Competent Authority Report

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

Cyphenothrin (PT 18)

CAS-No. 39515-40-7

Sumitomo Chemical (U.K.) PLC

DOCUMENT III-A

Study summaries

Section A6.5

Toxicology section

Rapporteur: Hellas

November 2017

6.5 Chronic toxicity

		1. REFERENCE	Official use only
1.1	**Reference**	Reference : A6.5/01 Authors : [redacted] Title: [redacted]: Combined oncogenicity and toxicity study in rats Laboratory : Life Sciences Research Ltd , UK Unpublished Report no : [redacted] Date: September 28 1988	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Sumitomo	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Not applicable	X
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Amber viscous liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	F-344	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and Female	
3.2.5	Age/weight at study initiation	4 – 6 weeks 84 – 120 g (males) and 73 – 102g (females)	
3.2.6	Number of animals per group	160 (80 male and 80 female)	
3.2.6.1	at interim sacrifice (54 weeks)	20 (10 male and 10 female) for toxicity study	
3.2.6.2	at terminal sacrifice (104 weeks)	40 (20 male and 20 female) for toxicity study 100 (50 male and 50 female) for oncogenicity study	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**		

3.3.1	Duration of treatment	104 weeks	
3.3.2	Frequency of exposure	Daily in feed	
3.3.3	Postexposure period	None	
3.3.4	Oral		
3.3.4.1	Type	Dietary	
3.3.4.2	Concentration	0, 100, 300 and 1000 ppm	X
3.3.4.3	Vehicle	None	
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 – twice daily	
3.4.1.2	Mortality	Yes – twice daily	
3.4.2	Body weight	Yes - weekly intervals for the first 14 weeks, and subsequently at two-weekly intervals	
3.4.3	Food consumption	Yes - the weight of food consumed by each cage of rats was calculated weekly from measurements of the amount of food offered and food remaining including that scattered; this was expressed as g/rat/week. Group mean food conversion ratios, that is, the amount of food consumed per unit gain in bodyweight, were calculated at weekly intervals for the first 14 weeks of treatment	
3.4.4	Water consumption	Yes Water intake was assessed by daily visual inspection of the water bottles. Accurate measurements were performed, over 24-hour periods, in Weeks 1, 4, 8, 13, 26, 52, 78 and 104.	
3.4.5	Opthalmoscopic examination	Yes - Before commencement of treatment, both eyes of all rats assigned to the study were examined using a Fisons binocular indirect ophthalmoscope after the instillation of 0.5% tropicamide During Weeks 26, 51, 78 and 103 the eyes of all surviving rats from Groups 1 and 4 of each replicate of the Oncogenicity Study were examined; the examination was extended to Groups 2 and 3 of the Oncogenicity Study for Week 103 only	
3.4.6	Haematology	Yes Before commencement of treatment blood samples were withdrawn from the retro-orbital sinus of ten male and ten female rats taken from the same batch as those on study. These rats were then killed by inhalation of carbon dioxide and discarded without necropsy. During Weeks 26. 51, 79, and 103 of treatment, blood samples were withdrawn from the retro-orbital sinus of five males and five females from each group of each replicate of the Toxicity Study. The following parameters were examined: Packed cell volume (PCV), Haemoglobin concentration (Hb), Erythrocyte count (RBC), Leucocyte count (WBC), Platelet count, Reticulocyte, Mean cell volume (MCV), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC)	

3.4.7	Clinical chemistry	Yes At the same intervals as for haematology further blood samples were taken using lithium heparin as anticoagulant. Samples were obtained from the same animals used for haematological analysis, under the same conditions. After centrifugation the plasma was examined in respect of: Glucose concentration, Alkaline phosphatase activity (AP), Alanine amino-transferase activity (ALT), Aspartate amino-transferase activity (AST), Total protein concentration, albumin (A) concentration, globulin (G) concentration, A/G ratio, Sodium (Na) and Potassium (K) concentrations, Calcium concentration (Ca), Phosphorus (inorganic) concentration (P), Urea concentration, Total bilirubin concentration, Total cholesterol concentration, Creatinine concentration, Creatinine phosphokinase activity (CPK), Chloride concentration (Cl), Lactate dehydrogenase activity (LDH)
3.4.8	Urinalysis	Yes During Weeks 26, 51, 79 and 103, of treatment urine samples were collected from, as far as possible, the animals used to provide samples for haematology and blood chemistry investigations. Water was removed from cages at 12.30 hours; the rats were placed individually into metabolism cages at 17.00 hours, with water and food removed. Urine was collected until 09 hours the following morning and examined in respect of the following: Appearance, Volume, pH, Specific gravity (SG), Glucose, Protein, Ketones, Blood, Bilirubin, Urobilin, Total reducing substances, Nitrite Micropsy of the sediments
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Adrenal glands, Pituitary gland, Brain, Spleen, Heart, Testes, Kidneys, Thyroid glands (with parathyroids), Liver, Ovaries, Uterus with cervix
3.5.2	Gross and histopathology	Yes Prior to commencement of the necropsy the clinical history of each animal was studied. The gross necropsy included examination of the external surfaces including all natural orifices, the cranial, thoracic and abdominal cavities and an examination of the residual carcase. In the course of the necropsy the external and cut surfaces of the tissues and organs were examined. All details of abnormalities were recorded. Oesophagus, Adrenals, Ovaries, Aorta (thoracic arch), Pancreas, Brain, Pituitary, Caecum, Presumptive tumours, Carcase, Prostate, Colon, Rectum, Duodenum, Salivary glands (submandibular), Epididymides, Sciatic nerves, Seminal vesicles, Femoral Bone and marrow (with joint), Skeletal muscle, Skin, Spinal cord, Heart, Spleen, Hardarian Glands, Sternum (with marrow), Eye and optic nerve, Ileum, Jejunum, Stomach, Kidneys, Liver, Testes, Thymus, Lungs (with main stem bronchi), Thyroid (with parathyroids), Lymph nodes, Trachea, Urinary bladder, Mammary glands and Uterus with cervix
3.5.3	Other examinations	
3.5.4	Statistics	Tests for the significance of difference between each treatment group and the corresponding controls were conducted as follows: For bodyweight gain and haematology data (where applicable), a series of Student's t-tests was performed using a pooled within-treatment error variance. A least significant difference was calculated

		at the 0.1%, 1% and 5% levels of significance. Inter-group differences in mean absolute or bodyweight-relative organ weights were assessed using Dunnett's test at the 1% and 5% levels of significance. Time-to-event analysis of mortality (Main Study animals only) was by Cox's test applied both as an overall test for homogeneity of survival curves and for pairwise comparison against control. Tarone's extension of Cox's test (Biometrika, 62, 679-684, 1975) was used to examine linear trend on dose and to assess deviation from linearity. Fisher's Exact Probability test was applied as a two-tailed test, where appropriate, to the distribution of macroscopic pathological entities. The probability levels quoted are 0.1% 1% and 5%. Bonferroni's correction which allows for simultaneous statistical inference (Gart, Chu and Tarone, 3 Natl, Cancer Inst. 62 957, 1979) was also applied to assist interpretation. The Fisher's Exact Probability Test was used to analyse non-neoplastic and neoplastic histopathological findings using the two- and one-tailed tests respectively. This test was only applied when inspection of the data suggested that the difference between control and treated groups might be statistically significant ($P < 0.05$). In the case of neoplastic findings, only increases in incidences were examined (one-tailed test). Bonferroni's correction was applied as above.	
3.6	**Further remarks**		
		4. RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Unaffected by treatment	
4.1.2	Mortality	Unaffected by treatment	
4.2	**Body weight gain**	Female rats receiving 1000 ppm of cyphenothrin gained less weight than their controls over the first 78 weeks of treatment. All other groups were unaffected by treatment See table A.6.5-1	X
4.3	**Food consumption and compound intake**	Female rats receiving 1000 ppm of cyphenothrin gained less weight than their controls over the first 78 weeks of treatment. All other groups were unaffected by treatment	
4.4	**Ophthalmoscopic examination**	Unaffected by treatment	
4.5	**Blood analysis**		
4.5.1	Haematology	Unaffected by treatment	
4.5.2	Clinical chemistry	Unaffected by treatment	
4.5.3	Urinalysis	Unaffected by treatment	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Unaffected by treatment	
4.6.2	Gross and histopathology	Unaffected by treatment	
4.7	**Other**		

5. APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of eighty male and eighty female F-344 rats received cyphenothrin (95.1%) continuously, via the diet provided *ad libitum* at concentrations of 100, 300 or 1000 ppm. A similarly constituted group received diet containing no cyphenothrin and served to generate contemporaneous control data. Ten of the above 80 animals in each group and sex were sacrificed for interim study after 54 weeks of treatment. Surviving animals were sacrificed after 104 weeks had been completed.	
5.2	**Results and discussion**	Survival was unaffected by treatment with cyphenothrin Female rats receiving 1000 ppm of cyphenothrin gained less weight than their controls over the first 78 weeks of treatment. Female rats receiving 1000 ppm consumed slightly less food than their controls. Food conversion ratios over the first 14 weeks was unaffected by treatment. The dosages achieved during the first week of treatment ranged from 13 to 129 mg/kg/day approximately. Dosages fell as the study progressed such that by Week 104 the range of dosages achieved was approximately 4 to 45 mg/kg/day. Water consumption was unaffected by treatment. No toxicologically-significant ophthalmic changes were observed for animals receiving cyphenothrin The chemical and cellular composition of the blood and urine were unaffected by treatment. Organ weight analysis did not reveal any inter-group differences that were considered to be related to treatment with cyphenothrin. There were no macroscopic changes which were attributed to treatment with cyphenothrin None of the histopathological changes seen was considered to be related to treatment. On the basis of the evidence generated in this study, it is concluded that the continuous administration of cyphenothrin to F-344 rats for 2 years, at dietary levels of up to 1000 ppm, did not influence the incidence of any tumour. Some evidence of an effect of treatment was observed for female rats which had received 1000 ppm. The no-effect level was 1000 ppm (providing 48 mg/kg/day*) for males and 300 ppm (providing 18 mg/kg/day*) for females. * These are the overall dosages calculated for animals of the Oncogenicity Study.	
5.3	**Conclusion**		
5.3.1	LO(A)EL		
5.3.2	NO(A)EL	NOEL was 300 ppm (8 mg/kg/day) based on reduced bodyweight and food consumption at 1000 ppm	X
5.3.3	Other		

5.3.4 Reliability 2

5.3.5 Deficiencies This study was not conducted to a recognized OECD guideline.

Table A6.5-1 Bodyweight changes – group mean values (grammes)

	Controls	100ppm	300ppm	1000 ppm
Males – Oncogenicity Study				
weeks				
0 - 70	380	388	380	375
70 - 104	-49	-53	-70	-58
0 - 104	327	327	307	316
Females – Oncogenicity Study				
weeks				
0 - 78	233	231	227	221*
78 - 104	8	12	14	12
0 - 104	241	244	238	232
Males – Toxicity Study				
weeks				
0 - 70	387	394	382	377
70 - 104	-66	-57	-72	-66
0 - 104	321	335	303	310
Females – Toxicity Study				
weeks				
0 - 78	232	227	226	227
78 - 104	14	19	17	~~18~~ 8
0 - 104	241	246	240	236

* Significantly different from controls $p < 0.01$

EVALUATION BY COMPETENT AUTHORITIES

EVALUATION BY RAPPORTEUR MEMBER STATE

Date November, 2017

Materials and methods

Point 3.3.4.2: The RMS considers that the average amount of compound ingested at each dose group, as determined based on food consumption data, should be presented in the report. The following table should be added:

Table A.6.5-2: Average amount of ingested cyphenothrin for 104 weeks in the Oncogenicity Study.

Dose level (ppm)	Compound ingested (mg/Kg bw/day)	
	Males	Females
0	0	0
100	4.84	5.89
300	14.49	17.77
1000	48.16	58.52

Point 4.2: Regarding Table A.6.1-1, there is a typographical error and the table is amended accordingly.

Point 5.3.5: The RMS wants to indicate that although the study was not conducted according to a recognized OECD guideline, it is generally in compliance to the OECD 453 (combined chronic toxicity/carcinogenicity studies) which corresponds to the EU testing method B.33, with the exception of some limitations (see remarks below, comment to point 2.3).

Results and Discussion

The applicant's version is generally acceptable. However, the RMS considers the following points.

Point 4.6.1: The RMS considers that more information with regard to organ weight values should be presented in the report, as follows:

a) **Toxicity study (54 weeks – n=10):** Increased absolute and relative kidney weight in males of 1000ppm; higher relative liver weight in males and females of 1000ppm. The statistically significant increase in relative-to-body liver weight at 54 weeks although not remarkable (< 10%) was considered as related to treatment since after prolonged exposure to cyphenothrin (104 weeks) relevant histopathology was evident (increased incidence of liver/bile duct hyperplasia in females – see comment in Point 4.6.2).

There was no associated histopathological change however.

Table A.6.5-3: Organ weight values (Toxicity study, 54 weeks)

Organ	Sex	0	100 ppm	300 ppm	1000 ppm
Absolute organ weight values (g)					
Kidneys	M	3.32	3.27	3.34	3.53*
	F	2.05	2.09	2.05	2.05
Thyroid+ parathyroid	M	0.017	0.016	0.016	0.014*
	F	0.014	0.010 **	0.012	0.013
Brain	M	2.02	2.01	2.01	1.99
	F	1.82	1.81	1.84	1.81
Pituitary	M	0.008	0.009	0.008	0.008
	F	0.009	0.010	0.010	0.009
Relative-to-body organ weight values (%)					
Kidneys	M	0.729	0.736	0.745	0.812**
	F	0.805	0.822	0.803	0.829
Liver	M	3.56	3.77	3.74	3.83*
	F	3.53	3.61	3.59	3.74*

*=0.05 level
**= 0.01 level

b) Toxicity study (104 weeks – n=20): absolute and relative kidney weight in females of 1000ppm was lower than control. Relative kidney weight females 300ppm was lower than control. Absolute and relative thyroid/parathyroid gland weight in females of 100 or 300 ppm was lower than control.

Table A.6.5-4: Organ weight values (Toxicity study, 104 weeks)

Organ	Sex	0	100 ppm	300 ppm	1000 ppm
Absolute organ weight values (g)					
Kidneys	M	4.37	4.37	4.21	4.02
	F	3.07	2.93	2.82	2.79 *
Thyroid+ parathyroid	M	0.029	0.027	0.024	0.023
	F	0.022	0.018*	0.016**	0.019
Relative-to-body organ weight values (%)					
Kidneys	M	1.080	1.069	1.074	1.023
	F	0.978	0.892	0.882*	0.878*
Thyroid+ parathyroid	M	0.0071	0.0067	0.0061	0.0060
	F	0.0069	0.0054 **	0.0049**	0.0059
Liver	M	5.27	4.68	4.84	4.77
	F	4.66	4.20	4.07	4.19

*=0.05 level
**= 0.01 level

c) Oncogenicity study (104 weeks – n=50): absolute and relative pituitary gland weight in females in all treatment groups was lower than the control with no dosage-relationsip. Absolute and relative thyroid/parathyroid gland weight in females of 300 and 1000 ppm was lower than control. Absolute kidney weight was lower in females of 1000ppm. No associated histopathological changes were found.

Table A.6.5-5: Organ weight values (Oncogenicity study, 104 weeks)

Organ	Sex	0	100 ppm	300 ppm	1000 ppm
Absolute organ weight values (g)					
Pituitary	M	0.015	0.014	0.014	0.015
	F	0.014	0.010*	0.009**	0.010*
Kidneys	M	4.30	4.21	4.24	4.19
	F	2.93	2.87	2.80	2.76*
Thyroid+ parathyroid	M	0.031	0.029	0.027	0.027
	F	0.023	0.022	0.017**	0.017**
Relative-to-body organ weight values (%)					
Pituitary	M	0.0037	0.0035	0.0036	0.0037
	F	0.0043	0.0031**	0.0027**	0.0033**
Kidneys	M	1.038	1.014	1.111	1.069
	F	0.925	0.896	0.880	0.892
Thyroid+ parathyroid	M	0.0074	0.0069	0.0071	0.0069
	F	0.0073	0.0072	0.0053**	0.0055**
Liver	M	4.66	4.53	4.93	5.02
	F	4.31	4.22	4.06	4.23

*=0.05 level
**= 0.01 level

Point 4.6.2: A slight increase in the incidence of liver / bile duct hyperplasia in female rats treated with 1000 ppm cyphenothrin for 104 weeks (Oncogenicity study) was noted (Table A.6.5-6). Although this finding did not follow a dose-related pattern, it was only observed in females and did not reach statistical difference compared to the control, the RMS considers that a relation to cyphenothrin administration cannot be excluded.

Table A.6.5-6: Liver histopathology (Oncogenicity Study, 104 weeks)

	Sex	0	100 ppm	300 ppm	1000 ppm
Liver (bile duct hyperplasia)	M	33(30)	30(30)	33(31)	34(32)
	F	37(16) 43%	41(15) 37%	34(13) 38%	**35(23)** **66%**

Number of animals tested (number of animals affected)

During the commenting period of the draft CAR, the applicant noted that bile duct hyperplasia is often observed as one of aging findings and thus did not consider that the slight difference without toxicological significance is treatment related. The eCA responded that although bile duct hyperplasia may be noted in aging rats (control incidence: 43%), the incidence of the effect at 1000 ppm was substantially increased (66%). Taking also into consideration the increased relative-to-body liver weight after 54 weeks of exposure, the eCA considered that relation to cyphenothrin administration could not be excluded.

Point 4.5.1: Relative to the values for the controls, the following changes were evident in animals receiving 1000 ppm after 25 weeks of treatment; lower PCV in males, lower haemoglobin concentrations in males and females and lower RBC in females. Generally, these were considered to be small differences and not of toxicological significance.

Conclusion

LOAEL = 1000 ppm (equivalent to 48.16 mg/Kg bw/day in males and 58.52 mg/Kg bw/day in females) based on body weight and food consumption reduction in females, as well as the increase in relative-to-body liver weight in both sexes at 54 weeks, followed by increased liver/bile duct hyperplasia in females at 104 weeks.

NOAEL = 300 ppm (equivalent to 14.49 mg/Kg bw/day in males and 17.77 mg/Kg bw/day in females)

The applicant has considered that the daily consumption of 300 ppm corresponds to a 8 mg/kg b.w./day dose. Nevertheless, the RMS considers that the average amount of compound ingested at each dose group, as determined based on food consumption data, should be used.

Other conclusions: In general, it seems that females are more sensitive to cyphenothrin administration than males. Target organ: liver.

Reliability 2

Acceptability Acceptable

Remarks

Point 2.1: Although the study was not conducted according to a recognized OECD guideline, it is generally in compliance to the OECD 453 (combined chronic toxicity/carcinogenicity studies) which corresponds to the EU testing method B.33, with the exception of some limitations (see comment to point 2.3).

Point 2.3:

1. In addition to the time points of blood sampling, an additional blood sampling should have been performed at 13 weeks.
2. Regarding Haematology, there was no measurement of the blood clotting potential. Moreover, no differential blood count was performed on samples of animals in the highest dose and control group.
3. Regarding Clinical Chemistry, there was no measurement of the ODC (ornithine decarboxylase) activity and blood urea nitrogen.