frequency of benign or malignant tumours, or those rats with one or more types of neoplasms did not reveal any treatment-related effects.

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

Animals killed at termination: Groups 2 and 3 (only liver, thyroids, tissue masses and presumptive tumours examined)

Non-neoplastic findings

There was a wide range of banal degenerative and inflammatory changes similar in type and incidence to those commonly found in Wistar rats of this age at Life Science Research, which were not considered to be related to treatment.

Neoplastic findings

There was a wide range of neoplasms present, none of which was considered to be related to treatment. The most frequent neoplasms were mammary gland benign fibro-epithelial tumours and pituitary adenomas.

Analysis of frequency of benign or malignant tumours, or those rats with one or more types of neoplasms did not reveal any treatment-related effects.

4.13 OTHER EXAMINATIONS

Not applicable

4.14 Time to tumours

Not applicable (oral administration)

4.15 Other

Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 MATERIALS AND METHODS

Diets containing permethrin at concentrations sufficient to provide dosages of 10, 50 or 250 mg/kg body weight/day were fed to groups of 60 male and 60 female Wistar rats for 103 consecutive weeks. An identical group of rats received diet without permethrin, and served as negative controls.

A further 15 males and 15 females were appended to, and received the same treatment as, each of the above four groups; so far as possible, blood and urine samples for laboratory study were withdrawn from these animals only.

Main study animals were used for observational purposes and for histopathology. Satellite animals were used to provide, as far as possible, only blood and urine samples. Satellite animals that died during the treatment period, or those that were killed terminally, were necropsied, and tissues were taken into fixative but not examined histologically.

Serial observations included clinical signs, mortality, body weight, food consumption, intake of test compound, water consumption, ophthalmoscopy, haematology, clinical chemistry and urinalysis. Terminal observations included macroscopic examinations, organ weight analysis and histopathology.

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

5.2 RESULTS AND DISCUSSION	From the 90th week of treatment until termination, a low incidence of generalised body tremor was seen among rats receiving permethrin at 250 mg/kg/day. There were no other signs of reaction to treatment at any dosage. At 250 mg/kg/day, permethrin exerted significant adverse effect upon survival in males, but not in females. At 10 or 50 mg/kg/day, permethrin was without effect on survival in either sex. In respect of the rate of body weight gain and the amounts of food and water consumed, treated and control rats remained essentially identical throughout the treatment period. Ophthalmoscopic examination revealed no treatment-related abnormalities. The observed cellular and chemical composition of the blood and urine were not affected by treatment. The group distribution of macropathological entities observed at necropsy displayed no relation to dosage. In males that received permethrin at 250 mg/kg/day, liver weight was significantly higher than in controls, both in absolute terms and after relation to body weight. Microscopic examination of a wide range of tissues revealed a dosage-related increase in the incidence of periacinar hepatocytic hypertrophy, affecting the two upper dosage groups only. Treatment with permethrin also associated with reductions in the incidence and degree of geriatric nephropathy and parathyroid hyperplasia. There was no evidence of any neoplastic response to treatment.
5.1 Conclusion	
LO(A)EL	50 mg/kg bw/day, based on histopathological evidence of hepatic work hypertrophy.
NO(A)EL	10 mg/kg bw/day.
Other	It was concluded that the main effects of permethrin, administered at 250 mg/kg/day, comprised a moderate decrease in the survival of males only, and indications of hepatic work hypertrophy in both sexes. There was histopathological evidence of the latter change at 50 mg/kg/day, while rats receiving 10 mg/kg/day remained in all respects indistinguishable from controls.
5.3.4 Reliability	2
5.3.5 Deficiencies	Yes; not GLP.

Section A6.7 **6.7(1) Carcinogenicity – oral (rat)**

Annex Point IIA6.7

Key Study

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*30/11/05*
Materials and Methods	***2.3*** *There was no high dose satellite group included for pathological evaluation.* ***3.1*** *This is only correct if we are to assume that the TS described as 21Z is the same as 21Z73 (that identified in Section 2)?* ***3.1.2*** *To what exactly does 'specification' refer?* ***3.1.2.1*** *This is only correct if we are to assume that the TS described as 21Z is the same as 21Z73 (that identified in Section 2)?* ***3.1.2.2*** *This is only correct if we are to assume that the TS described as 21Z is the same as 21Z73 (that identified in Section 2)?* ***3.3.11*** *The diet provided was Spratt's Laboratory Diet No. 2.* *State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version.*
Conclusion	*Adopt applicant's version.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_7(1)-1a. Table for Haematology

Treatment Week	Dosage (mg/kg/day)	Sex	Parameter	Deviation	p value
6	250	♀	Hb	+	<0.05
	250	♀	MCV	+	<0.05
	250	♂	PT	+	<0.001
27	250	♂	RBC	-	<0.01
	250	♂	MCV	+	<0.05
29	10	♂	Hb	+	<0.05
	50	♂	Hb	+	<0.01
51	250	♂	PT	+	<0.001
78	250	♀	Total WBC	+	<0.05
103	10	♂	RBC	+	<0.05
	50	♂	RBC	+	<0.05
	10	♂	MCV	-	<0.001
	50	♂	MCV	-	<0.001
	250	♂	Total WBC	+	<0.01
	250	♂	N	+	<0.05
	250	♂	L	+	<0.05
	250	♂	Platelets	+	<0.01
	250	♀	Platelets	-	<0.05

Table A6_7(1)-1b. Table for Clinical Chemistry

Treatment Week	Dosage (mg/kg/day)	Sex	Parameter	Deviation	p value
6	10	♂	Glucose	-	<0.01
	50	♂	Glucose	-	<0.05
	250	♂	Glucose	+	<0.01
27	250	♀	Urea	-	<0.05
	250	♀	Glucose	-	<0.05
	250	♂	SAP	-	<0.05
	250	♀	SGPT	-	<0.01
	250	♀	SGOT	-	<0.01
	250	♂	Total proteins	+	<0.01
	250	♂	Albumin	+	<0.01
	250	♀	Albumin	-	<0.05
	250	♂	α1 Globulin	-	<0.01
	250	♀	α1 Globulin	+	<0.001
	250	♀	Na	-	<0.01
	250	♀	K	-	<0.01
29	50	♂	Urea	-	<0.001
	10	♀	Urea	-	<0.001
	50	♀	Urea	-	<0.001
	50	♀	Glucose	-	<0.05
	10	♀	SAP	-	<0.05
	50	♀	SAP	-	<0.05
	50	♂	SGPT	-	<0.05
	10	♀	SGPT	-	<0.01
	50	♀	SGPT	-	<0.05
	50	♂	Total proteins	+	<0.001
	10	♂	K	-	<0.05
	10	♀	K	+	<0.01
	50	♀	K	+	<0.001
78	250	♂	AP	-	<0.05
	250	♀	Na	+	<0.001
103	250	♂	Glucose	+	<0.05
	250	♀	Glucose	-	<0.05
	10	♀	SAP	-	<0.05
	50	♂	Albumin	-	<0.01
	250	♂	Albumin	-	<0.01
	10	♀	β Globulin	-	<0.05
	50	♀	β Globulin	-	<0.05
	10	♂	β Globulin	+	<0.01
	250	♂	α1 Globulin	+	<0.05
	10	♂	α2 Globulin	-	<0.01
	50	♂	α2 Globulin	-	<0.05
	250	♂	α2 Globulin	-	<0.05
	250	♀	γ Globulin	+	<0.01
	50	♂	Na	-	<0.001
	250	♂	Na	-	<0.001
	250	♂	K	-	<0.05

Table A6_7(1)-2c. Results of Carcinogenicity study

Parameter	control data				low dose		medium dose		high dose		dose-response + / -	
	historical		study									
	m	f	m	f	m	f	m	f	m	f	m	f
	If differing numbers of animals are examined, give number affected/number of animals examined for each individual finding.											
Number of animals examined			60	60	60	60	60	60	60	60		
Mortality			35	22	47	20	39	18	48	20	+	-
clinical signs			-	-	-	-	-	-	+	+	+	+
body weight gain											-	-
food consumption											-	-
clinical chemistry											-	-
haematology											-	-
urinalysis											-	-
Overall tumour incidence:												
No. of animals with neoplasms			38	47	27	50	37	46	31	45	-	-
No. of animals with benign neoplasms											-	-
No. of animals with malignant neoplasms											-	-
No. of animals with > 1 neoplasm			16	18	11	24	24	25	8	22	-	-
Liver												
tumour a*												
tumour x*												
non-neoplastic changes			-	-	-	-	+	+	+	+	+	+

Section A6.7 **6.7(2) Carcinogenicity– oral (rat, mouse)**
Annex Point IIA6.7

Key Study

		Official use only
1 REFERENCE		
1.1 Reference	Ishmael J & Litchfield MH; 1988; Chronic Toxicity and Carcinogenic Evaluation of permethrin in Rats and Mice; Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ, England; *Fundam. Appl. Toxicol.* **11**, 308-322; 1988.	
1.2 Data protection	No	
1.2.1 Data owner	Public domain	
1.2.2 COMPANIES WITH LETTER OF ACCESS	Not applicable	
1.2.3 Criteria for data protection	No data protection claimed	
2 GUIDELINES AND QUALITY ASSURANCE		
2.1 GUIDELINE STUDY	No; no guidelines available.	
2.2 GLP	Yes	
2.3 Deviations	No	X
3 MATERIALS AND METHODS		
3.1 Test material	Permethrin 40% *cis*/60% *trans*	
3.1.1 Lot/Batch number	Not reported	
3.1.2 pecification	Deviating from specification given in section 2 as follows	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	≥ 93.9% (nominal *cis*:*trans* ratio of 40:60)	
3.1.2.3 Stability	The dietary concentrations and the *cis*:*trans* isomer content of permethrin were analysed periodically throughout the study by gas chromatography. The concentrations of permethrin fed to mice generally were within ± 10% of the required levels and the *cis*:*trans* content was within ± 5%.	
3.2 Test Animals		
3.2.1 Species	Rat, mouse	
3.2.2 Strain	Rat: Alpk:AP (Wistar-derived) albino Mouse: Alpk:AP Swiss-derived	
3.2.3 SOURCE	Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ, England	
3.2.4 Sex	♂ and ♀	

Section A6.7 Annex Point IIA6.7	**6.7(2) Carcinogenicity– oral (rat, mouse)**	
	Key Study	
3.2.5 Age/weight at study initiation	Rat: 4 to 5 weeks of age/65-80 g weight range at study initiation Mouse: 4 to 5 weeks of age/18-22 g weight range at study initiation	
3.2.6 NUMBER OF ANIMALS PER GROUP	Rat: 96 main study; 12 interim sacrifice at 52 weeks Mouse: 100 main study; 40 interim sacrifice at 26 and 52 weeks (20 at each time-point)	
3.2.6.1 at interim sacrifice	Rat: 12 animals/group/sex Mouse: 10 animals/group/sex	
3.2.6.2 at terminal sacrifice	Rat: 24 animals/group/sex Mouse: 25 animals/group/sex	
3.2.7 Control animals	Yes	
3.3 Administration /Exposure	*Oral*	
3.3.1 Duration of treatment	Rat: 104 weeks Mouse: 98 weeks (lifetime study: 80% mortality)	
3.3.2 Interim sacrifice(s)	Rat: after 52 weeks Mouse: after 26 and 52 weeks	
3.3.3 Final sacrifice	Rat: after 104 weeks Mouse: after 98 weeks	
3.3.4 Frequency of exposure	Daily	
3.3.5 Postexposure period	Not applicable	
	Oral	
3.3.6 Type	In food	
3.3.7 CONCENTRATION	Rat: food ..... 0, 500, 1000, 2500 ppm food ..... 0, 25, 50, 125 mg/kg bw food consumption per day ..... ad libitum Mouse: food ..... 0, 250, 1000, 2500 ppm food ..... 0, 38, 150, 380 mg/kg bw food consumption per day ..... ad libitum	X X
3.3.8 Vehicle	Not applicable	
3.3.9 Concentration in vehicle	Not applicable	
3.3.10 Total volume applied	Not applicable	
3.3.11 Controls	Plain diet	
3.4 Examinations		

Section A6.7 **6.7(2) Carcinogenicity– oral (rat, mouse)**
Annex Point IIA6.7

		Key Study	
3.4.1 Body weight	Yes		
3.4.2 Food consumption	Yes		
3.4.3 WATER CONSUMPTION	No		
3.4.4 Clinical signs	Yes		
3.4.5 Macroscopic investigations	No		X
3.4.6 Ophthalmoscopic examination	No		
3.4.7 Haematology	Yes		
	Number of animals:	Rat: 8 animals/sex/group Mouse: survivors of designated 10 animals/sex/group	
	Time points:	Rat: Pre-experiment and at Weeks 4, 13, 26, 39, 52, 65, 91, 104 Mouse: Weeks 26, 52	X
	Parameters:	Haemoglobin concentration, packed cell volume, erythrocyte count, total and differential leukocyte count, platelet count, prothrombin time Other: Kaolin-Cephalin indices; bone marrow smears (Weeks 52 and 104 in rats; Week 52 in mice)	
3.4.8 Clinical Chemistry		Yes	
	Number of animals:	Rat: 8 animals/sex/group Mouse: survivors of designated 10 animals/sex/group	
	Time points:	Rat: Pre-experiment and at Weeks 4, 13, 26, 39, 52, 65, 91, 104 Mouse: Weeks 26, 52	X
	Parameters:	Glucose, blood urea nitrogen, alanine aminotransferase (alanine transaminase: ALT), aspartate aminotransferase (aspartate transaminase: AST)	
	Other	Not applicable	
3.4.9 Urinalysis	No		
	Number of animals:	Not applicable	
	Time points:	Not applicable	
	Parameters:	Not applicable	
	Other	Not applicable	

Section A6.7 **6.7(2) Carcinogenicity– oral (rat, mouse)**
Annex Point IIA6.7

		Key Study
3.4.10 Pathology	Yes	
3.4.10.1 Organ Weights	Yes	
	from:	all surviving animals, at interim sacrifice, at terminal sacrifice
	Organs:	Liver, kidneys, testes, spleen, brain, heart
		Other: Lung
3.4.10.2 Histopathology	Yes	
	from:	all dose groups (including animals that died or were killed when moribund)
	from:	all surviving animals
		at interim sacrifice
		at terminal sacrifice
	Organs:	Brain, spinal cord, pituitary, thyroid, parathyroid, thymus, salivary glands, stomach, jejunum, liver, pancreas, kidneys, adrenals, spleen, heart, lungs, gonads (testes, ovaries), uterus/cervix, female mammary gland, urinary bladder, lymph node, peripheral (sciatic) nerve, skin.
		Other: Epididymis, all gross abnormalities
Other examinations	Hepatic aminopyrine-*N*-demethylase activity Smooth endoplasmic reticulum proliferation.	
3.5 Statistics	Body weight gains, food consumption, food utilisation, and haematological and biochemical values were analysed by analysis of variance and by Student's *t* test. Organ weights were compared by analysis of variance and analysis of covariance on body weight and by Student's *t* test. Mortality rates were compared using the Logrank test (Peto and Pike, 1973). Tumour incidence was initially analysed by Fisher's Exact Test. The data were considered separately for males and females, each treated group being compared with the corresponding control group. Where appropriate, tumour incidence was further considered by a Logrank analysis which allowed for differences in mortality between groups and the context of observation, i.e. whether incidental or non-incidental, of each tumour (Peto *et al*, 1980).	
3.6 Further remarks	Examination details may be incomplete (e.g. organs examined histopathologically) due to abbreviation of original study methodology for publication purposes.	
	4 RESULTS AND DISCUSSION	

Section A6.7 **6.7(2) Carcinogenicity– oral (rat, mouse)**
Annex Point IIA6.7

Key Study

4.1 Body weight	Rat: There was a small decrease in body weight gain in the permethrin treated groups, during the first 6 weeks of study, which was not strictly dose-related. After this initial period, all the treated male and female groups grew similarly or better than the control groups and there was no evidence for a compound-related effect. Mouse: The male and female mice fed 2500 ppm permethrin grew less well than the controls in the earlier part of the study, the differences from control attaining statistical significance on occasions. After 52 weeks the weight gain was similar to controls, although body weight generally remained lower than that of controls for the remainder of the study. The male and female mice fed 1000 ppm permethrin also gained less weight than controls in the earlier stages of the study but the differences were not statistically significant. The mice given 250 ppm permethrin grew similarly to the controls throughout the study.
4.2 Food consumption	Rat: There were no consistent dose-related deviations for food consumption in either sex, the amounts eaten by treated animals being similar to that eaten by the controls. Mouse: There were no dose-related changes except that permethrin-treated male mice ate more than the controls up to Week 12.
4.3 Water consumption	Not reported
4.4 Clinical signs	Rat: Slight whole body tremors associated with hypersensitivity to localised noise and disturbance, and piloerection were noted during the routine clinical examinations in all male and female rats fed 2500 ppm permethrin during the first 2 weeks of the study. Slight whole body tremors were also noted in one female rat in Week 8 and one male rat in Week 44 from the 2500 ppm permethrin group. These compound-related findings were not seen in rats at the lower dose levels. A yellow staining of the fur in the genital area, and brown staining of the tail occurred in rats of all groups but was more pronounced both in severity and numbers affected in those fed permethrin. There was no evidence to indicate that these observations were associated with urinary obstruction or diarrhoea. Other clinical findings were not related to the administration of permethrin. There were very few mortalities up to Week 52. From that point onward the mortalities in the males given 2500 ppm permethrin remained somewhat higher than in the control males although the difference did not attain statistical significance. No apparent compound-related changes were observed in the females and the mortality incidence in the treated groups generally remained below the control values over the latter half of the study.

Section A6.7 Annex Point IIA6.7	**6.7(2) Carcinogenicity– oral (rat, mouse)**
	Key Study
	Mouse: The general health and condition of the animals remained good throughout the treatment period. The few clinical abnormalities which were observed were distributed across all groups and there was no evidence of any changes due to permethrin. The mortality rate at 2500 ppm permethrin was slightly greater than in the other groups but did not attain statistical significance compared with controls.
4.5 MACROSCOPIC INVESTIGATIONS	Not reported
4.6 Ophthalmoscopic examination	Not applicable
4.7 Haematology	Rat: The results at 52 weeks are typical of those obtained for the haematological assays undertaken during the study. Occasional values in treated groups were statistically significant from the control group values but there was no evidence for dose-related effects. Mouse: The results at 52 weeks for rats are typical of those obtained for the haematological assays undertaken during the study. Occasional values in treated groups were statistically significant from the control group values but there was no evidence for dose-related effects.
4.8 Clinical Chemistry	Rat: The results at 52 weeks are typical of those obtained for the blood biochemistry assays undertaken during the study. Occasional values in treated groups were statistically significant from the control group values but there was no evidence for dose-related effects. There was evidence for a reduction in the clotting factor indices at 52 weeks in the males given 1000 or 2500 ppm permethrin. However, at 104 weeks there was a small decrease in the prothrombin time only in the males given 2500 ppm permethrin. All bone marrows examined appeared normal except in one 2500 ppm permethrin male which had an increased myeloid:erythroid ratio. Mouse: The results at 52 weeks for rats are typical of those obtained for the blood biochemistry assays undertaken during the study. Occasional values in treated groups were statistically significant from the control group values but there was no evidence for dose-related effects.
4.9 Urinalysis	Not reported
4.10 Pathology	Not reported

Section A6.7 **6.7(2) Carcinogenicity– oral (rat, mouse)**
Annex Point IIA6.7

Key Study

4.11 ORGAN WEIGHTS

Rat: The organ weights showed no indication of a treatment-related effect except for the liver. Liver weight was increased above control values in all treated groups of both sexes at Week 104 and in all female groups and the 2500 ppm permethrin male group at Week 52.

Mouse: The liver weight of permethrin-treated mice increased in all male groups and the two higher treatment groups of the females at Week 52 and in the female 2500 ppm permethrin group at Week 98. Apart from a small decrease in male kidney weight in all treatment groups at termination, there were no other apparently treatment-related changes in the other organs weighed.

4.12 Histopathology

Rat:

Non-neoplastic lesions

Effects attributed to permethrin administration were confined to the liver. At 52 weeks there was an apparent increase in the incidence of vacuolated hepatocytes in the mid-zonal and centrilobular areas in the 2500 ppm permethrin males. To quantify this effect liver sections were re-examined "blind" and the number of vacuolated hepatocytes in five high-power fields counted. The mean count for the 2500 ppm permethrin males was approximately 10 times greater than that of the controls and other treatment groups. No differences between groups were noted in the livers of female rats. At the terminal kill at Week 104 increased hepatocyte vacuolation was seen in both male and female rats fed 2500 ppm permethrin although the effect was variable and not all rats were equally affected. The vacuoles predominantly contained lipid but some were considered to be of anoxic type. At 104 weeks centrilobular hypertrophy associated with increased cytoplasmic eosinophilia was found in all treated groups with the highest incidence at 2500 ppm permethrin.

Detailed histological and ultrastructural examination of the sciatic nerves did not reveal abnormalities attributable to permethrin administration. The other non-neoplastic abnormalities observed were generally those expected in animals of this age and of this strain.

Neoplasia

The types of tumours observed were those generally expected in this strain with pituitary and mammary tumours predominating. Liver tumours were not seen in any group and the incidence of lung tumours was very low and showed no relationship to treatment. There was a higher incidence of mammary fibroadenoma in the 2500 ppm females compared to the controls but the difference was not statistically significant.

It was concluded that permethrin had no effect on the incidence of any particular tumour type nor on the overall incidence of tumour-bearing rats.

Section A6.7
Annex Point IIA6.7

6.7(2) Carcinogenicity– oral (rat, mouse)

Key Study

Mouse:

Non-neoplastic lesionsIncreased eosinophilia of centrilobular hepatocytes was seen in the liver of both sexes of the 2500 ppm permethrin group at 26 and 52 weeks and in this group and the 1000 ppm permethrin group at the terminal kill.Detailed histological and ultrastructural examination of the sciatic nerves did not reveal abnormalities attributable to permethrin administration. The other non-neoplastic abnormalities observed were generally those expected in animals of this age and of this strain. Apart from the liver changes described above the only other change apparently related to treatment was a decrease in the degree of vacuolation of the renal proximal tubular epithelium of males. This was seen from Week 26 onward and affected mainly the 2500 ppm permethrin group.

NeoplasiaThe main types of neoplasia were liver, lung and lymphoreticular tumours. There was a slight increase in the incidence of hepatic tumours in male mice receiving 2500 ppm permethrin but this was not statistically significantly different from controls by either the Fisher's exact or Logrank tests. A fairly high incidence of lung adenoma was seen in all groups, with a slightly higher incidence than controls for the 2500 ppm group. Using Fisher's exact test (5% level, one-sided) the difference between the control and 2500 ppm permethrin groups was not significant for either sex. With the Logrank test the increase was statistically significant for the 2500 ppm permethrin males (5% level) but not for females. There was no significant increase in the incidence of unusual tumour types in any of the permethrin-treated groups nor of the overall incidence of tumour-bearing mice.

4.13 OTHER EXAMINATIONS

Rat:

Hepatic aminopyrine-*N*-demethylase activity

The hepatic APDM activity was increased in all treated groups of both sexes at Weeks 52 and 104 with the largest increase at the top dose.

Smooth endoplasmic reticulum proliferation

Electron microscopic examination showed hepatic SER proliferation in most of the permethrin-treated rats at Week 52 and for those in the two higher dose groups at Week 104. Quantitation of the SER confirmed these findings and showed that the highest results occurred in the top dose group.

Mouse:

Hepatic aminopyrine-*N*-demethylase activity

Similar findings to those in the rat study although the treatment-related responses were less marked than in the rat.

Smooth endoplasmic reticulum proliferation

Similar findings to those in the rat study although the treatment-related responses were less marked than in the rat.

Section A6.7 Annex Point IIA6.7	6.7(2) Carcinogenicity– oral (rat, mouse)
	Key Study
4.14 TIME TO TUMOURS	Not reported
4.15 Other	Not applicable

Section A6.7
Annex Point IIA6.7

6.7(2) Carcinogenicity– oral (rat, mouse)

Key Study

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Permethrin was supplied by Plant Protection Division, Imperial Chemical Industries PLC, Jealott's Hill Research Station, Bracknell, Berkshire, UK, as technical material of not less than 93.9% purity and with a nominal *cis*:*trans* ratio of 40:60. The concentrations of permethrin fed to rats or mice generally were within ± 10% of the required levels and the *cis*:*trans* content was within ± 5%.

Specific pathogen free Alpk:AP (Wistar-derived) albino rats and Swiss-derived mice were supplied from the breeding colonies at Alderley Park, Cheshire, UK, at 4 to 5 weeks of age. There were four groups of 96 rats (48 males and 48 females) maintained for 104 weeks on diets containing 0, 500, 1000, or 2500 ppm permethrin. An additional 12 rats per sex per group were designated for an interim kill at 52 weeks.

Four groups of 100 mice (50 males and 50 females) were maintained for a lifetime study (80% mortality) on diets containing 0, 250, 1000, or 2500 ppm permethrin. Satellite groups of 40 mice (20 males and 20 females) were designate for interim kills at 26 weeks and 52 weeks (10 per sex per group at each time-point).

All animals on both studies were examined daily and abnormalities in clinical condition or behaviour were recorded. Individual body weights were recorded at the start of the study, weekly for the first 12 weeks and then at 2-weekly intervals throughout the study. Estimates of food consumption were recorded weekly for the first 12 weeks and then for approximately 1 week per month for the remainder of the study.

For rats, tail vein blood samples for haematological (8 per sex per group) and clinical chemistry (8 per sex per group) determinations were taken pre-experimentally and at Weeks 4, 13, 26, 39, 65, 78, and 91. At the 52- and 104-week kills blood samples were taken by cardiac puncture immediately before autopsy. For mice, blood samples were taken by cardiac puncture from the survivors of the designated 10 males and 10 females per group at the interim kills of 26 and 52 weeks.

Section A6.7 Annex Point IIA6.7	**6.7(2) Carcinogenicity– oral (rat, mouse)**
	Key Study
	Necropsies were performed on all rats and mice that either died, were killed when moribund, or survived to the end of the studies. A comprehensive selection of tissues, including all gross abnormalities, was fixed in neutral-buffered formol saline or formol-sublimate and processed for histological examination. Histological slides were examined from all animals and all lesions were recorded. Gross observations were correlated with microscopic findings. Samples of liver from up to six animals per sex per group were also taken for electron microscopic examination from both rats and mice at termination, from mice at the 25-week and rats at the 52-week kills. For the rats, smooth endoplasmic reticulum (SER) was quantified in centrilobular hepatocytes. Samples of liver from four of these rats per sex per group at 52 and 104 weeks were assayed for hepatic aminopyrine-*N*-demethylase (APDM) activity. Samples of liver were also assayed for APDM activity from four or five mice at 26 weeks and 52 weeks. The weights of the following organs were recorded in rats and mice at the scheduled kills: heart, lung, kidney, testis, spleen, liver, and brain.
5.2 RESULTS AND DISCUSSION	Changes of toxicological significance were confined to the dose level of 2500 ppm permethrin in both species. Tremors and hypersensitivity to noise were noted in rats at this dose during the first 2 weeks of study but such signs were not seen in mice. Pathological examination of the central and peripheral nervous systems did not reveal abnormalities attributable to permethrin administration. The effect on mice at 2500 ppm permethrin was shown by decreased body weight gain. Liver hypertrophy, associated with increase in liver weight, microsomal enzyme activity, and proliferation of smooth endoplasmic reticulum occurred in the rat with similar but less marked changes in the mouse. This was considered to be an adaptive response of no toxicological significance. No evidence of a carcinogenic effect was seen in the rat study. In the mouse study a slight elevation in benign lung tumour incidence in males only at 2500 ppm Permethrin was observed but was not considered to represent a carcinogenic effect.
5.3 CONCLUSION	
5.3.1 LO(A)EL	Rat: 2500 ppm ≡ 125 mg/kg bw/day, based on tremors and hypersensitivity to noise during the first 2 weeks of study. Mouse: 2500 ppm ≡ 380 mg/kg bw/day, based on decreased body weight gain.
5.3.2 NO(A)EL	Rat: 1000 ppm ≡ 50 mg/kg bw/day Mouse: 1000 ppm ≡ 150 mg/kg bw/day

Section A6.7 **Annex Point IIA6.7**	**6.7(2) Carcinogenicity– oral (rat, mouse)**
	Key Study
5.3.3 Other	Pathological examination of the central and peripheral nervous systems did not reveal abnormalities attributable to permethrin administration. Liver hypertrophy, associated with increase in liver weight, microsomal enzyme activity, and proliferation of smooth endoplasmic reticulum occurred in the rat with similar but less marked changes in the mouse. This was considered to be an adaptive response of no toxicological significance. No evidence of a carcinogenic effect was seen in the rat study. In the mouse study a slight elevation in benign lung tumour incidence in males only at 2500 ppm permethrin was observed but was not considered to represent a carcinogenic effect.
5.3.4 RELIABILITY	2
5.3.5 Deficiencies	Yes; public domain reporting of these GLP-compliant studies has been somewhat abbreviated, however this does not detract from the quality of the conduct of the studies or the conclusions drawn.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*7/12/05*
Materials and Methods	***2.3*** *Only 48 animals/sex/treatment were used (as opposed to min of 50); neither is there a high dose satellite group included for pathological evaluation.* ***3.3.7*** *This appears to be an approximate conversion, no precise value seems to be given in the publication.* ***3.4.5*** *It is reported that gross abnormalities were fixed for histological examination, therefore indicating that such observations were made. However, none of the findings was reported.* ***3.4.7*** *Samples were taken at week 78 also.* ***3.4.8*** *Samples were taken at week 78 also.* *Applicants version is acceptable.*
Results and discussion	*Adopt applicant's version.*
Conclusion	*Adopt applicant's version.*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A6.7 Annex Point IIA6.7	**6.7(2) Carcinogenicity– oral (rat, mouse)**
	Key Study
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6 7(2)-1a. Table for Haematology – Rat

HEMATOLOGICAL PARAMETERS AT 52 WEEKS ON RATS FED CONTROL OR PERMETHRIN DIETS[a]

Dietary permethrin (ppm)	Hemoglobin (g/dl)	Packed cell volume	Red blood cells ($\times 10^{12}$/liter)	White blood cells ($\times 10^{9}$/liter)	Platelets ($\times 10^{9}$/liter)
		Male			
0	15.0	0.42	8.6	6.0	980
500	15.2	0.42	8.5	6.1	940
1000	14.3*	0.40*	8.2	6.2	936
2500	14.9	0.42	8.6	6.6	909
Approximate 95% confidence limits	±0.4	±0.01	±0.3	±1.0	±89
		Female			
0	14.9	0.41	7.8	5.4	754
500	14.5	0.40	7.7	4.2	796
1000	14.7	0.41	7.8	4.5	689
2500	14.4	0.41	7.7	4.2	743
Approximate 95% confidence limits	±0.4	±0.01	±0.3	±1.0	±90

[a] Mean results for seven or eight rats per group.
* Significantly different from control group mean at 5% level (*t* test).

Table A6 7(2)-1b. Table for Clinical Chemistry – Rat

BLOOD BIOCHEMISTRY PARAMETERS AT 52 WEEKS ON RATS FED CONTROL OR PERMETHRIN DIETS[a]

Dietary permethrin (ppm)	Blood urea (mg/100 ml)	Blood glucose (mg/100 ml)	Plasma alanine transaminase (mU/ml)	Plasma aspartate transaminase (mU/ml)
		Male		
0	30	106	11.4	34
500	29	110	10.1	39
1000	30	105	11.6	34
2500	28	105	10.8	33
Approximate 95% confidence limits	±4	±11	±3.1	±19
		Female		
0	39	97	11.8	64
500	42	97	15.1	90
1000	53*	102	10.6	46
2500	41	103	13.2	46
Approximate 95% confidence limits	±4	±11	±3.1	±19

[a] Mean results for seven or eight rats per group.
* Significantly different from control group mean at 5% level (*t* test).

Table A6_7(2)-2a. Results of Carcinogenicity study – Rat

SITE/NATURE/INCIDENCE OF NEOPLASMS IN RATS FED CONTROL AND PERMETHRIN DIETS FOR 2 YEARS

	Male				Female			
Dose (ppm)	0	500	1000	2500	0	500	1000	2500
Number of rats examined	60	60	60	60	59	60	60	60
Adrenal gland								
Phaeochromocytoma	2	4	6	4	0	1	1	0
Cortical adenoma	0	1	1	0	1	0	0	2
Brain								
Meningioma	1	1	2	3	1	0	0	2
Glioma	1	0	1	3	1	0	1	1
Lung								
Adenoma	0	0	0	1	1	0	1	0
Hemopoietic								
Lymphoma (generalized)	1	0	0	2	0	2	0	0
Lymphoma (localized)	2	2	1	1	2	2	2	2
Myeloid leukemia	0	1	1	0	0	0	0	0
Hemangioma (spleen and lymph nodes)	5	3	1	3	0	3	3	2
Pancreas								
Islet cell adenoma	1	1	1	0	2	0	1	1
Exocrine adenoma	1	2	1	1	0	0	0	0
Pituitary								
Adenoma	14	15	11	15	34	36	34	33
Skin/subcutis								
Carcinoma (all types)	3	1	3	2	0	0	0	0
Benign tumors (all types)	5	6	5	6	1	1	1	1
Sarcomas	1	2	0	3	0	0	0	1
Thyroid gland								
C-cell adenoma	8	3	2	6	6	4	9	5
Follicular adenoma	0	2	1	0	0	3	0	0
Mammary gland								
Carcinoma	0	0	0	0	8	4	3	7
Fibroadenoma	0	0	0	0	11	9	11	19
Adenoma/papilloma	1	0	1	0	0	2	3	3
Uterus/cervix								
Sarcoma	—	—	—	—	1	2	1	0
Carcinoma	—	—	—	—	1	0	2	0
Papilloma/adenoma	—	—	—	—	1	0	1	0
Polyp	—	—	—	—	3	3	1	3
Testis								
Mesothelioma	3	0	1	0	—	—	—	—
Leydig cell tumor	1	1	2	0	—	—	—	—
Miscellaneous								
Schwannoma—spinal cord	0	0	0	0	1	0	0	0
Sarcoma—heart	0	0	0	0	0	0	0	1
Carcinoma—kidney	1	0	1	0	0	0	0	0
Lipoma—kidney	0	0	0	1	0	0	0	0
Liposarcoma—kidney	0	1	0	0	0	0	0	0
Squamous carcinoma—thymus	0	0	1	0	0	1	1	1
Squamous carcinoma—oral cavity	0	0	0	1	0	0	0	0
Adenoma—salivary gland	0	0	0	0	0	0	0	1
Papilloma—stomach	0	0	1	0	0	1	0	0
Leiomyoma—stomach	0	0	0	0	0	1	0	0
Leiomyoma—jejunum	0	0	0	0	0	0	1	0
Adenoma—parathyroid	0	0	1	0	0	0	0	0
Granulosa/thecal cell tumor—ovary	—	—	—	—	2	2	2	0
Tubular adenoma—ovary	—	—	—	—	0	1	1	0
Sarcoma—epididymis/vas deferens	0	0	1	1	—	—	—	—
Leiomyoma—epididymis/vas deferens	0	0	0	1	—	—	—	—
Lipoma—abdomen	1	0	1	0	1	0	0	0
Sarcoma—thorax	1	0	0	1	0	0	0	0
Undiagnosed—spleen	0	0	0	0	1	0	0	0
Number of rats with neoplasms	34	35	35	35	43	49	41	43

Table A6_7(2)-2b. Results of Carcinogenicity study - Mouse

SITE/NATURE/INCIDENCE OF NEOPLASMS IN MICE FED CONTROL AND PERMETHRIN DIETS FOR 98 WEEKS

	Male				Female			
Dose (ppm)	0	250	1000	2500	0	250	1000	2500
Number of mice examined	70	69	70	70	70	69	70	69
Liver								
Hepatocellular adenoma	10	7	6	13	2	0	2	1
Hepatocellular carcinoma	1	3	3	3	1	2	0	0
Type uncertain	0	1	0	1	0	0	0	1
Lung								
Adenoma	11	6	13	17	11	8	10	15
Adenocarcinoma	0	0	0	0	0	1	1	1
Lymphoreticular								
Malignant lymphoma	4	10	7	7	20	17	18	13
Mast cell tumor	1	0	0	0	0	0	1	0
Kidney								
Adenoma	4	1	0	0	0	0	0	0
Pituitary								
Adenoma	0	2	1	0	19	17	13	10
Vascular								
Hemangioma	5	0	1	1	6	4	5	4
Hemangiosarcoma	0	0	0	0	1	2	0	0
Miscellaneous								
Phaeochromocytoma—adrenal gland	0	0	1	0	1	0	2	1
Adenoma—harderian gland	2	1	0	0	1	0	1	0
Adenoma—parathyroid gland	0	0	0	0	1	0	0	0
Adenoma—thyroid gland	0	0	0	0	0	0	1	0
Papilloma—stomach	0	0	1	0	1	2	1	0
Carcinoma—stomach	0	0	0	0	0	2	0	1
Undiagnosed—stomach	0	0	0	0	1	0	1	0
Leiomyoma—bladder	1	0	0	0	0	0	0	0
Papilloma—skin	0	0	0	0	0	0	0	1
Fibroma—skin	0	0	0	0	1	0	0	0
Sarcoma—skin	0	0	0	0	1	1	0	1
Leydig cell tumor—testis	1	4	2	1	—	—	—	—
Sarcoma—epididymis	2	0	3	0	—	—	—	—
Carcinoma—preputial gland	0	1	0	0	—	—	—	—
Meningioma—brain	0	1	0	0	0	0	0	0
Glioma—brain	0	0	1	0	0	0	1	0
Adenoma—mammary gland	0	0	0	0	0	0	2	0
Carcinoma—mammary gland	0	0	0	0	3	0	1	0
Granulosa cell tumor—ovary	—	—	—	—	1	0	0	1
Cystadenoma—ovary	—	—	—	—	1	0	0	0
Adenoma—ovary	—	—	—	—	1	0	0	0
Polyp—uterus	—	—	—	—	0	0	2	0
Fibroma—uterus	—	—	—	—	0	1	0	0
Sarcoma—uterus	—	—	—	—	2	2	1	0
Carcinoma—uterus	—	—	—	—	0	0	0	1
Carcinoma—salivary gland	0	1	0	0	0	0	0	0
Sarcoma—thorax	0	0	0	0	0	1	0	0
Carcinoma—origin uncertain	0	0	0	0	0	1	0	0
Number of mice with neoplasms	25	26	28	30	44	41	39	33

Section A6.8.1 **6.8.1(1) Teratogenicity Study – oral (rat)**

Annex Point IIA6.8.1

	Key Study	Official use only
	1 REFERENCE	
1.1 Reference	[redacted]; 1976a; Foetal Toxicity Study of 21Z73 (NRDC 143) in the Rat; [redacted]; unpublished Report No. BPAT 74/10; 03.1976.	
1.2 Data protection	Yes	
1.2.1 Data owner	Sumitomo Chemical (UK) PLC	
1.2.2 Companies with letter of access	Bayer Environmental Science	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	No; no guidelines available.	
2.2 GLP	No; GLP was not compulsory at the time the study was performed.	
2.3 Deviations	No	X
	3 MATERIALS AND METHODS	
3.1 Test material	As given in section 2 (name used in study report: 21Z73 (NRDC 143))	
3.1.1 Lot/Batch number	Not reported	
3.1.2 pecification	As given in section 2	
3.1.2.1 Description	As given in section 2	
3.1.2.2 Purity	As given in section 2	
3.1.2.3 Stability	Not applicable (daily doses by gavage)	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	Wistar	
3.2.3 SOURCE	[redacted]	
3.2.4 Sex	♀	
3.2.5 Age/weight at study initiation	Approximately 3 months of age/170-240 g	
3.2.6 NUMBER OF ANIMALS PER GROUP	Group 1, Untreated: 22 Group 2, Vehicle control (corn oil): 23 Group 3, 200 mg/kg permethrin: 23	
3.2.7 Control animals	Yes	

Section A6.8.1 **6.8.1(1) Teratogenicity Study – oral (rat)**

Annex Point IIA6.8.1

	Key Study	
3.2.8 Mating period	Females were examined daily by vaginal smear to establish oestrus cycles. On the day of pro-oestrus females were paired with males of the same strain and age. The day of mating, confirmed by the presence of sperm in the vagina, was designated Day 0 of pregnancy.	
3.3 Administration/ Exposure	*Oral*	
3.3.1 Duration of exposure		
	rat: day 6-16 post mating	
3.3.2 Postexposure period	4 days	
	Oral	
3.3.3 Type	Gavage	
3.3.4 Concentration	Gavage 200 mg/kg bw	
3.3.5 Vehicle	Corn oil	
3.3.6 Concentration in vehicle	Not reported	
3.3.7 Total volume applied	10 mL/kg bw	
3.3.8 Controls	Untreated and vehicle controls	
3.4 Examinations		
3.4.1 Body weight	Yes	
3.4.2 Food consumption	No	
3.4.3 Clinical signs	Yes	
3.4.4 Examination of uterine content		
	Number of corpora lutea	
	Number of implantations	X
3.4.5 EXAMINATION OF FOETUSES		
3.4.5.1 General	Litter Size, Nr. of live/dead Foetuses, Foetal Weight, Sex Ratio	
3.4.5.2 Skeleton	Yes	
3.4.5.3 Soft tissue	Yes	
3.5 Further remarks		
	4 RESULTS AND DISCUSSION	
4.1 Maternal toxic Effects	There was no significant effect of treatment on weight gain, behaviour or well-being.	

Section A6.8.1 **6.8.1(1) Teratogenicity Study – oral (rat)**

Annex Point IIA6.8.1

Key Study

4.2 Teratogenic / embryotoxic effects

One foetus in Group 3 (200 mg/kg permethrin) showed major external abnormalities including brachymelia and tetradactyly. These abnormalities were accompanied by protruding tongue, mild cervical oedema and reduced body weight. This foetus was further examined for skeletal morphology. This finding was singular in a litter of 9. No similar finding was observed in other dams in this group. The finding was therefore considered unrelated to treatment. No other external abnormalities were observed.

On internal skeletal examination, one foetus (referred to above) showed clear signs of structural abnormality. Subsequent investigation showed further evidence of deformation, including narrow skull, fused and pointed lower jaw, dysgenic pectoral and pelvic girdles. Except for this singular find there were no further occurrences of abnormality or any significant changes in the frequencies of minor anomalies to indicate any effect of treatment on skeletal morphogenesis.

4.3 OTHER EFFECTS

Two females in Group 3 (200 mg/kg permethrin) died, on Days 16 and 17 of pregnancy. Both females were pregnant. One was devoured by cage-mates. The second showed no indication of cause of death.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 MATERIALS AND METHODS

Virgin female rats of the Wistar strain were examined daily by vaginal smear to establish oestrus cycles. On the day of pro-oestrus females were paired with males of the same strain and age. The day of mating, confirmed by the presence of sperm in the vagina, was designated Day 0 of pregnancy.

22/23 mated female rats were assigned to each of three study groups: Group 1, untreated environmental control; Group 2, corn oil vehicle control; Group 3, 200 mg/kg permethrin. Dosing by gavage occurred on Days 6-16 (inclusive) of pregnancy.

Females were weighed on Days 0, 6, 17 and 20, and observed daily for changes in behaviour and well-being. Animals which died during the study were examined internally to elucidate the cause of death. On Day 20 of pregnancy, females were killed by chloroform and were dissected. The numbers of corpora lutea, implantations, live foetuses, early deaths, late deaths and foetal abnormalities were recorded. Mothers were examined for any abnormal condition which may have contributed to impairment of foetal growth or development.

Section A6.8.1 **6.8.1(1) Teratogenicity Study – oral (rat)**

Annex Point IIA6.8.1

Key Study

Each foetus was assigned an order number in the uterus, weighed, sexed and examined for external evidence of abnormality. All foetuses were further examined, by one of three investigative procedures, allocation being randomly determined:

a) Open dissection, for examination of the organs of neck, thorax and abdomen.

b) Wilson's section technique for examination of serial sections, cut free-hand.

c) Staples and Schnell's Alizarin red S staining method, for examination of skeletal morphology.

Analysis of variance was carried out between groups for foetal characteristics.

Section A6.8.1 **6.8.1(1) Teratogenicity Study – oral (rat)**

Annex Point IIA6.8.1

Key Study

5.2 RESULTS AND DISCUSSION

There was no significant effect of treatment on maternal weight gain, behaviour or well-being. Two females in Group 3 (200 mg/kg permethrin) died, on Days 16 and 17 of pregnancy. Both females were pregnant. One was devoured by cage-mates. The second showed no indication of cause of death.

There were no significant differences between the groups with respect to the frequency distribution of implantations or corpora lutea/implantation ratios. There was no significant effect of treatment on numbers of live and normal foetuses. By implication this means that similarly there was no difference between groups regarding foetal deaths and abnormalities. Further analysis confirmed this. Chi-squared analysis of foetal deaths as proportions of implants and abnormalities as proportions of live implants showed no significant differences between the groups. There was no significant effect of treatment on litter size or foetal weight. There was no apparent effect of treatment on foetal sex ratio.

One foetus in Group 3 (200 mg/kg permethrin) showed major external abnormalities including brachymelia and tetradactyly. These abnormalities were accompanied by protruding tongue, mild cervical oedema and reduced body weight. This foetus was further examined for skeletal morphology. This finding was singular in a litter of 9. No similar finding was observed in other dams in this group. The finding was therefore considered unrelated to treatment. No other external abnormalities were observed.

On internal examination by open dissection and Wilson's technique, no major abnormalities or significant changes in the frequencies of minor anomalies were found to indicate any effect of treatment. On internal skeletal examination, one foetus (referred to above) showed clear signs of structural abnormality. Subsequent investigation showed further evidence of deformation, including narrow skull, fused and pointed lower jaw, dysgenic pectoral and pelvic girdles. Except for this singular find there were no further occurrences of abnormality or any significant changes in the frequencies of minor anomalies to indicate any effect of treatment on skeletal morphogenesis.

5.3 CONCLUSION

The considerable dose presently reported produced no effect on maternal body weight or well-being throughout pregnancy and no effect on the numbers of live foetuses, foetal deaths or abnormalities. Examination of foetuses did not reveal any significant abnormalities attributable to treatment. No foetal abnormalities are likely to be produced by doses up to the equivalent of 200 mg/kg in the rat.

5.3.1 LO(A)EL maternal toxic effects

> 200 mg/kg

5.3.2 NO(A)EL maternal toxic effects

200 mg/kg

Section A6.8.1 **6.8.1(1) Teratogenicity Study – oral (rat)**

Annex Point IIA6.8.1

	Key Study
5.3.3 LO(A)EL embryotoxic / teratogenic effects	> 200 mg/kg
5.3.4 NO(A)EL embryotoxic / teratogenic effects	200 mg/kg
5.3.5 Reliability	2
5.3.6 Deficiencies	Yes; the study is a limit test at 200 mg/kg, whereas guideline requirements are for a limit test at 1000 mg/kg, however, such a high dose would significantly exceed the oral LD_{50} value for permethrin in corn oil of 480 mg/kg.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*8/12/05*
Materials and Methods	***2.3*** *Administration of a single dose only.* ***3.4.4*** *In addition, early deaths and late deaths were recorded separately.*
Results and discussion	*Adopt applicant's version.*
Conclusion	*Adopt applicant's version.*
Reliability	*3*
Acceptability	*Acceptable.*
Remarks	*It is highly irregular to conduct a study with only one treatment group (other than if it is a limit dose test). As the LD50 for permethrin is 480 mg/kg bw, the limit dose test (at 1000mg/kg bw) would not be practical in this case. However, given the age of the study, and taking into account that it was not formally complying with any guidelines, we can view it in a different way. As pointed out by the applicant, 200 mg/kg bw is a considerable dose and it produced no effect on maternal body weight or well-being throughout pregnancy and no effect on the numbers of live foetuses, foetal deaths or abnormalities. Examination of foetuses did not reveal any significant abnormalities attributable to treatment either. Therefore, all we can conclude from this study is that both the NOAEL and the LOAEL exceed 200 mg/kg bw. The data requirements state that where only one study is submitted, the preferred species is the rabbit. Therefore, the rabbit study can be the definitive and this can be of supplementary value.*
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A6.8.1 **6.8.1(1) Teratogenicity Study – oral (rat)**

Annex Point IIA6.8.1

	Key Study
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_8(1)-1. Table for Teratogenic effects (separate data for all dosage groups)

Maternal effects

Modify if necessary and give historical data if available

Maternal and Foetal Analysis at Caesarian section on day 20 of Pregnancy in Rats
Dosed orally with 21273 on days 6 to 16 of Pregnancy.

		Group 1 Environmental Control			Group 2 Corn Oil Control			Group 3 200 mg/kg			Pooled Variance		
			%age	s.e.		%age	s.e.		%age	s.e.		1 v 2	2 v 3
MATERNAL INFORMATION	Number of dams mated	22			23			23					
	Number of deaths	0			0			2					
	Number pregnant at Caesarian	22			23			21					
	Mean weight at mating (g)*	197.05	100		193.91	100		198.81	100				
	Mean weight gain - dosing (g)	54.77	27.80	2.914	53.04	27.35	2.510	51.92	26.12	3.675	202.9	n.s	n.s
	Mean weight gain - pregnancy (g)	117.05	59.40	3.382	107.61	55.49	3.568	113.37	57.12	4.434	317.2	n.s	n.s
	Total number C.L.	234			237			239					
	Mean number C.L.	10.64		0.358	10.30		0.285	11.38		0.554	**	n.s	n.s

* Used as base for subsequent % calculations. ** variances unequal.
C.L. = Corpora lutea.

Table A6_8(1)-2. Table for Teratogenic effects (separate data for all dosage groups)

Litter response (Caesarean section data)

Modify if necessary and give historical data if available

Maternal and Foetal Analysis at Caesarian section on day 20 of Pregnancy in Rats
Dosed orally with 21273 on days 6 to 16 of Pregnancy.

		Group 1 Environmental Control			Group 2 Corn Oil Control			Group 3 200 mg/kg			Pooled Variance		
			%age	s.e.		%age	s.e.		%age	s.e.		1 v 2	2 v 3
	Mean number of implantations	10.05		0.311	9.26		0.463	10.14		0.444	3.75	n.s	n.s
	Total number of implantations*	221	100		213	100		213	100				
FOETAL INFORMATION	Total number dead	0	0		0	0		0	0				
	Total number early deaths	7	3.17		7	3.29		6	2.82				
	Total number late deaths	0	0		0	0		0	0				
	Foetal loss	7	3.17		7	3.29		6	2.82				
	Total number live foetuses*	214	96.83		206	96.71		207	97.18				
LITTER INFORMATION (live foetuses)	Total number normal	214	100		206	100		206	99.52				
	Total number malformed	0	0		0	0		1	0.47				
	Mean number live	9.73		0.362	8.96		0.455	9.86		0.438	3.90	n.s	n.s
	Mean number normal	9.73		0.362	8.96		0.455	9.81		0.445			
	Mean number malformed	0			0			0.05					
	Mean weight litter (g)	35.82		1.466	33.41		1.711	37.37		1.760	59.92	n.s	n.s
	Mean weight normal foetus (g)	3.68		0.055	3.75		0.055	3.81		0.045	0.06	n.s	n.s

* Used as base for subsequent % calculations. ** variances unequal.
C.L. = Corpora lutea.