Section A6.3.3 **6.3.3 Repeated dose toxicity (Inhalation)**

Annex Point IIA6.3

Key Study

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24/11/05*
Materials and Methods	***3.1*** *Permethrin tech is the stated TS, however no code number is given to correspond to that which is described in Section 2.* ***3.1.2*** *What exactly does 'specification' refer to?* ***3.4.5*** *No reference is made to an ophthalmoscopic examination being done in the report.* ***4.4*** *No specific mention is made to any ophthalmoscopic examination results. Not a requirement of such a study, therefore of no particular consequence.* *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 or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	LO(A)EL: 42.2 mg m^{-3} NO(A)EL: 6.1 mg m^{-3} Other conclusions: *Adopt applicant's version*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ... ***(specify)***
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 6.3.3_3.3.4.2 Respirability of chamber aerosol

		% of total permethrin deposited on each stage				
		Stage 1	Stage 2	Stage 3	Stage 4	Filter
Exposure level[1]		5.9 µm	1.7 µm	0.65 µm	0.45 µm	<0.45 µm
6.1 mg m^{-3}	Mean[2]	0.0	23.19	64.81	12.00	0.0
	SD	0.0	4.362	5.350	5.778	0.0
42.2 mg m^{-3}	Mean[1]	0.76	13.37	60.37	25.44	0.05
	SD	1.674	2.703	7.463	7.273	0.183
583 mg m^{-3}	Mean[1]	1.46	27.18	58.51	12.86	0.0
	SD	0.751	5.249	5.694	2.463	0.0

1 Control animals were not exposed to aerosol
2 Determined once per exposure period, 15 determinations

Table 6.3.3_4.5.1 Haematology

TABLE 6

Haematology - group mean values following 13 exposures

Group:	1	2	3	4
Compound:	Air control		Permethrin technical	
Concentration (mg/m^3):	-	6.1	42.2	583

(Bled 5 February 1980)

Group		PCV %	Hb g/100ml	RBC mill./cmm	MCHC %	MCV cμ	MCH μμg	WBC 1000/cmm						Platelets 1000/cmm	Thrombotest secs.
								Total	N	L	E	B	M		
1♂	Mean	47.4	14.26	7.14	30.2	66.3	19.99	10.24	1.62	8.58	0.02	0.00	0.02	702.0	24.2
	SD.	3.8	0.57	0.35	1.4	3.1	0.75	1.99	0.68	1.55	0.05	0.00	0.03	77.9	2.3
2♂	Mean	45.8	13.58*	6.74	29.7	68.0	20.16	10.68	1.87	8.74	0.07	0.00	0.00	730.0	24.2
	SD	1.5	0.19	0.21	0.6	2.1	0.47	2.69	0.99	1.78	0.11	0.00	0.00	36.7	2.5
3♂	Mean	46.8	14.02	7.00	30.0	66.9	20.06	14.02*	1.90	12.07*	0.02	0.00	0.03	482.0*	25.7
	SD	1.9	0.34	0.36	0.8	2.2	0.77	3.00	0.32	3.25	0.04	0.00	0.07	120.5	2.1
4♂	Mean	46.2	13.60*	6.84	29.5	67.7	19.90	10.98	1.37	9.57	0.05	0.00	0.00	648.0	30.0**
	SD	1.3	0.40	0.30	1.1	3.6	0.56	1.70	0.13	1.65	0.10	0.00	0.00	210.2	3.8

Group		PCV %	Hb g/100ml	RBC mill./cmm	MCHC %	MCV cμ	MCH μμg	WBC 1000/cmm						Platelets 1000/cmm	Thrombotest secs.
								Total	N	L	E	B	M		
1♀	Mean	46.2	13.58	6.76	29.4	68.5	20.13	12.12	1.16	10.88	0.08	0.00	0.00	740.0	21.6
	SD	2.3	0.71	0.52	0.4	3.7	0.87	2.23	0.51	2.35	0.12	0.00	0.00	114.7	1.9
2♀	Mean	47.2	13.84	6.80	29.3	69.4	20.36	12.46	1.13	11.30	0.03	0.00	0.00	744.0	19.8
	SD	0.8	0.18	0.19	0.4	1.9	0.35	2.60	0.45	2.78	0.06	0.00	0.00	15.2	1.7
3♀	Mean	46.0	13.04	6.42	28.4	71.8	20.36	11.32	1.11	10.12	0.08	0.00	0.00	694.0	19.0*
	SD	3.4	0.59	0.47	1.5	4.5	0.98	1.55	0.37	1.80	0.08	0.00	0.00	77.0	1.0
4♀	Mean	46.0	13.96	6.78	30.3	67.9	20.60	10.54	1.16	9.28	0.09	0.00	0.00	720.0	19.4*
	SD	1.6	0.66	0.38	0.5	1.9	0.60	2.37	0.48	2.28	0.13	0.00	0.00	112.5	1.1

Notes:

SD = Standard deviation

* Significantly different from Group 1 (Control) at P <0.05
** Significantly different from Group 1 (Control) at P <0.01

Table 6.3.3_4.5.2 Clinical chemistry

TABLE 7

Blood chemistry - group mean values following 13 exposures

Group:	1	2	3	4
Compound:	Air control		Permethrin technical	
Concentration (mg/m³):	-	6.1	42.2	583

(Bled 5 February 1980)

Group		Urea mg%	Glucose mg%	Serum Protein g% Total	Serum Protein g% Alb.	A/G ratio	SAP mU/ml	SGPT mU/ml	Na mEq /l	K mEq /l	Ca mEq /l	Cl mEq /l	P mEq /l	Cholesterol mg%	Creatinine mg%
1♂	Mean	38.2	82.6	6.82	4.04	1.462	63.0	28.0	145.2	4.46	5.08	103.6	4.54	34.4	0.58
	SD	3.4	6.0	0.23	0.15	0.143	10.0	4.6	1.1	0.13	0.11	1.7	0.26	5.6	0.8
2♂	Mean	38.8	77.2	6.72	3.92	1.404	70.8	27.4	143.8	4.66	5.10	102.6	4.72	38.0	0.58
	SD	7.9	5.1	0.16	0.04	0.088	12.4	2.9	0.8	0.13	0.07	0.9	0.27	9.8	0.04
3♂	Mean	38.2	79.0	6.58	3.98	1.539	53.4	31.0	142.8***	4.64	5.12	103.0	4.84	42.8	0.58
	SD	5.6	5.3	0.23	0.08	0.131	13.4	5.5	1.3	0.25	0.08	1.6	0.34	9.3	0.04
4♂	Mean	38.8	65.6***	6.64	4.06	1.581	66.2	27.6	143.8	4.52	5.18	104.0	4.94*	44.6	0.64
	SD	7.9	4.9	0.09	0.15	0.153	3.2	3.2	1.3	0.24	0.08	1.6	0.26	12.0	0.05

Group		Urea mg%	Glucose mg%	Serum Protein g% Total	Serum Protein g% Alb.	A/G ratio	SAP mU/ml	SGPT mU/ml	Na mEq /l	K mEq /l	Ca mEq /l	Cl mEq /l	P mEq /l	Cholesterol mg%	Creatinine mg%
1♀	Mean	56.2	90.8	6.68	4.06	1.559	34.8	20.2	142.4	4.34	5.12	103.8	4.26	32.4	0.78
	SD	9.4	8.9	0.24	0.05	0.142	5.6	2.2	1.1	0.24	0.13	2.5	0.22	5.3	0.08
2♀	Mean	49.8	88.8	6.68	4.12	1.615	33.8	22.0	142.8	4.46	5.24	104.6	4.64	33.8	0.66*
	SD	9.9	5.0	0.08	0.15	0.136	4.4	2.8	2.0	0.25	0.18	2.2	0.34	6.3	0.05
3♀	Mean	60.8	77.2*	6.42	3.88*	1.545	29.4	19.6	140.6	4.30	5.00	102.0	4.32	31.0	0.78
	SD	13.9	9.6	0.33	0.04	0.178	5.5	2.6	1.1	0.19	0.12	0.7	0.41	3.0	0.08
4♀	Mean	43.2	75.4**	6.70	3.96	1.450	37.8	22.8	141.6	4.00*	4.92	101.2*	4.32	34.0	0.66*
	SD	5.1	7.6	0.23	0.15	0.110	7.9	1.8	3.6	0.16	0.08	1.6	0.16	6.6	0.09

Notes:

SD = Standard deviation

* Significantly different from Group 1 (Control) at P <0.05
** Significantly different from Group 1 (Control) at P <0.01
*** Significantly different from Group 1 (Control) at P <0.001

Table 6.3.3_4.5.3 Urinalysis

TABLE 8

Urinalysis - group mean values following 13 exposures

Group: 1 2 3 4

Compound: Air control Permethrin technical

Concentration (mg/m³): - 6.1 42.2 583

Week 4

Group		Volume mls	pH	SG	Protein mg/100 ml
1d	Mean	3.52	6.40	1047	42.0
	SD	1.08	0.34	4.4	31.1
2d	Mean	4.12	6.52	1046	70.0
	SD	1.50	0.20	9.2	33.2
3d	Mean	2.72	6.24	1055	58.0
	SD	0.58	0.15	4.7	25.9
4d	Mean	4.32	6.34	1046	94.0**
	SD	1.26	0.24	3.0	8.9

Week 4

Group		Volume mls	pH	SG	Protein mg/100 ml
1♀	Mean	3.24	6.12	1048	0.0
	SD	1.14	0.16	7.4	0.0
2♀	Mean	2.68	6.42	1048	0.0
	SD	1.23	0.33	7.7	0.0
3♀	Mean	2.96	6.30	1045	0.0
	SD	0.96	0.23	4.6	0.0
4♀	Mean	1.92	5.98	1058*	0.0
	SD	0.54	0.36	3.6	0.0

Notes:

SD = Standard deviation

* Significantly different from Group 1 (Control) at P <0.05

** Significantly different from Group 1 (Control) at P <0.01

Table 6.3.3_4.6.1 Organ weights

TABLE 9

Organ weights - group mean values

Group: 1 2 3 4

Compound: Air control Permethrin technical

Concentration (mg/m³): - 6.1 42.2 583

Group	Group size	Body-weight (g)	Brain (g)	Pituitary (mg)	Heart (g)	Lungs (g)	Liver (g)	Spleen (g)	Prostate (g)	Kidney (g)	Thyroid (mg)	Adrenals (mg)	Gonads ♂ (g)	Gonads ♀ (mg)	Uterus (g)	Thymus (mg)
Means adjusted					2 A		A			A						A
1♂	5	287.6	1.78	14.4	1.11 (1.08)	1.32	12.6 (12.1)	0.70	0.37	2.22 (2.18)	20.2	56.0	3.76	-	-	0.50 (0.47)
2♂	5	310.4	1.74	13.8	1.09 (1.12)	1.40	13.5 (14.2)	0.72	0.38	2.41 (2.46)	20.2	49.4	3.68	-	-	0.52 (0.55)
3♂	4/5[1]	295.2	1.78	11.4	1.07 (1.06)	1.36	12.1 (12.0)	0.70	0.39	2.25 (2.24)	18.2	38.9	3.65	-	-	0.47 (0.46)
4♂	5	297.6	1.72	9.8**	1.14 (1.14)	1.26	14.1** (14.1)	0.62	0.32	2.28 (2.28)	16.6	47.2	3.68	-	-	0.47 (0.47)
Residual variance		469.15	0.006	5.50	0.012 (0.014)	0.036	0.55 (1.62)	0.024	0.014	0.035 (0.040)	29.98	173.38	0.056	-	-	0.006 (0.009)

Notes:

1 See Appendix 7
2 Where organ weight has been adjusted for the bodyweight at necropsy, A, the unadjusted mean and residual variance are given in brackets.

Significance level in comparison with Group 1 (Control): ** P <0.01

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Table 6.3.3_4.6.1 Organ weights (cont'd)

TABLE 9

(continued)

Group	Group size	Body-weight (g)	Brain (g)	Pitui-tary (mg)	Heart (g)	Lungs (g)	Liver (g)	Spleen (g)	Prost-tate (g)	Kid-ney (g)	Thy-roid (mg)	Adre-nals (mg)	Gonads		Uterus (g)	Thymus (mg)
													♂ (g)	♀ (mg)		
Means adjusted			A[2]			A	A			A						
1♀	5	208.8	1.74 (1.74)	13.8	0.90	1.17 (1.18)	8.91 (8.96)	0.58	-	1.65 (1.66)	16.2	61.0	-	84.6	0.52	0.44
2♀	4/5[1]	211.2	1.73 (1.72)	12.8	0.78(*)	1.17 (1.20)	8.63 (8.78)	0.58	-	1.60 (1.62)	17.5	70.2	-	85.8	0.45	0.39
3♀	5	208.0	1.72 (1.72)	13.8	0.82	1.02(*) (1.02)	8.15 (8.16)	0.62	-	1.64 (1.64)	17.2	67.2	-	90.0	0.62	0.40
4♀	5	202.6	1.67 (1.68)	16.4	0.74*	1.12 (1.08)	9.88* (9.66)	0.54	-	1.65 (1.62)	18.4	75.4*	-	91.0	0.58	0.42
Residual variance		132.68	0.0053 (0.0060)	5.35	0.008	0.009 (0.018)	0.376 (0.593)	0.007		0.012 (0.015)	29.32	87.18	-	110.13	0.024	0.006

Notes:

1 See Appendix 7
2 Where organ weight has been adjusted for the bodyweight at necropsy, A, the unadjusted mean and residual variance are given in brackets.

Significance level in comparison with Group 1 (Control) * P <0.05

Using method of LSD but not confirmed by Williams' test: (*) P <0.05

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Section A6.4.1 — 6.4.1(1) Subchronic oral toxicity test – Rat

Annex Point IIA6.4.1

Key Study

			Official use only
		1 REFERENCE	
1.1	Reference	[redacted]; 1976; 21Z73, Rat Oral 90 Day Study; [redacted]; unpublished Report No. HEFG 76-1; 25.02.1976.	
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	
		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 WP	
3.1.2	Specification	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	Permethrin was found to be stable in diet for more than 30 days; food in the hoppers in the animal cages was changed twice weekly; 10% w/v dietary premix was prepared every 2-3 weeks	
3.2	Test Animals	Non-entry field	
3.2.1	Species	Rat	
3.2.2	Strain	Wistar	
3.2.3	Source	[redacted]	

Section A6.4.1 **6.4.1(1) Subchronic oral toxicity test – Rat**

Annex Point IIA6.4.1

		Key Study	
3.2.4	**Sex**	♂ and ♀	
3.2.5	**Age/weight at study initiation**	64-166g	
3.2.6	**Number of animals per group**	20 per treatment group, 16 per recovery group	X
3.2.7	**Control animals**	Yes	
3.3	**Administration/ Exposure**	Oral/Inhalation/dermal/intraperitoneal/intravenous/intratracheal	X
3.3.1	**Duration of treatment**	90 days	
3.3.2	**Frequency of exposure**	daily	
3.3.3	**Postexposure period**	36 days	
3.3.4	**Oral**		
3.3.4.1	**Type**	in food	
3.3.4.2	**Concentration**	food 0, 200, 600, 2000 and 4000 ppm food 0, 17.0, 49.9, 179.6 and 357.4 mg/kg bw ♂ food 0, 18.5, 56.2, 176.5 and 356.7 mg/kg bw ♀ food consumption per day ad libitum	
3.3.4.3	**Vehicle**	not applicable	
3.3.4.4	**Concentration in vehicle**	not applicable	
3.3.4.5	**Total volume applied**	not applicable	
3.3.4.6	**Controls**	plain diet	
3.4	**Examinations**		
3.4.1	**Observations**		
3.4.1.1	**Clinical signs**	Yes; daily.	
3.4.1.2	**Mortality**	Yes; daily.	
3.4.2	**Body weight**	Yes; weekly.	
3.4.3	**Food consumption**	Yes; weekly.	
3.4.4	**Water consumption**	No; some problems in the watering system were experienced at the start of the trial.	

Section A6.4.1 **6.4.1(1) Subchronic oral toxicity test – Rat**

Annex Point IIA6.4.1

		Key Study
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	Yes number of animals: 6 animals of each sex/group, except at 90 days when an additional 10 animals of each sex/group were examined time points: 14, 28, 56, 90 days (representing the dosing phase) and 126 days (36 day recovery phase) Parameters: packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total white cell (WBC) and differential counts were determined.
3.4.7	Clinical Chemistry	Yes number of animals: 6 animals of each sex/group, except at 90 days when an additional 10 animals of each sex/group were examined time points: 14, 28, 56, 90 days (representing the dosing phase) and 126 days (36 day recovery phase) Parameters: fasting serum glucose (GLUC), urea nitrogen (UN), alkaline phosphatase (AP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total protein (TP), albumen (ALB), sodium (Na) and potassium (K).
3.4.8	Urinalysis	Yes number of animals: composite urine samples time points: collected overnight under conditions of food and water deprivation on days 90 (representing the dosing phase) and 126 (36 day recovery phase) Parameters: protein, pH, glucose, ketones, blood, osmolarity.
3.5	Sacrifice and pathology	
3.5.1	Organ Weights	Yes organs: adrenals, brain, heart, kidney, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroids, uterus

Section A6.4.1 **6.4.1(1) Subchronic oral toxicity test – Rat**

Annex Point IIA6.4.1

Key Study

3.5.2	**Gross and histopathology**	Yes high dose group and controls (10 ♂, 10 ♀), other dose groups only if effects organs: adrenals, aorta, bladder (urinary), bone marrow, brain, colon, duodenum, eyes, heart, kidney, liver, lungs, lymph node (mesenteric), oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, small intestine, spleen, stomach, testes, thymus, thyroids, tongue, uterus (other tissues preserved: bone, caecum, epididymis, ileum, jejunum, mammary tissue, skin, trachea, vagina)
3.5.3	**Other examinations**	Oestrus cycle
3.5.4	**Statistics**	Standard (e.g. bodyweights, 't' test; oestrus cycle, 't' test)
3.6	**Further remarks**	The average food conversion rate was calculated weekly.

4 RESULTS AND DISCUSSION

4.1	**Observations**	
4.1.1	**Clinical signs**	Symptoms comprising hypersensitivity were seen in the ♂ and ♀ rats given 4000 ppm permethrin. No adverse effects were seen in the other groups.
4.1.2	**Mortality**	No dose-associated deaths occurred.
4.2	**Body weight gain**	Only the ♂ rats of the 4000 ppm group showed a reduction in bodyweight gain during the dosing period. No other adverse effects were detected.
4.3	**Food consumption and compound intake**	No important differences in food intake between dosed and control groups were detected. The average compound intake during the trial was:

Permethrin ppm	mg/kg/day	
	♂	♀
200	17.0	18.5
600	49.9	56.2
2000	179.6	176.5
4000	357.4	356.7

4.4	**Ophthalmoscopic examination**	Not applicable
4.5	**Blood analysis**	
4.5.1	**Haematology**	No important differences between dosed and control groups were detected. There was a slight transient leucopoenia in the early stages of the trial in the 4000 ppm group.

Section A6.4.1 **6.4.1(1) Subchronic oral toxicity test – Rat**

Annex Point IIA6.4.1

Key Study

4.5.2	**Clinical chemistry**	No important differences between dosed and control groups were detected at any dose.
4.5.3	**Urinalysis**	No important differences between dosed and control groups were detected at any dose.
4.6	**Sacrifice and pathology**	
4.6.1	**Organ weights**	No consistent dose-related changes were detected in absolute and relative weights of the majority of organs. The liver weights of ♂ and ♀ rats of the 4000 ppm group showed a slight but significant increase at 90 days which had disappeared by the end of the recovery period (126 days).
4.6.2	**Gross and histopathology**	No dose-associated changes were detected post mortem and no important dose-associated changes were detected in the histopathology of the 4000 ppm group.
4.7	**Other**	No important differences in food conversion between dosed and control groups were detected. No important differences in the lengths of the oestrus cycle detected between dosed and control groups.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of 36 (18♂ and 18♀/group) weanling rats were offered diets containing 0, 200, 600, 2000 and 4000 ppm permethrin for 90 consecutive days. At 90 days groups of 20 (10♂ and 10♀/group) rats were killed and subjected to full post mortem procedures, whilst the surviving animals were offered untreated diet for a further 36 days, this being the 'recovery' phase. During the trial animals were observed for signs of toxicity and bodyweights, food and compound intake were recorded weekly and sets of laboratory investigations including haematology and blood chemistry were performed on samples taken at 14, 28, 56, 90 and 126 days. At 90 and 126 days groups of animals were killed, post mortem and histopathological examinations performed and organ weights recorded.

Section A6.4.1 **6.4.1(1) Subchronic oral toxicity test – Rat**

Annex Point IIA6.4.1

Key Study

5.2 Results and discussion

Mortalities

No dose-associated deaths occurred.

Clinical toxicity

Symptoms comprising hypersensitivity were seen in the ♂ and ♀ rats given 4000 ppm Permethrin. No adverse effects were seen in the other groups.

Bodyweights

Only the ♂ rats of the 4000 ppm group showed a reduction in bodyweight gain during the dosing period. No other adverse effects were detected.

Food consumption

No important differences in food intake between dosed and control groups were detected.

Compound intake

The average compound intake during the trial was:

Permethrin ppm	mg/kg/day	
	♂	♀
200	17.0	18.5
600	49.9	56.2
2000	179.6	176.5
4000	357.4	356.7

Food conversion

No important differences in food conversion between dosed and control groups were detected.

Oestrus cycle

No important differences in the lengths of the oestrus cycle detected between dosed and control groups.

Haematology

No important differences between dosed and control groups were detected. There was a slight transient leucopoenia in the early stages of the trial in the 4000 ppm group.

Blood chemistry

No important differences between dosed and control groups were detected at any dose.

Urine analysis

No important differences between dosed and control groups were detected at any dose.

Post mortem findings

No dose-associated changes were detected.

Organ weights

No consistent dose-related changes were detected in absolute and relative weights of the majority of organs. The liver weights of ♂ and ♀ rats of the 4000 ppm group showed a slight but significant increase at 90 days which had

Section A6.4.1 **6.4.1(1) Subchronic oral toxicity test – Rat**

Annex Point IIA6.4.1

Key Study

5.3	**Conclusion**	Non-entry field
5.3.1	**LO(A)EL**	4000 ppm ≡ 355 mg/kg bw/day, based on hypersensitivity, slight transient leucopoenia and slight but significant increase in liver weight in ♂ and ♀, and reduction in bodyweight gain in ♂.
5.3.2	**NO(A)EL**	2000 ppm ≡ 175 mg/kg bw/day
5.3.3	**Other**	There were no histopathological changes found that could be related to the administration of high doses of permethrin in the diet.
5.3.4	**Reliability**	2
5.3.5	**Deficiencies**	Yes. No ophthalmoscopy was conducted as required by test guideline EC B. 26, however, no dose-associated changes were detected post mortem and no important dose-associated changes were detected in the histopathology of the 4000 ppm group. No specific functional observations were conducted as required by test guideline EC B. 26, however, clinical symptoms comprising hypersensitivity were seen in the ♂ and ♀ rats given 4000 ppm Permethrin, whilst no adverse effects were seen in the other groups. Some problems in the watering system were experienced at the start of the trial and the effects were reflected in reduced bodyweights of the ♀ 200 ppm, ♂ 2000 ppm and ♂ 4000 ppm groups. The adverse effects of the watering failure on these groups was resolved by about Day 14 of the trial.

Section A6.4.1 **6.4.1(1) Subchronic oral toxicity test – Rat**

Annex Point IIA6.4.1

Key Study

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29th April 2009
Materials and Methods	3.2.6 The notifier states that 20 animals per group plus a recovery group of 16 were used at each dose. However, the study states group sizes of 24 (12 male and 12 female) and 12 (6 male and 6 female) for used for treatment and recovery groups respectively. 3.3 The route of administration was oral via diet.
Results and discussion	Adopt applicant's version
Conclusion	LO(A)EL: 4000 ppm approximatelly 356 mg/kg bw/d. NO(A)EL: 2000 ppm approximatelly 175 mg/kg bw/d. Other conclusions:
Reliability	*2*
Acceptability	Acceptable
Remarks	The study broadly complies with OECD 408. However, it is lacking in information regarding the stability of the substance in the feed. A statement is made "that feed was made up every 2-3 weeks" and that "it was stable for more than 30 days". However this information is not backed up with analytical data. Information regarding the identity and purity of the substance is scant in the study. However, the ID code is referenced in the substance ID section A2. No ophthalmoscopy was conducted as required by test guideline.
	COMMENTS FROM ... *(specify)*
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 A6.4.1	6.4.1(1) Subchronic oral toxicity test – Rat
Annex Point IIA6.4.1	

	Key Study
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_4(1)-1. Results of haematology (white cell parameters)

Day of trial	Sex	14** days			28** days			56** days			90** days			120** days			90 day ** Terminal		
21273 ppm in diet		WBC	L	N	WBC	L	N	WBC	L	N	WBC	L	N	WBC	L	N	WBC	L	N
200	♂	D+ ns	-	-	-	-	-	-	D+ ns	I+ ns	-	-	-	-	-	-		-	D+
	♀	D+ s	-	-	D+ ns	-	-	-	-	-	-	I+ ns	D+ ns	-	-	-	-	-	-
600	♂	D+ ns	-	-	D+ s	D+ s	D+ ns	-	D+ ns	D ns	-	-	-	-	-	I+ ns	-	-	-
	♀	D++ s	-	I+ ns	D+ s	-	-	D+ ns	-	-	D+ ns	I+	-	D++ s	D+ s	-	-	-	-
2000	♂	-	I+ ns	-	D++ s	D+ s	D ns	I+ ns	D+ ns	I+ ns	-	-	-	-	-	-	-	-	D+ ns
	♀	-	D++ s	I++ s	-	D+ ns	I+ ns	-	-	-	-	-	-	D+ ns	D+ ns	-	-	-	-
4000	♂	D+ ns	-	-	D+ s	D+ ns	D+ ns	I+ ns	D+ s	I++ s	-	-	-	-	-	-	-	I+ ns	D+ s
	♀	D++ ns	D+ s	I++ s	D+ ns	-	I+ ns	-	I+ s	D+ ns	D+ ns	-	D+ ns	I+ ns	-	I+	-	-	-

Key: D = decrease
I = increase
\+ = slight
++ = moderate
\- = result similar to control
s = significant p<0.05

ns = non significant p>0.05
* = 6♂ + 6♀/group
** = 10♂ + 10♀/group
WBC = white blood cell
L = lymphocytes
N = neutrophils

Table A6_4(1)-2. Results *(clinical signs, body weight, white cell count, liver weight)* of repeated dose toxicity study

Parameter	Control		low dose		medium doses		high dose		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	10	10	10	10	10	10	10	10		
Mortality										
clinical signs* hypersensitivity	0/10	0/10	0/10	0/10	0/10	0/10	10/10	10/10	+	+
body weight							↓		+	
food consumption										
clinical chemistry*										
haematology* white cell count							↓	↓	+	+
urinalysis*										
Liver organ weight*							↑	↑	+	+
gross pathology*										
microscopic pathology*										
Organ y										

** specify effects; for different organs give special findings in the order organ weight, gross pathology and microscopic pathology if there are effects*

[a] ***give number of animals affected/total number of animals, percentage, or just ↑ or ↓ for increased or decreased***

Section A6.4.1
Annex Point IIA6.4.1

6.4.1(2) Subchronic oral toxicity test – dog

	Key Study	Official use only
	1 REFERENCE	
1.1 Reference	[redacted]; 1978; Permethrin Oral Administration to Dogs for 6 Months; [redacted]; unpublished Report No. HEFG 78-14; 01.12.1978.	
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	X
3.1.1 Lot/Batch number	Batch ZJ	
3.1.2 Specification	*As given in section 2*	X
3.1.2.1 Description	Liquid	
3.1.2.2 Purity	94.5%	
3.1.2.3 Stability	As given in section 2	X
3.2 Test Animals		
3.2.1 Species	Dog	
3.2.2 Strain	Beagle	
3.2.3 Source	[redacted]	
3.2.4 Sex	♂ and ♀	
3.2.5 AGE/WEIGHT AT STUDY INITIATION	20-22 weeks	
3.2.6 Number of animals per group	8	
3.2.7 Control animals	Yes	
3.3 Administration/ Exposure	*Oral*	

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(2) Subchronic oral toxicity test – dog**	
	Key Study	
3.3.1 Duration of treatment	180 days	
3.3.2 Frequency of exposure	daily	
3.3.3 Postexposure period	not applicable	
3.3.4 Oral		
3.3.4.1 Type	capsule (gelatin)	
3.3.4.2 Concentration	capsule 0, 10, 50 and 250 mg/kg bw	
3.3.4.3 Vehicle	not applicable	
3.3.4.4 Concentration in vehicle	not applicable	
3.3.4.5 Total volume applied	not applicable	
3.3.4.6 Controls	not reported (incidence of vomiting suggests empty capsule)	X
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes; daily.	
3.4.1.2 Mortality	Yes; daily.	
3.4.2 Body weight	Yes; twice weekly.	
3.4.3 Food consumption	Yes; daily, excluding weekends and Bank holidays when all animals were fed approximately 400 g of fresh diet/day but the residue was not weighed.	
3.4.4 WATER CONSUMPTION	No	
3.4.5 Ophthalmoscopic examination	Yes; days -6, 28, 91 and 173.	
3.4.6 Haematology	Yes number of animals: all animals time points: days -14, -7, 0, 14, 56, 112 and 180 Parameters: packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), differential white blood cell count, prothrombin.	

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(2) Subchronic oral toxicity test – dog**
	Key Study
3.4.7 Clinical Chemistry	Yes number of animals: all animals time points: days -14, -7, 0, 14, 56, 112 and 180 Parameters: glucose, urea, sodium (Na^+), potassium (K^+), bilirubin (BILI), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (AP), creatine phosphokinase (CPK), total protein, albumin, α-, β_1-, β_2-, and γ-globulin.
3.4.8 Urinalysis	Yes number of animals: all animals time points: end of study Parameters: nitrite, pH, blood, glucose, ketones, urobilinogen, bilirubin, protein, appearance.
3.5 SACRIFICE AND PATHOLOGY	
3.5.1 ORGAN WEIGHTS	Yes organs: brain, liver, pituitary, spleen, heart, lungs, adrenals, testis, ovaries, thyroids, kidneys
3.5.2 Gross and histopathology	Yes **all dose groups; for nerve and muscle tissue, high dose group and controls, other dose groups only if effects** organs: pituitary, thyroid, heart, lungs, pyloric stomach, duodenum, colon, mesenteric lymph node, liver, spleen, pancreas, kidney, adrenal, urinary bladder, prostate, uterus, testis, ovaries, bone, gall bladder, tongue, salivary gland, thymus, trachea, abdominal skin, mammary gland, aortic arch, oesophagus, jejunum, ileum, caecum, skeletal muscle, costochondral junction, sternum, parathyroid, epididymis, vagina, cervical lymph node, ureter, oviduct, eyes, brain, trigeminal ganglia, dorsal root ganglia, posterior thigh muscle, lumbrical muscle and the following nerves - sciatic, ulnar, radial, posterior tibial, superficial fibular and plantar
3.5.3 OTHER EXAMINATIONS	
3.5.4 Statistics	Standard
3.6 Further remarks	<u>Electrocardiography</u> Yes number of animals: all animals time points: days -35, 91 and 174 <u>Plasma antipyrine determinations</u> Yes number of animals: all animals time points: end of study

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(2) Subchronic oral toxicity test – dog**	
	Key Study	
	4 RESULTS AND DISCUSSION	
4.1 Observations		
4.1.1 Clinical signs	Isolated cases of vomiting were seen in a few dose and control animals and one animal was treated for demodectic mange. No dose related signs of clinical toxicity were seen.	
4.1.2 Mortality	No mortalities at any dose.	
4.2 Body weight gain	No statistically significant changes were seen in the dosed groups compared with the controls.	
4.3 Food consumption and compound intake	Statistically significant changes in food consumption occurred on isolated occasions in the 50 mg/kg and 250 mg/kg groups but these were not considered to be of toxicological importance.	
4.4 Ophthalmoscopic examination	No toxicologically important changes were seen.	
4.5 Blood analysis		
4.5.1 Haematology	Statistically significant changes were seen in the following parameters: PCV (10 mg/kg), MCV (10 mg/kg), lymphocytes (50 and 250 mg/kg), neutrophils (50 and 250 mg/kg), band neutrophils (50 and 250 mg/kg). None of these changes were considered to be of toxicological importance.	
4.5.2 Clinical chemistry	Statistically significant changes occurred in the following parameters during the dosing period; glucose (10 mg/kg), urea (10 mg/kg), sodium (250 mg/kg), potassium (10 mg/kg), bilirubin (50 and 250 mg/kg), total protein (10, 50 and 250 mg/kg), albumin (250 mg/kg), β_1-globulin (10 mg/kg) and β_2-globulin (250 mg/kg). None of these changes appeared to be dose- or time-related or were of sufficient magnitude to be of toxicological importance. Similar significant but minor changes were occasionally observed before the animals were dosed.	X
4.5.3 Urinalysis	The only difference between the dosed and control groups was a slight lowering of the pH in the dosed groups.	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	<u>Absolute organ weights</u> No statistically significant changes occurred between any of the dosed groups and the controls. <u>Relative organ weights</u> Statistically significant increases occurred in the heart weight for the 50 mg/kg group, liver weight for the 50 and 250 mg/kg groups and kidneys in all dosed groups. The magnitude of these changes does not increase with the dose level and in every case is not more than 17% above the control value and are therefore not considered to be of toxicological significance.	X
4.6.2 Gross and histopathology	No changes were found in any of the dosed groups which could be considered to be caused by dosing with permethrin.	

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(2) Subchronic oral toxicity test – dog**	
	Key Study	
4.7 Other	Electrocardiography No toxicologically important changes were seen. Plasma antipyrine elimination A non-significant increase in the elimination rate of antipyrine was seen in the 50 and 250 mg/kg groups. This is probably due to inter-animal variation and is not considered to be important.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Groups of 8 Beagle dogs were given an oral dose of 10, 50 or 250 mg/kg permethrin (94.5% w/v, *cis:trans* 25:75) daily for 6 months. The animals were weighed twice weekly and the dose of compound calculated according to bodyweight. The required quantity of compound was weighed into size 000 gelatin capsules, and administered orally once daily. A similar group of animals were kept as controls. Toxicological examinations included clinical signs, mortality, bodyweight, food consumption, ophthalmoscopy, electrocardiography, haematology, clinical chemistry, urinalysis, plasma antipyrine elimination, organ weights, gross pathology and histopathology.	
5.2 Results and discussion	No toxicologically important changes were found in any of the following parameters: clinical signs, mortality, bodyweight, ophthalmoscopy, electrocardiography, plasma antipyrine elimination, absolute organ weights, gross pathology and histopathology. Statistically significant changes occurred in food intake (isolated occasions in the 50 mg/kg and 250 mg/kg groups), but these were not considered to be of toxicological importance. Statistically significant changes occurred in some haematological parameters (packed cell volume (10 mg/kg), mean corpuscular volume (10 mg/kg), lymphocytes (50 and 250 mg/kg), neutrophils (50 and 250 mg/kg), band neutrophils (50 and 250 mg/kg)), but none of the changes observed showed any time-related trends or were of sufficient magnitude to be of toxicological importance.	X
	Statistically significant changes also occurred in some clinical chemistry parameters (glucose (10 mg/kg), urea (10 mg/kg), sodium (250 mg/kg), potassium (10 mg/kg), bilirubin (50 and 250 mg/kg), total protein (10, 50 and 250 mg/kg), albumin (250 mg/kg), globulin fractions β_1 (10 mg/kg) and β_2 (250 mg/kg)), but none of these changes appeared to be dose- or time-related or were of sufficient magnitude to be of toxicological importance.	X
	Statistically significant changes occurred in relative organ weights for liver (50 and 250 mg/kg), heart (50 mg/kg) and kidneys (10, 50 and 250 mg/kg), but the magnitude of these changes does not increase with the dose level and in every case is not more than 17% above the control value. None of these changes were considered to be of toxicological importance.	

Section A6.4.1 **Annex Point** **IIA6.4.1**	**6.4.1(2) Subchronic oral toxicity test – dog**	
	Key Study	
5.3 Conclusion		
5.3.1 LO(A)EL	> 250 mg/kg (highest dose tested)	X
5.3.2 NO(A)EL	250 mg/kg (highest dose tested)	X
5.3.3 Other		
5.3.4 Reliability	2	
Section A6.4.1 **Annex Point** **IIA6.4.1**	**6.4.1(1) Repeated dose toxicity – oral (dog)** *Specify section no. and heading, route and species*	
5.3.5 Deficiencies	Yes; a few tissues were not found when the tissues were 'blocked' for histology or were damaged during histology preparation, however, no dose related abnormalities were seen in any of the examined tissues.	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24/11/05*
Materials and Methods	***3.1*** *Permethrin tech is the stated TS, however no code number is given to correspond to that which is described in Section 2.* ***3.1.2*** *What exactly does 'specification' refer to?* ***3.1.2.3*** *Stability is not given in Section 2.* ***3.3.4.6*** *What does this entry mean?* *Otherwise, the applicants version is acceptable.*
Results and discussion	***4.5.2*** *Bilirubin levels were decreased in the 10 mg/kg bw group also.* *Otherwise, adopt applicant's version.* ***4.6.1*** *The applicant considers that because the magnitude of the relative weight changes in liver, kidney and heart does not increase with the dose level and in every case is not more than 17% above the control value, that therefore these observations are not considered to be of toxicological significance. However, it cannot be ignored that data from other studies demonstrate that increased liver weight is a classic effect following permethrin administration and therefore changes such as these have toxicological significance.* ***5.2*** *As pointed out above, bilirubin levels were decreased in the 10 mg/kg bw group also.* *See comment at 4.6.1 above also.*
Conclusion	***5.3.1*** *LO(A)EL: 50mg/kg bw* ***5.3.2*** *NO(A)EL: 10 mg/kg bw*
Reliability	*2*
Acceptability	*Acceptable*

Remarks	*The authors of the report have omitted to include information on the 'Special Histopathological Examination of the Nervous System' which is to be found at the back of the report (P.81 – 88). In their results, they report that no evidence was found of damage to peripheral nerve fibres in proximal or distal trunks, or in motor and sensory endings, nor were lesions seen in the brain, spinal cord and trigeminal and dorsal root ganglia. This is useful information and should have been reported as part of the results in the main body of the text.* *In the data requirements, there is a stated requirement for studies to be **usually** conducted in 2 species, one rodent and one non-rodent. There are two 90 day rat studies submitted, as well as a 90 day, 6 month and 1 year study in the dog. As only the 6 month and 1 year **dog** studies are reported in the key study format, are we to presume that the applicant is making the case that the dog is the more sensitive species (which appears to be the case)? This should have been stated and explained by the applicant.*
	COMMENTS FROM ... *(specify)*
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 A6.4.1
Annex Point
IIA6.4.1

6.4.1(3) Subchronic oral toxicity test – oral (dog)

Key Study

Official use only

	1 REFERENCE
1.1 Reference	[redacted]; 1982; Permethrin: One Year Oral Dosing Study in Dogs; [redacted]; unpublished Report No. CTL/P/647; 24.02.1982.
1.2 Data protection	Yes
1.2.1 Data owner	Syngenta Crop Protection 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
	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
	MATERIALS AND METHODS
3.1 Test material	Permethrin
3.1.1 Lot/Batch number	Batch BX 108136E, Lots 8-12, 14-20.
3.1.2 Specification	Deviating from specification given in section 2 as follows
3.1.2.1 Description	Red-brown viscous liquid
3.1.2.2 Purity	92.5% (isomer ratio nominally 32.3% *cis*, 60.2% *trans*)
3.1.2.3 Stability	Permethrin concentration was within ± 10% of nominal concentration for the greater majority of solutions. Only four solutions gave values outside of this range, the maximum deviation being 16% of nominal concentration. Repeat analysis of the first batch of solutions showed that permethrin was chemically stable in corn oil for up to 10 weeks (seven days supply of capsules was prepared for each animal after its weekly weighing).
3.2 Test Animals	
3.2.1 Species	Dog
3.2.2 Strain	Beagle
3.2.3 Source	[redacted]
3.2.4 Sex	♂ and ♀
3.2.5 AGE/WEIGHT AT STUDY INITIATION	15-22 weeks

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(3) Subchronic oral toxicity test – oral (dog)**	
	Key Study	
3.2.6 Number of animals per group	12 (6 ♂ and 6 ♀)	
3.2.7 Control animals	Yes	
3.3 Administration/ Exposure	Oral	
3.3.1 Duration of treatment	52 weeks	
3.3.2 Frequency of exposure	daily	
3.3.3 Postexposure period	not applicable	
3.3.4 Oral		
3.3.4.1 Type	capsule (gelatine, 10 mL)	
3.3.4.2 Concentration	capsule 0, 5, 100 and 1000 (reduced from 2000 after 2 days) mg/kg bw	
3.3.4.3 Vehicle	corn oil	
3.3.4.4 Concentration in vehicle	5 mg/kg/day dose level: 10.8 g technical material made up to one litre with corn oil; 100 mg/kg/day dose level: 216.2 g technical material made up to one litre with corn oil; 1000 mg/kg/day dose level: 720.7 g technical material made up to one litre with corn oil.	
3.3.4.5 Total volume applied	0.5 mL/kg bw for the control, low and middle doses; 1.5 mL/kg bw for the top dose (3 mL/kg bw when the 2000 mg/kg bw dose level was used during first 2 days)	
3.3.4.6 Controls	0.5 mL/kg bw corn oil	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes; at least twice daily (in the morning and at the end of the working day). All dogs were given a full clinical examination by a veterinarian pre-experimentally and after 13, 26 and 39 weeks, and terminally (the examination included auscultation of the chest and ophthalmoscopy).	
3.4.1.2 Mortality	Yes; at least twice daily (in the morning and at the end of the working day).	
3.4.2 Body weight	Yes; weekly (all weighing was done before giving the main meal).	X
3.4.3 Food consumption	Yes; daily (prior to giving the next main meal).	
3.4.4 WATER CONSUMPTION	No	

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(3) Subchronic oral toxicity test – oral (dog)**
	Key Study
3.4.5 Ophthalmoscopic examination	Yes; pre-experimentally, after 13, 26 and 39 weeks, and terminally.
3.4.6 Haematology	Yes number of animals: all animals time points: pre-experimentally, weeks 4, 8, 12, 16, 20, 26, 39 and 52 (all samples were obtained prior to giving the main meal) Parameters: haemoglobin, haematocrit, red cell count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, total white cell count, platelet count, differential white cell count, kaolin-cephalin and prothrombin times. Bone marrow aspirates were obtained from all animals by iliac-crest puncture in week 26 and at termination and stained with a Romanowsky stain prior to examination.
3.4.7 Clinical Chemistry	Yes number of animals: all animals time points: pre-experimentally, weeks 4, 8, 12, 16, 20, 26, 39 and 52 (all samples were obtained prior to giving the main meal) Parameters: plasma urea, glucose, triglycerides, albumin, total protein, plasma cholesterol, calcium, plasma potassium, plasma alkaline phosphatase, alanine transaminase, aspartate transaminase, creatine kinase activities
3.4.8 Urinalysis	Yes number of animals: all animals time points: pre-experimentally, weeks 8, 16, 26, 39 and 50 (the collection period was approximately 18 hours; water, but not food, was available during the collection period) Parameters: glucose, ketones, bilirubin, urobilinogen, pH, specific gravity, protein. A microscopic examination of the centrifuged urine deposits from all animals was performed pre-experimentally and in weeks 8, 26 and 50 on the same samples taken for biochemical analysis. The samples were examined for the presence or absence of crystals and sperm; erythrocytes, leucocytes, squamous epithelial cells, small epithelial cells and casts were counted.
3.5 Sacrifice and pathology	
3.5.1 Organ weights	Yes (the left and right components of paired organs being weighed separately) organs: gonads, spleen, adrenals, kidneys, liver, thymus, heart, lungs (left and right combined with 15 tracheal rings attached), brain, pituitary, thyroids

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(3) Subchronic oral toxicity test – oral (dog)**
	Key Study
3.5.2 Gross and histopathology	Yes **all dose groups** organs: pituitary, submandibular salivary gland, heart, thymus, thyroids, parathyroids, lungs, main stem bronchi, trachea, oesophagus, stomach, duodenum, ileum, jejunum, caecum, colon, adrenals, pancreas, gall bladder, liver, kidneys, urinary bladder, ureter, aorta, testes, epididymis, prostate, overies, uterus, cervix, spleen, mesenteric, prescapular and bronchial lymph nodes, voluntary muscle (biceps femoris), mammary gland (females only), brain, spinal chord, sciatic and posterior tibial nerves, rib including costo-chondral junction and bone marrow and all abnormal tissues. Eyes were fixed in Davidson's Solution and skin, from the inguinal region, in Bouin's Solution.
3.5.3 Other examinations	Not applicable
3.5.4 Statistics	Standard
3.6 Further remarks	Examination of the nervous system Yes number of animals: control and high dose groups time points: pre-experimentally and in weeks 13, 26, 39 and terminally (several high dose dogs were examined during the course of the study at times when gross neurological abnormalities were displayed) tests/observations: observation of gait, observation of posture, flexor reflexes, extensor reflexes, patellar reflexes, anal reflex, panniculus reflex, tests of cranial nerve function (pupillary light reflexes, corneal reflexes, palpebral reflexes, blink reflexes, gag reflex), postural reactions (extensor postural thrust, hopping reaction, visual placing reaction, tactile placing reaction, righting reaction, optic righting reaction), attitudinal reactions (tonic neck reactions, tonic optic reactions), assessment of temperament (postural, attitudinal and righting reactions were tested only in animals showing irregularities of posture or gait) Absorption of test substance Yes **number of animals**: all animals **time points**: in week 26 **Parameter**: analysis of urine samples for the investigation of the absorption of the test compound RESULTS AND DISCUSSION
4.1 Observations	

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(3) Subchronic oral toxicity test – oral (dog)**
	Key Study
4.1.1 Clinical signs	There were no clinical abnormalities at the 5 and 100 mg permethrin/kg/day dose levels that were attributable to treatment. Two days after commencing the study, two of the four dogs that had been introduced into the experiment at 2000 mg permethrin/kg/day exhibited clinical effects of a severity that necessitated the reduction of the level of administration to 1000 mg permethrin/kg/day. Subsequently the 1000 mg permethrin/kg/day dose level at times also produced severe neurotoxic effects in several dogs but only after some days or weeks of administration. The effects seen at this top dose level included inco-ordination, whole body tremors, convulsions and nervousness. Excessive salivation was noted in many animals given 1000 mg permethrin/kg/day, most frequently just prior to dosing though direct contact of the compound with the mouth (when there was spillage from the capsules) also caused it. Vomiting was seen in most dogs given 1000 mg permethrin/kg/day at various times during the study though it was most common within the first few days of dosing. Over the course of the study, four of the high dose males showed a general loss of condition. One in particular became very thin and had a minimal weight gain over the course of the study. With two exceptions of dogs showing clinical abnormalities which were incidental to treatment, all dogs maintained good clinical health, though one control female became obese as the study progressed.
4.1.2 Mortality	There were no mortalities.
4.2 Body weight gain	Reduced bodyweight gain was seen in dogs of both sexes given 1000 mg permethrin/kg/day; actual bodyweight losses occurred initially in some females. Though there were differences to control in the weight gain of males given 5 and 100 mg permethrin/kg/day, these were not dose related and were accounted for by the difference in initial weight. The final bodyweights of dogs in these two groups were almost identical to the control group. The weight gains of females in the 100 mg permethrin/kg/day group were statistically significantly reduced in weeks 12-36 and thereafter the difference to control was maintained until the end of the study. In the female 5 mg permethrin/kg/day group, a reduced growth rate was apparent from approximately week 16 but this was statistically significantly different from the control weight gain only in weeks 24 and 28.

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(3) Subchronic oral toxicity test – oral (dog)**
	Key Study
	For females, the analyses were repeated excluding all the data for one control animal (which had shown exceptional weight gain for its height and became obese) and all the data after week 12 for one 5 mg permethrin/kg/day animal (which was unwell with suspected polyarteritis (unassociated with treatment) which adversely affected growth from at least week 23). The reduced weight gain of the 100 mg permethrin/kg/day group was still apparent, though statistical significance was attained only in weeks 16 and 24. However, the weight gain of the 5 mg permethrin/kg/day group was now considered comparable with that of the control group at all time periods.
4.3 Food consumption and compound intake	Food was left uneaten, either occasionally or more consistently, by several dogs including the controls at various times during the study. However, at the 1000 mg permethrin/kg/day dose level there was evidence in some dogs of temporarily reduced food consumption being related to severe neurological effects; in addition, decreased food intake of at least three other high dose level dogs was possibly a direct result of permethrin administration. One female (5 mg permethrin/kg/day) frequently left food uneaten from week 17 until the end of the study, with adverse effects on bodyweight as a result; this dog was known to be unwell from week 23 onwards. There was no explanation for the reduced food consumption shown at times by other dogs in the control, 5 and 100 mg permethrin/kg/day groups. As test compound administration was oral via capsule, test compound intake was not affected by food consumption.
4.4 Ophthalmoscopic examination	The sclera of the right eye of one male control animal were reddened, accompanied by a discharge and blepharospasm, in week 40. The condition was successfully treated.
4.5 Blood analysis	

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(3) Subchronic oral toxicity test – oral (dog)**

Key Study

4.5.1 Haematology	Statistically significant differences between the control and treatment group means were occasionally found for most blood parameters. However, the only consistent trends were seen in red cell count, mean cell volume, platelet count and prothrombin time. The red cell count was slightly reduced in week 4 in males treated with 100 mg permethrin/kg/day, in week 20 in the female 1000 mg permethrin/kg/day group and in week 39 in females treated with 100 mg permethrin/kg/day. The mean cell volume was slightly increased in weeks 4, 8 and 20 in the male 1000 mg permethrin/kg/day group and in the female 1000 mg permethrin/kg/day group in week 20. There was a slight increase in mean cell volume of females given 100 mg permethrin/kg/day in week 26. Slight to moderate increases in platelet count were seen at various time periods in animals of both sexes in the 100 and 1000 mg permethrin/kg/day groups. In addition, there were slight increases in the male animals treated with 5 mg permethrin/kg/day. Changes in prothrombin time were restricted to the 1000 mg permethrin/kg/day group. A slight increase in the prothrombin time of both sexes was noted in week 16 and in the females only in weeks 20, 39 and 52. All bone marrows appeared normal.
4.5.2 Clinical chemistry	Some changes occurred with several parameters, mainly at the top dose level, and although of minor significance, they may have been related to treatment. A slight decrease in the plasma potassium level of the 1000 mg permethrin/kg/day males was noted at most time periods. There were slight decreases in the plasma calcium level of dogs of both sexes given 1000 mg permethrin/kg/day, which were statistically significantly different from the control mean at all time periods in the males and in weeks 4, 8, 20 and 52 in the females. There was a dose-related increase in the plasma alkaline phosphatase activity of both sexes receiving 100 and 1000 mg permethrin/kg/day. The increase was seen throughout the course of the study at the 1000 mg permethrin/kg/day level and from weeks 4 and 16 in males and females respectively given 100 mg permethrin/kg/day. No differences from control were noted in the 5 mg permethrin/kg/day group apart from a slight rise for females at week 52.

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6.4.1(3) Subchronic oral toxicity test – oral (dog)

Key Study

There was evidence of slightly decreased plasma cholesterol level in the male 1000 mg permethrin/kg/day group from week 12 onwards (statistically significantly different to the control mean at several time periods). Much of this decrease was influenced by the consistently lower plasma cholesterol levels of two dogs. No real trends of cholesterol decrease were apparent in the females given 1000 mg permethrin/kg/day nor in the lower dose levels for males or females.

Increased plasma triglycerides level was evident in males given 1000 mg permethrin/kg/day in the early part of the study, i.e. weeks 8-20. There was no evidence of increased plasma triglycerides level in females at this dose level.

There were decreases in the plasma albumin level of male and female dogs in the 100 and 1000 mg permethrin/kg/day groups throughout the course of the study. These were statistically different from the control mean at most time periods, most frequently in the males.

Plasma total protein was decreased in the male 100 and male and female1000 mg permethrin/kg/day groups at most time periods. The differences to control were statistically significant at all time periods for the overall means of the 1000 mg permethrin/kg/day group.

Isolated statistically significant differences to control in several other parameters were noted but these were usually due to unusual values of individual animals and therefore not considered to be of importance.

4.5.3 URINALYSIS

There were no treatment-related changes in any parameters. All the glucose, ketones and urobilinogen results were negative or trace (acceptable for the dog) throughout the study. No treatment-related abnormalities were noted during microscopic examination of urine centrifuged deposits.

4.6 Sacrifice and pathology

4.6.1 Organ weights

There was a marked increase in the liver weight of males and females given 100 and 1000 mg permethrin/kg/day. The liver weights of dogs given 5 mg permethrin/kg/day were unaffected.

Though the weight of the adrenals of the male treated groups appeared increased, this was a consequence of slightly low mean control weight rather than a treatment-related effect.

A statistically significantly reduced heart weight was evident in males given 1000 mg permethrin/kg/day but this was possibly due to the lower bodyweight of this group.

The mean thyroids weight in all male treated groups and in females given 100 and 1000 mg permethrin/kg/day was increased. However, the changes were slight and not dose-related.

There were no other differences to control in the remaining organ weights.

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6.4.1(3) Subchronic oral toxicity test – oral (dog)

Key Study

4.6.2 GROSS AND HISTOPATHOLOGY

Macroscopic findings

The only treatment-related findings were slight swelling and enlargement of the liver in three males receiving 1000 mg permethrin/kg/day. In one of these, there was also a reduction in size of the thymus, prostate gland, testes, mesenteric and pre-scapular lymph nodes. A reduced amount of sub-lumbar fat in this dog was consistent with the poor condition observed clinically.

Unilateral renal agenesis was found in two treated females. The animals were litter mates, suggesting a hereditary basis. This condition has been reported in the Beagle.

Microscopic findings

Treatment-related changes occurred in the adrenal glands and the liver.

1) Adrenal glands: Two types of changes were observed in the adrenals of dogs receiving 100 and 1000 mg permethrin/kg/day. The first type of lesion consisted of focal inflammation, associated with degenerative changes in five males and four females given 1000 mg permethrin/kg/day and in one male in the 100 mg permethrin/kg/day group. One further female given 1000 mg permethrin/kg/day showed focal inflammation only. The zona fasciculate was most consistently involved but similar changes were seen in the zona reticularis in a few animals. The zona arcuata (glomerulosa) and adrenal medulla were not affected.

Degenerative changes in the zona fasciculata consisted of increased cytoplasmic eosinophilia, cytoplasmic vacuolation and shrinkage and nuclear swelling. In a few animals there were foci of necrosis within larger areas of milder degenerative change. The associated inflammatory response was variable, even between different areas in the same gland. It was absent in a few small lesions but generally consisted of a light to moderate infiltrate of mixed inflammatory cells. Lymphocytes predominated but a few neutrophils were usually also present. Dense accumulations of inflammatory cells consisted entirely of lymphocytes.

Multinucleate giant cells were present in some degenerate foci and also as isolated groups not associated with other inflammatory changes. Nuclei were aligned at the perimeter of these cells and the cytoplasm had a degenerate appearance containing many vacuoles and cholesterol clefts. Giant cell formation probably resulted from fusion of macrophages following phagocytosis of degenerate zona fasciculata cells. The presence of vacuoles and cholesterol clefts reflects the high lipid content of the zona fasciculata cells.

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6.4.1(3) Subchronic oral toxicity test – oral (dog)

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Fibroblast proliferation was present in many lesions, with cords of fibroblasts extending from areas of degeneration between columns of zona fasciculata cells. Use of a specific strain (Picro -sirius Red Modification of Van Gieson's Stain) showed that collagen fibres were very slightly increased in number and thickness in these areas. These changes represent an active reparative phase. Mature fibrous tissue scarring, suggesting older healed lesions was not found.

The second change, noted in three males and one female dosed with 1000 mg permethrin/kg/day and one male and one female in the 100 mg permethrin/kg/day group consisted of swelling and vacuolation of cells in the zona reticularis, extending into the zona fasciculata in a few animals. Use of Oil Red O Stain in selected cases showed that the change was due to increased cytoplasmic accumulation of neutral lipid. There was no consistent association between this change and the inflammatory and degenerative change noted above.

2) Liver: A change referred to as cellular swelling was seen in the livers of three males and one female treated with 100 mg permethrin/kg/day and four males and five females treated with 1000 mg permethrin/kg/day.

It consisted of slight to moderate enlargement of hepatocytes, sometimes resulting in sinusoidal obliteration. Affected cells showed dense cytoplasmic eosinophilia at the periphery and around the nucleus while the remainder of the cytoplasm had a 'ground glass' appearance. In these dogs the change was approximately uniform throughout the liver. All lobular zones were equally affected and minor variations in severity across a section were unrelated to any anatomical feature.

One male receiving 5 mg permethrin/kg/day also showed a similar but less extensive change in which only centrilobular zones were affected.

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6.4.1(3) Subchronic oral toxicity test – oral (dog)

Key Study

3) Other Organs: No consistent treatment-related changes were seen in any other organ. No lesions were detected in the central or peripheral nervous system which could account for the neurological abnormalities observed clinically.
Gross reduction in size of the prostate in one male receiving 1000 mg permethrin/kg/day was due to immaturity. This may have been related to the general poor condition of the dog, although testes and epididymis were normal and contained mature spermatozoa (no other treated animal, including one in worse clinical condition, showed any prostatic abnormality). This animal also showed moderate thymic involution. Premature thymic involution occurs in response to stress. It is possible that stress associated with the effect of permethrin on the nervous system was responsible for this change, although again it was not seen in other top dose animals which showed similar or more severe nervous signs. As these were incidental findings their significance must remain uncertain.
A number of congenital and acquired lesions were detected in other organs with incidence and severity unrelated to treatment

4.7 Other

Examination of the nervous system

Neurological examinations undertaken whilst dogs were showing muscle tremors and incoordination sometimes revealed exaggerated flexor reflexes and an absent or depressed pupillary reflex. In addition, following a normal patellar reflex, the digits were sometimes seen to adduct and abduct two or three times. On one occasion each, four dogs given 1000 mg permethrin/kg/day displayed either an exaggerated flexor reflex or depressed pupillary reflex without showing any of the other typical neurotoxic effects. Apart from these instances, neurological examinations of apparently clinically normal dogs revealed no abnormalities.

Absorption of test substance

The presence of 3-(4′hydroxyphenoxy)benzoic acid, a metabolite of permethrin, in the urine of all treated dogs demonstrated the absorption of the test substance. There was a dose-related increase in the concentration and amount of the metabolite excreted. None was detected in control dog urine.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 MATERIALS AND METHODS

Four groups of beagle dogs, each containing six males and six females, received permethrin orally by gelatine capsule for 52 weeks at dose levels of 0, 5, 100 and 1000 mg/kg/day. Bodyweights and food consumption were measured and the dogs were observed for clinical and behavioural abnormalities. A variety of haematological and biochemical investigations was made at intervals throughout the study. At termination, all dogs were subjected to macroscopic and microscopic pathological examinations and a selection of organs was weighed.

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(3) Subchronic oral toxicity test – oral (dog)**
	Key Study
5.2 Results and discussion	Clinical effects, which included convulsions, muscle tremor and incoordination, sometimes associated with loss of condition, were seen frequently in dogs dosed with 1000 mg permethrin/kg/day. The bodyweight gains of dogs of both sexes in the 1000 mg permethrin/kg/day group and of females given 100 mg permethrin/kg/day were reduced. Focal degeneration with inflammatory changes was seen in the adrenal cortex of both sexes treated with 1000 mg permethrin/kg/day and in one male treated with 100 mg permethrin/kg/day. Swelling and vacuolation of cells in the zona reticularis due to increased lipid accumulation was also seen at these two dose levels. There was no consistent association between the two types of adrenal changes in terms of their severity and occurrence and there was evidence that these effects occurred only after a prolonged period of permethrin administration. The liver weight of dogs given 100 and 1000 mg permethrin/kg/day was increased above control level and was accompanied by hepatic cellular swelling. These findings were considered to represent an adaptive response and not a toxicological effect and were consistent with observed increases in plasma alkaline phosphatase activity.
5.3 CONCLUSION	
5.3.1 LO(A)EL	100 mg/kg, based on histopathological changes in the adrenals in males and females, and reduced bodyweight gain in females.
5.3.2 NO(A)EL	5 mg/kg
5.3.3 Other	The dose level of 1000 mg permethrin/kg/day was overtly toxic to dogs, resulting in neurological abnormalities which were associated in some animals with poor clinical condition. Reduced bodyweight gain occurred in dogs of both sexes given 1000 mg permethrin/kg/day and in females at 100 mg permethrin/kg/day. Histopathological changes in the adrenals provided further evidence of toxic effect at 100 and 1000 mg permethrin/kg/day. Adaptive hepatic changes, characteristic of synthetic pyrethroid administration occurred at the two higher dose levels. It was concluded that the oral administration of 5 mg permethrin/kg/day for one year was without toxicological effect in dogs.
5.3.4 Reliability	2
5.3.5 Deficiencies	Not GLP
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*29/11/05*

Section A6.4.1 Annex Point IIA6.4.1	**6.4.1(3) Subchronic oral toxicity test – oral (dog)**
	Key Study
Materials and Methods	***3.4.2*** *Bodyweights were also recorded one day prior to commencement of dosing.* *Otherwise, applicant's version is acceptable.*
Results and discussion	*Adopt applicant's version.*
Conclusion	***5.3.1*** *Liver weight increase in both sexes, accompanied by hepatic cellular swelling was also observed in the 100 mg/kg/day group.* *LO(A)EL: 100 mg/kg/day* *NO(A)EL: 5 mg/kg day*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ... *(specify)*
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_4_3(3)-1. Female Group Mean Bodyweight Gain (kg) Excluding Selected Animals
by Week

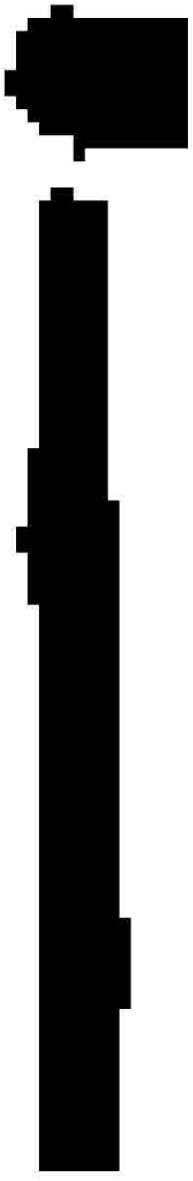
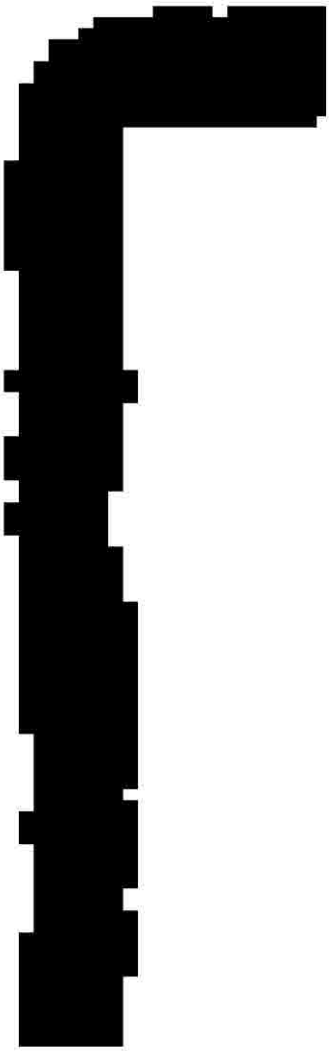


Table A6_4_3(3)-2. Summary of Treatment-Related Lesions in Adrenal Glands



Section A6.4.2	A6.4.2 Subchronic Dermal toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	This study is usually required when the dermal route of exposure is significant and the compound is known to be toxic by the dermal route and can penetrate through intact skin. This study with permethrin is not required on the following basis; • Although the dermal route of exposure is the most significant route of exposure in professional wood preservation use, there is evidence to indicate that significant amounts of permethrin can not pass through intact skin (1.24% dermal adsorption). • Acute dermal toxicity studies showed no toxic effects up to and including the highest dose tested (See Section 6.1.2). • It is also possible to calculate the route-to-route exposure from available oral toxicity studies and using dermal penetration studies (Section 6.2) as there are no specific effects observed following dermal exposure in animals. Therefore an accurate and realistic determination of dermal toxicity can be derived from available sub-chronic oral exposure studies and *in vitro* dermal penetration studies.	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	*24/11/05*	
Evaluation of applicant's justification	*Where are the **in vitro** dermal penetration studies referred to here?*	
Conclusion	*Is there an **actual** requirement for a multiple dose study for Biocides (unlike PPPs)? Or, is there a problem with the data that exists - being derived from a human volunteer study (6.2?), and therefore any reference values derived from it would not be 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		

Section A6.4.3	A6.4.3 Subchronic Inhalation toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [X] Other justification [ ]	
Detailed justification:	According to the Technical Notes for Guidance on data requirements for active substances and biocidal products, these studies are required for active substances that have the following characteristics: • For volatile substances and gases (vapour pressure > 1 x 10^{-2} Pa) • In cases where inhalation exposure is significant, an inhalation study is required instead of an oral study This study with permethrin is not required on the following basis; Engineering controls significantly reduce or even eliminate wood preservative plant operator exposure to the product. The vapour pressure (2 µPa), Saturated Vapour Concentration (2.6 × 10^{-9} ppm) and Henry's constant (1.87 × 10^{-6} atm-m3/mole) of permethrin indicate that losses to air will be negligible in the timber treatment process. The acute inhalation LC50 (>23.5 mg l^{-1}) indicates permethrin to be of negligible toxicity by this exposure route. No significant inhalation exposure will occur to passers-by at the treatment plant or the general public through use of treated timber. A Tier 1 (unrefined) model of the exposure via inhalation (Document IIB) indicates total exposure to permethrin *via* spraying of 510 mg per day. Of this, 0.415 mg is *via* inhalation, the remainder being by other exposure routes, primarily dermal. A Tier II refinement gives 43.6 mg total and 0.134 mg inhalation. Therefore, in comparison to the modelled exposure *via* the dermal route, exposure *via* inhalation is not significant.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*24/11/05*	
Evaluation of applicant's justification	*Accept applicant's justification.*	
Conclusion	*Applicant's justification is acceptable.*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	

Section A6.4.3	A6.4.3 Subchronic Inhalation toxicity
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 A6.5 6.5(1) chronic toxicity – oral (rat)

Annex Point IIA6.5

	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
	2 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 A6.5 6.5(1) chronic toxicity – oral (rat)

Annex Point IIA6.5

	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 A6.5 6.5(1) chronic toxicity – oral (rat)

Annex Point IIA6.5

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 A6.5 6.5(1) chronic toxicity – oral (rat)

Annex Point IIA6.5

		Key Study
3.4.10 PATHOLOGY	Yes	
3.4.10.1 Organ Weights	Yes	
	from:	all surviving animals, at terminal sacrifice
	Organs:	Adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, uterus
		Other: lungs, pituitary gland, thyroid
3.4.10.2 HISTOPATHOLOGY	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	

Section A6.5 **6.5(1) chronic toxicity – oral (rat)**

Annex Point IIA6.5

Key Study

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 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 A6.5 **6.5(1) chronic toxicity – oral (rat)**

Annex Point IIA6.5

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. 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.

Section A6.5 6.5(1) chronic toxicity – oral (rat)

Annex Point IIA6.5

Key Study

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.
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 [redacted]. There were no trends and it was considered that the results did not reflect a response to treatment with permethrin.

Section A6.5 **6.5(1) chronic toxicity – oral (rat)**

Annex Point IIA6.5

Key Study

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 A6.5 **6.5(1) chronic toxicity – oral (rat)**

Annex Point IIA6.5

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

Section A6.5 **6.5(1) chronic toxicity – oral (rat)**

Annex Point IIA6.5

Key Study

In males given the highest dosage, liver weight was significantly higher than in controls. 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 A6.5 **6.5(1) chronic toxicity – oral (rat)**

Annex Point IIA6.5

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.

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.

Section A6.5 6.5(1) chronic toxicity – oral (rat)

Annex Point IIA6.5

Key Study

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.

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.

Section A6.5 **6.5(1) chronic toxicity – oral (rat)**

Annex Point IIA6.5

Key Study

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.

5.3 CONCLUSION

5.3.1 LO(A)EL	50 mg/kg bw/day, based on histopathological evidence of hepatic work hypertrophy.
5.3.2 NO(A)EL	10 mg/kg bw/day.
5.3.3 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.

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)?*

Section A6.5 **6.5(1) chronic toxicity – oral (rat)**

Annex Point IIA6.5

	Key Study
	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	

Section A6.5 6.5(1) Carcinogenicity/chronic toxicity – oral (rat)

Annex Point IIA6.5

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 A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

	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 Specification	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 ♀	
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	

Section A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

	Key Study	
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	
3.1.6.2 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		
3.4.1 Body weight	Yes	
3.4.2 FOOD CONSUMPTION	Yes	

Section A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

		Key Study	
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	
3.4.10 PATHOLOGY	Yes		
3.4.10.1 Organ Weights	Yes		

Section A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

		Key Study
	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 A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

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.

Section A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

Key Study

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.
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 PHTHALMOSCOPIC 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

Section A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

Key Study

4.10 Pathology	Not reported
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.

Section A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

Key Study

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.

Mouse:

Non-neoplastic lesions
Increased 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.

Neoplasia
The 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.

Section A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

Key Study

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.

4.14 Time to tumours Not reported

4.15 Other Not applicable

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.

Section A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

Key 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.
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.

Section A6.5 **6.5(2) Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

	Key Study
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
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	

Section A6.5 6.5(2) Chronic toxicity – oral (rat, mouse)

Annex Point IIA6.5

	Key Study
	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 A6.5 **6.5(2) Carcinogenicity/Chronic toxicity – oral (rat, mouse)**

Annex Point IIA6.5

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	0.43	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	38
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	33*	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).

Section A6.5 6.5(2) Carcinogenicity/Chronic toxicity – oral (rat, mouse)

Annex Point IIA6.5

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

Section A6.5 **6.5(2) Carcinogenicity/Chronic toxicity – oral (rat, mouse)**
Annex Point IIA6.5

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

Annex Point IIA6.6.1

		Official use only
	Key Study	
	1 REFERENCE	
1.1 Reference	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.	
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	
	2 MATERIALS AND METHODS	
3.1 Test material	As given in section 2	
3.1.1 Lot/Batch number	Lot No. 8E8026 and 8I8012	
3.1.2 Specification	As given in section 2	X
3.1.2.1 Description	Slightly viscous suspension	
3.1.2.2 Purity	Lot No. 8E8026, 94.1-96.3%; Lot No. 8I8012, 95.91-97.3%.	
3.1.2.3 Stability	Not applicable (short-term administration)	
3.2 Study Type	Bacterial reverse mutation test	
3.2.1 Organism/cell type	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100, TA 1538	
3.2.2 Deficiencies / Proficiencies	Histidine amino acid deficient	
3.2.3 METABOLIC ACTIVATION SYSTEM	S9 mix, rat, liver, induced, Aroclor 1254, 500 mg/kg i.p.	
3.2.4 Positive control	2-Aminoanthracene (2AA), +S9, TA98, TA100, TA1538 Propane sultone (PS), -S9, TA1535 9-Aminoacridine (9AAD), -S9, TA1537	
3.3 Administration / Exposure; Application of test substance		

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

Annex Point IIA6.6.1

	Key Study
3.3.1 Concentrations	0, 0.5, 2.6, 13.0, 25.0, 50.0 µL/plate ± S9 (equivalent to 0, 0.8, 4.3, 21.7, 41.7, 83.3 µL/mL in the pre-incubation test)
3.3.2 WAY OF APPLICATION	plate incorporation, pre-incubation
3.3.3 Pre-incubation time	plate incorporation: not applicable pre-incubation: 20 minutes
3.3.4 Other modifications	Not applicable
3.4 Examinations	see tables in appendix for examinations and results
3.4.1 Number of cells evaluated	Results expressed as revertants observed per 10^8 cells plated.
	3 RESULTS AND DISCUSSION
4.1 GENOTOXICITY	
4.1.1 without metabolic activation	No
4.1.2 with metabolic activation	No
4.2 Cytotoxicity	Yes, 50.0 µL/plate
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	Permethrin was tested in the Ames Salmonella/mammalian microsome plate incorporation and pre-incubation mutagenesis assays using TA 98, TA 100, TA 1535, TA 1537, and TA 1538 tester strains. Each assay was performed in the presence and absence of metabolic activation.
5.2 RESULTS AND DISCUSSION	In both assays at the doses tested (plate incorporation: 0.5 µL/plate-50.0 µL/plate; pre-incubation: 0.8 µL/mL-83.3 µL/mL) no gene mutations were found to occur under any metabolic activation condition.
5.3 Conclusion	The results of the Salmonella plate incorporation and pre-incubation mutagenesis assays indicate that permethrin does not cause a significant increase in the reversion index of any of the tester strains with or without metabolic activation by Aroclor induced rat liver microsomes.
5.3.1 Reliability	2
5.3.2 Deficiencies	Not GLP