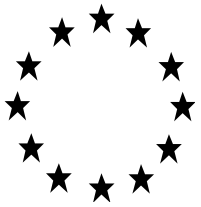
Competent Authority Report

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

Sections A6.3-A6.4

Toxicology section

Rapporteur: Hellas

November 2017

6.3 Short-term repeated dose toxicity

6.3.1 Repeated dose toxicity (oral)

			Official use only
		1. REFERENCE	
1.1	**Reference**	Reference : A6.3.1.01 Authors : [redacted] Title: A five week sub acute feeding Toxicity Study of [redacted] in Rats Laboratory : Sumitomo Chemical Co Ltd Unpublished Report no : [redacted] Date : September 3, 1984	
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 existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No - but broadly follows OECD 407	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	Yellow brown oily 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	CD	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	5 weeks 136 - 154g (male) and 104 - 117g (female	
3.2.6	Number of animals per group	12	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral (dietary)	

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3.3.1	Duration of treatment	35 days	
3.3.2	Frequency of exposure	daily	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	0, 100, 300, 1000 or 2000 ppm *ad libitum*	X
3.3.4.3	Vehicle	None	
3.3.4.4	Concentration in vehicle	Not applicable	
3.3.4.5	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes	
3.4.1.2	Mortality	Yes	
3.4.2	Body weight	Yes	
3.4.3	Food consumption	Yes	
3.4.4	Water consumption	Yes	
3.4.5	Opthalmoscopic examination	Yes	
3.4.6	Haematology	Yes Number of animals: All animals (60) Time points: End of study Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, MCV	
3.4.7	Clinical chemistry	Yes Number of animals: : All animals (60) Time points: End of study Parameters: Albumin, alkaline phosphatase, blood urea nitrogen, cholesterol, creatine phosphokinase, cholinesterase, creatinine, glucose, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, leucine aminopeptidase, lactic dehydrogenase, total protein, albumin/globulin ratio, sodium, potassium, chlorine, calcium, triglycerides, phospholipids.	
3.4.8	Urinalysis	Yes Number of animals: All animals (60) Time points: Weeks 4 or 5 Parameters: pH, protein, glucose, blood, bilirubin and ketone bodies, protein, leukocytes, erythrocytes, crystals, casts, epithelian cells, sperm	X
3.5	**Sacrifice and pathology**		
3.5.1	Organ weights	Yes Organs: liver, kidneys, adrenals, testes, uterus, spleen, brain, heart, pituitary, thyroid, lungs	X

3.5.2	Gross and histopathology	Yes High dose groups and control: Organs: brain, spinal cord, pituitary, thyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, uterus, prostate, urinary bladder, lymph nodes, peripheral nerve, femur/bone marrow, skin, eyes, testes/ovaries, epididymides, tongue and gross abnormal lesions Intermediate dose: Brain, lungs, heart, spleen, liver, kidneys, testes/ovaries, pituitary, thyroid, adrenals and gross abnormal lesions	X
3.5.3	Other examinations	Opthamology	
3.5.4	Statistics	With respect to bodyweight, food consumption, water intake, organ weight, haematolical examination and biochemical examination data differences in variance were analysed by F test and when homoscedatic Student's t-test and when not by Fishcer Behrens test. For gross pathological and histopathological findings x^2 tests were conducted	
3.6	**Further remarks**	-	
		4. RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	In the 2000 ppm group, tremor and hypersensitivity to external stimulation such as touch and/or sound were observed from day 2 of administration in all males and females, but the severity of the symptoms showed a trend of gradual decrease during the administration period. Also in the 1000 ppm group a slight tremor was noticed in the morning of day 2 in all animals but it had disappeared by the afternoon of the same day.	
4.1.2	Mortality	On day 28 of administration one male died in the 100 ppm group. However, due to severe autolysis the casue of death could not be clarified.	
4.2	**Body weight gain**	Table A.6.3.1-1 shows the changes in body weight for each group throughout the administration period. In the 2000 pm group, significant low values were observed in both males and females throughout the administration period. As for body weight gain in 5 weeks after administration, significant low values were noticed in the 2000 ppm in both males and females. Depressed body weight was noticed in females of the 1000 ppm group but the difference was not statistically significant.	
4.3	**Food consumption and compound intake**	Table A.6.3.1-2 shows mean food consumption and mean intake of the test compound throughout the administration period. Significant decreases were observed in males of the 2000 ppm group in weeks 1 and 2 and in males of the 1000 ppm group and females in the 2000ppm group in week 1. Water intake was significantly low in both males and females of the 2000 ppm group during the first week.	
4.4	**Ophthalmoscopic examination**	Haemorrhaging in the *fundus occuli* was observed in 2 males in the 2000 ppm group. One female in this group had an indistinct *fundus occuli* figure in the left eye.	
4.5	**Blood analysis**		
4.5.1	Haematology	Slight, but statistically significant, changes were observed in erythrocyte count, haemocrit value, mean corpuscular volume, ratio of eosinophil count and ratio of monocyte count. However, there was no dose-dependency in any of these changes.	

4.5.2	Clinical chemistry	In males of the 2000 ppm group slightly high values in urea nitrogen and slightly low values in glucose and total protein were observed. Slightly high values were noticed in albumin/globulin ratio and glutamic pyruvic transaminase activity in males and females in the 1000 and 2000 ppm groups. In some other parameter, differences with statistical significane were obsered but there was no dose dependency in any of these changes.	
4.5.3	Urinalysis	No treatment related changes were seen	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	In males treated at 1000 and 2000 ppm, absolute pituitary weights were decreased by 8-14%. (Table A.6.3.1-3). In females treated at the top dose, relative thyroid, lung and liver weights were significantly increased In males treated at the top dose, relative adrenal weights were significantly increased, and relative thyroid weights were significantly increased at 1000 and 2000 ppm. (Table A.6.3.1-4) Increased relative organ weights were related to decreased body weight gain.	X
4.6.2	Gross and histopathology	There were no treatment-related gross pathological changes. Histopathological investigations included examination of the brain, spinal cord and sciatic nerve, but no treatment-related effects on these tissues were observed.	
4.7	**Other**		
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a 5 week feeding study (1982), CD (Charles River Japan) rats (12 rats/sex/dose) were fed diets containing 0, 100, 300, 1000 or 2000 ppm cyphenothrin. (93.6%)	
5.2	**Results and discussion**	Throughout the administration period, toxic symptoms such as tremor and hypersensitivity and a depression of body weight gain were observed in the 2000 ppm group in both males and females. Also in the 1000 ppm group a slight tremor was noticed in both males and females during the early stages of administration, and a reduction in body weight gain in females was observed. In the 2000 ppm group, decreases were noticed in food consumption and water intake in the early stages of administration in both males and females. In the 1000 ppm group, a decrease in food consumption was observed in males during the early stages of administration. No treatment related effects in urinanalysis, ophthalmology; haematology and biochemistry were observed. In males in receiving 1000 and 2000 ppm, low values in pituitary weights were observed. Slight increases in the relative thyroid weights were observed in males in the 1000 and 2000 ppm groups and also in females in the 2000 group. There were no treatment related pathological or histopathological effects The lowest no observed effect level (NOEL), based mainly on the overt signs of toxicity at the 1000 and 2000 ppm dose levels, was in males at 300 ppm equivalent to 30.9 mg kg^{-1} per day	

5.3 Conclusion

5.3.1	LO(A)EL	1000 ppm
5.3.2	NO(A)EL	300 ppm equivalent to 30.9 mg kg^{-1} per day
5.3.3	Other	
5.3.4	Reliability	2
5.3.5	Deficiencies	The study is not conducted to a recognized OECD Guideline

Table A6.3.1-1 Mean Body weight (g)

	Control		100ppm		300ppm		1000ppm		2000ppm	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Day 0	144.2	109.5	145.2	109.8	146.3	110.4	144.2	110.2	143.6	109.4
Day 3	162.0	121.9	160.6	120.1	162.3	122.5	159.3	119.5	145.2#	103.7#
Week 1	204.6	143.5	200.7	142.5	203.7	147.3	203.7	142.5	178.8#	131.8#
Week 2	261.0	163.8	253.0	164.7	260.4	167.8	249.2	162.5	223.5#	156.3*
Week 3	314.3	189.4	303.0	186.8	312.0	192.0	310.3	184.6	279.0#	177.3#
Week 4	352.6	204.3	340.4	203.5	346.9	208.7	344.8	197.5	316.4#	195.1*
Week 5	382.1	219.9	368.6	218.9	377.3	213.1	374.8	212.3	345.2#	206.2#

* - $p<0.05$; # - $p<0.01$

Table A6.3.1-2 Food consumption and compound intake

Dietary level ppm	**Average amount of consumed food (g/kg bodyweight/day)**		**Compound ingested (mg/kg bodyweight/day)**	
	Male	Female	Male	female
0	105	112	—	
100	103	112	10.3	11.2
300	103	112	30.9	33.6
1,000	104	114	104	114
2,000	98.6	107	197	214

Table A6.3.1-3 Absolute pituitary weights (g)

	Control	100ppm	300ppm	1000ppm	2000ppm
males	14.32	14.66	13.42	13.19*	12.80*
females	14.82	15.90	14.63	14.70	14.35

*- p<0.05

Table A6.3.1-4 Relative organ weights (mg %)

	Control	100ppm	300ppm	1000ppm	2000ppm
Adrenal					
males	14.94	15.02	13.79	14.71	16.64*
females	28.33	28.30	32.67	30.92	30.14
Thyroid					
Males	5.649	6.124	6.220	6.481	6.994#
Females	7.124	7.326	7.052	7.525	8.679#
Lung					
Males	0.3992	0.3891	0.4043	0.3898	0.4094
Females	0.4938	0.4916	0.5043	0.5061	0.5312#
Liver					
males	2.843	2.792	2.777	2.903	2.963
females	2.640	2.767	2.656	2.743	2.864#

* - p<0.05; # - p<0.01

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November, 2017
Materials and methods	Point 3.4.8: The word "protein" appears twice. Point 3.5.1: According to the study, the ovaries were weighted and not the uterus. Point 3.5.2: According to the study, the list of organs that were subjected to histopathology in the highest and control groups also includes the preputial gland, seminal vesicle and femoral muscle. These are not listed in this point.
Results and discussion	Point 4.2: There is a typographical error in Table A.6.3.1-1. According to the study, the mean body weight (g) of males treated with 100 ppm cyphenothrin is 253, instead of 233 (week 2). Point 4.3: There are typographical errors in Table A.6.3.1-2. According to the study, the average amount of consumed food (g/kg bw/day) by high dose males is 98.6, instead of 90.6. Moreover, the amount of compound ingested should be presented in mg/kg bw/day, instead of g/kg bw/day; and the value for male 300 ppm group should be 30.9 instead of 30 mg/kg bw/day. Point 4.6.1: There is a typographical error in Table A.6.3.1-4. According to the study the relative lung weight in mg (%) in the control group of females is 0.4938 and not 0.4943.
Conclusion	LOAEL: 1000 ppm (equivalent to 104 mg/kg bw/day), based on clinical signs consistent with synthetic pyrethroid toxicity (tremor), decreased body weight gain (females), decreased food consumption (males), decreased absolute pituitary weight (males), increased relative thyroid weight (males). NOAEL: 300 ppm (equivalent to 30.9 mg/kg bw/day) Other conclusions: In general, males seemed to be more severely affected than females.
Reliability	2
Acceptability	Acceptable, as a preliminary study.
Remarks	Point 2.1: It is noted that the appropriate experimental protocol is the OECD 407, equivalent to the EU testing method B.7. Point 2.3: 1. The duration of the study is 7 days longer (35 days) than the one required by the protocol (28 days). Nevertheless, this has not been considered as a significant deviation. 2. In Haematology, there was no measurement of the blood clotting potential. 3. In Clinical Chemistry, there was no measurement of urea levels. 4. In Organ Weights, there was no weighing of epididymides and thymus.

A6.3.1 Repeat dose toxicity (oral) 4 week study in the beagle

			Official use only
		1. REFERENCE	
1.1	**Reference**	Reference : A6.3.1/02 Authors : [redacted] Title: [redacted]. Preliminary toxicity study by oral (capsule) administration to beagle dogs for four weeks Laboratory : Life Sciences Research Ltd, UK Unpublished Report no : [redacted] Date : February 4, 1987	
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 existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No (No suitable OECD Guideline)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Not applicable	
		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	Viscous amber liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Beagle	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	27 – 31 weeks 11.1 – 12.0 kg (male) and 8.5 – 11.0 kg (female)	
3.2.6	Number of animals per group	1 male and 1 female	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Oral	

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3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	daily
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	Gelatine capsule
3.3.4.2	Concentration	10, 100 or 300mg/kg/day
3.3.4.3	Vehicle	None
3.3.4.4	Concentration in vehicle	None
3.3.4.5	Controls	None
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes All dogs - daily
3.4.1.2	Mortality	Yes Four dogs which were killed in extremis were subjected to the same terminal procedures as those that survived the treatment period
3.4.2	Body weight	Yes Each animal was weighed, before feeding, at weekly intervals during the accilmatisation period and at twice-weekly intervals during the treatment period. In addition, each dog was also weighed immediately before necropsy regardless of feeding cycle.
3.4.3	Food consumption	Yes daily
3.4.4	Water consumption	Yes Assessed but quantitative measurements were not performed.
3.4.5	Opthalmoscopic examination	No
3.4.6	Haematology	Yes Number of animals: All animals (6) Time points: Before commencement of the study, and before termination of treatment (before dosing), blood samples were withdrawn from the jugular vein of each dog, after overnight starvation. Parameters: Packed cell volume (PCV) Haemoglobin concentration (Hb) Erythrocyte count (RBC) Leucocyte count (WBC), — Leucocyte count (WBC), differential Platelet count Mean cell haemoglobin (MCH) Mean cell volume (MCV) Mean cell haemoglobin concentration (MCHC)
3.4.7	Clinical chemistry	Yes Number of animals: All animals (6)

		Time points: As for haematology Parameters: Alanine amino-transferase activity (ALT) Aspartate aniino-transferase activity (ALT) Alkaline phosphatase activity (AP) Creatinine concentration Glucose concentration Total bilirubin concentration Total protein concentration Electrophoretic protein fractions Sodium (Na) and Potassium (K) concentrations Chloride concentration (Cl) calcium concentration (Ca) Phosphorus (Inorganic) concentration (P)	 X X
3.4.8	Urinalysis	Yes Number of animals: All animals (6) Time points: Before commencement of the study and before termination of treatment Parameters: Appearance, Volume (overnight samples only), pH, Specific gravity (SG), Total reducing substances , Glucose, Ketones, Bilirubin, Urobilin, nitrite, blood	
3.5	**Sacrifice and pathology**		
3.5.1	Organ weights	Yes – all animals (6) Organs: Adrenals, Brain, Heart, Kidneys, Liver, Lungs (with bronchi), Ovaries, Pituitary, Prostate (with urethra sample), Spleen, Testes, Thyroid (with parathyroid), Uterus (with cervix).	
3.5.2	Gross and histopathology	Yes – all animals (6)	
3.5.3	Other examinations	Veterinary and neurological examinations before treatment and prior to termination. Veterinary examination Each animal was subjected to a rigorous veterinary examination in which particular attention was paid to: Teeth and gums, Mucous membranes and skin, Ears (external auditory canal), Superficial lymph nodes, Abdomen - including palpation, External genitalia and mammary glands, Chest - including auscultatlon of heart and lungs, Gait and stance - including palpation of limbs, General behaviour and appearance. Neurological examination The following reflexes were tested and observations performed: Cranial nerve reflexes: Pupillary light and consensual light, Palpebral - blink and corneal, Gag, General examination of the head to assess other cranial nerves Segmental reflexes: Flexor (withdrawal), including crossed extensor, Patellar, Extensor tone, Postural reactions: Placing reactions - visual and tactile, Extensor postural thrust, Righting reactions, Tonic neck reactions, Hopping reflex, General observations: Behavioural changes, Abnormalities of gait and stance, Presence of tremor or other dyskinesias.	

3.5.4	Statistics	No statistics applied as there was only one animal per group
3.6	**Further remarks**	
	4. RESULTS AND DISCUSSION	
4.1	**Observations**	
4.1.1	Clinical signs	Signs were seen from Day 1, predominantly in males that received 100 or 300 mg/kg/day, and included ataxia, severe tremors, emesis, salivation, peripheral vasodilation, prostration, inability to walk and twitching eyelids. In addition, convulsions or evidence of convulsions, mydriasis, restlessness, stiff or high stepping gait and panting were also occasionally observed at these dosages. Dogs that received 10 mg/kg/day showed occasional incidents of mydriasis, salivation, slight tremors, ataxia and twitching eyelids.
4.1.2	Mortality	All animals receiving 100 or 300 mg/kg/day were killed during the treatment period because of marked signs of reaction to treatment which Included ataxia, tremors and convulsions.
4.2	**Body weight gain**	Weight loss was observed in the few days before death in animals that received 100 mg/kg/day and the female that received 300 mg/kg/day.
4.3	**Food consumption and compound intake**	Slightly low food consumption was evident before death in the female that received 300 mg/kg/day.
4.4	**Ophthalmoscopic examination**	Not applicable
4.5	**Blood analysis**	
4.5.1	Haematology	No treatment related changes were seen
4.5.2	Clinical chemistry	Plasma phosphorus concentrations were low after 24 days of treatment in dogs receiving 10 mg/kg/day and in ante mortem samples from dogs that received 100 or 300 mg/kg/day, when compared with samples obtained from the same dogs before commencement. Also evident at 300 mg/kg/day were high aspartate amino-transferase activities and urea concentrations in both dogs and a low alanine arnino-transferase activity in the male only. High alkaline phosphatase, alanine arnino-transferase and aspartate amino-transferase activities were apparent in the male that received 100 mg/kg/day and a high urea concentration was seen in the female that received this dosage.
4.5.3	Urinalysis	No treatment related changes were seen
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	The male that received 100 mg/kg/day had high absolute and bodyweight-relative lung and liver weights and the female that received 300 mg/kg/day had high absolute and bodyweight-relative lung weights.
4.6.2	Gross and histopathology	At necropsy dark or congested lungs were noted in the female that received 300 mg/kg/day and the male that received 100 mg/kg/day.

4.7	**Other**	Veterinary examination before death generally confirmed the signs seen at routine observation in dogs that received 100 or 300 mg/kg/day, but depressed or absent gag reflex at 300 mg/kg/day and exaggerated response to sound and slow pupil response in the female at 100 mg/kg/day were also observed. Examination of surviving dogs did not reveal any treatment-related effects. Neurological examination before the death of the female that received 100 mg/kg/day confirmed the signs seen at routine observations or at veterinary examination and revealed slightly exaggerated corneal and tonic neck reflexes and a depressed extensor postural thrust reaction. A depressed extensor postural thrust reaction was also evident before the death of the female that received 300 mg/kg/day and in the surviving female receiving 10 mg/kg/day. The surviving male, receiving 10 mg/kg/day, had depressed gag and blink reflexes.

5. APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a 4 week Beagle dog study, animals (1/sex/dose) were administered, without using a vehicle, 10, 100 or 300 mg cyphenothrin kg/day by gelatin capsule. No controls were included in the study.
5.2	**Results and discussion**	Dogs treated at the low dose showed occasional signs of mydriasis, salivation, tremors, ataxia and twitching eyelids. Dogs treated at 100 and 300 mg/kg/day showed signs of toxicity from day one. These included salivation, ataxia, inability to walk, tremors, emesis, stiff gait, restlessness, subdued mood, twitching eyelids, mydriasis, convulsions, panting, prostration, peripheral vasodilation, chattering jaws and high stepping gait. Males were more severely affected than females. Veterinary examination prior to humane killing showed the gag reflex to be absent in the male and depressed in the female at the top dose. An exaggerated response to sound and slow pupil response were seen in the female that received 100 mg/kg/day. Body temperature was slightly high in the high dose male and female, and the female at 100 mg/kg/day, and this was considered to be associated with severe body tremor and convulsions. Neurological examination performed after three weeks confirmed the twitching facial muscles, exaggerated responses to sound, uncoordinated gait and muscle tremors in the female receiving 100 mg/kg/day. Other neurological irregularities considered to be treatment-related included slightly depressed extensor postural thrust reaction in all treated females, slightly depressed pupillary light reflex in the female receiving 100 mg/kg/day, slightly depressed blink reflex in the top dose female and blink and gag reflex in the male receiving 10 mg/kg/day, and exaggerated corneal and tonic reflexes in the female receiving 100 mg/kg/day. The male and female treated at 100 mg/kg/day were humanely killed on days 7 and 25 respectively. The male and female treated at 300 mg/kg/day were humanely killed on days 2 and 26 respectively. Body weight and food consumption were unaffected in dogs receiving 10 mg/kg/day. Body weight loss was observed in dogs treated at 100 mg/kg/day for several days prior to being killed. Body weight loss was observed in the female treated at the top dose, with a concomitant decrease in food consumption prior to being killed. Haematology results appeared to be unaffected by treatment. However, changes in blood chemistry were observed. On day 6, blood samples were taken, *ante mortem*, from the male treated at 100 mg/kg/day and displayed markedly elevated alkaline phosphate, alanine

aminotransferase and aspartate aminotransferase activities as well as elevated total bilirubin levels. On day 2, the male treated at 300 mg/kg/day was sampled, *ante mortem*, and showed elevated aspartate aminotransferase activities. On day 25, the female treated at 10 mg/kg/day had raised total bilirubin. On day 25, urea levels were raised in the female treated at 100 mg/kg/day and sampled *ante mortem*. On day 26, samples were taken, *ante mortem*, from the female treated at the high dose level, and displayed raised aspartate aminotransferase activities, urea levels and total bilirubin levels. There were no treatment-related urinalysis findings.

Macroscopic findings revealed wounds and abrasions in the male treated at 100 mg/kg/day and in the female treated at 300 mg/kg/day which were attributed to trauma sustained during convulsions. Congested lungs were noted in the female receiving 300 mg/kg/day and dark lungs were noted in the male receiving 100 mg/kg/day. These were considered to be associated with the convulsions seen before death and the high lung weights. No histopathological examination of animals was performed. However samples of tissue, including tissue from brain, spinal cord and sciatic nerve, were taken from each dog and held for possible future histopathological examination.

The no effect level could not be established since animals treated at the low dose showed occasional signs of mydriasis, salivation, twitching eyelids, tremors and ataxia

5.3	**Conclusion**	
5.3.1	LO(A)EL	10 mg kg^{-1} per day
5.3.2	NO(A)EL	None established
5.3.3	Other	
5.3.4	Reliability	1
5.3.5	Deficiencies	No

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November, 2017
Materials and methods	Point 3.4.7: The name of the second transaminase should read "Aspartate amino-transferase activity (AST)" instead of "Aspartate aniino-transferase activity (ALT)". Moreover, urea concentration was also measured, but is not mentioned in this report.
Results and discussion	The applicant's version is acceptable.
Conclusion	LOAEL = 10 mg/kg bw/day, based on clinical signs consistent with synthetic pyrethroid toxicity (mydriasis, salivation, tremors, ataxia and twitching eyelids), other neurological irregularities (slightly depressed extensor postural thrust reaction (female) and slightly depressed blink and gag reflex (male)), and clinical chemistry changes (raised total bilirubin, female). NOAEL < 10 mg/kg bw/day Other conclusions: - The RMS considers essential to indicate that no histopathological examination of animals was performed. - The RMS considers that the dose of 100 mg/kg bw/day exceeds by far the maximum tolerated dose (MTD) for cyphenothrin in dogs as evidenced by severe toxicity including mortality of all animals of both sexes.
Reliability	1
Acceptability	Acceptable, as a preliminary study.
Remarks	No further remarks.

6.3.2 Repeated dose toxicity (dermal)

	Justification for non-submission of data	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Given the fact that the compound is not toxic *via* the dermal route and that a repeated dose study in the rat by the oral route has been submitted, no further information should be required.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November, 2017	
Evaluation of applicant's justification	The applicant's version is acceptable.	
Conclusion	The applicant's version is acceptable.	
Remarks	No further remarks.	

6.3.3 Repeated dose toxicity (inhalation)

	Justification for non-submission of data	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Given the fact that the compound is not toxic *via* the inhalation route and that a repeated dose study in the rat by the oral route has been submitted, no further information should be required.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November, 2017	
Evaluation of applicant's justification	In Doc IIC as submitted by the applicant it is mentioned that: "*The aerosol will produce a droplet aerosol size median diameter of approximately 40µg, with less than 4 % of the droplets below 10µg in diameter, and a discharge rate of approximately 1.6 g of preparation per second (Unpublished, 1997)*". It is noted that the units should be µm and not µg. Considering the above statetement as valid, and taking into account that cyphenothrin is not a volatile substance (vapour pressure < 10^{-2} Pa, 25 ^{0}C), a repeated dose toxicity study by the inhalation route is not required. However, during the commenting period, the evaluation of the LC_{50} from acute inhalation was revised and classification as Acute Tox. 4 with H332 (Harmful if inhaled) was proposed. Following this conclusion, repeated-dose inhalation toxicity studies with cyphenothrin were requested by the eCA. The applicant provided the requested data so the waiving was no longer valid.	
Conclusion	In light of the repeated-dose inhalation toxicity studies provided by the applicant, the waiving was no longer valid.	
Remarks	No further remarks.	

SECTION A6.3.3/01 **REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION**
Annex Point IIA6.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	**Reference: A6.3.3/01** [redacted] (1983) Subacute Inhalation Toxicity of [redacted] in Rats. Laboratory of Biochemistry and Toxicology, Takarazuka Research Center, Sumitomo Chemical Co., LTD., Hyogo, Japan. September 19, 1983.	
1.2	**Data protection**	Yes	
1.2.1	Data owner		
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection		
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Methods used comparable to guidelines US EPA, Health Effects Test Guideline 82-4, Subdivision F and Equivalent to EC Guideline B.29, OECD Guideline for Testing of Chemicals, Number 412 Subacute Inhalation Toxicity: 28-Day Study (2009)	
2.2	**GLP**	Yes, Study was validated to indicate compliance with GLPs	
2.3	**Deviations**	Yes, exposures were 4 hours per day instead of 6 hours per day.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	Lot No. 81051	
3.1.2	Specification		
3.1.2.1	Description	Brownish yellow viscous oily liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rats	
3.2.2	Strain	Sprague Dawley	
3.2.3	Source	[redacted]	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	approximately 6 weeks	X
3.2.6	Number of animals per group	20 animals per group with 10 animals per sex per group	
3.2.7	Control animals	Yes, vehicle control animals were exposed to deodorized kerosene and negative control animals were assumed to be exposed to ambient air.	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	29 days	

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SECTION A6.3.3/01 REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION
Annex Point IIA6.3

3.3.2	Frequency of exposure	Daily 4 hour exposures for 29 days	X
3.3.3	Postexposure period	no	
3.3.4	**Inhalation**		
3.3.4.1	Concentrations	Nominal concentration 39.3; 117.9 and 275.2 [mg/m³] Analytical concentration 15.1; 54.0 and 152.0 [mg/m³]	X
3.3.4.2	Particle size	MMAD (mass median aerodynamic diameter) [µm] (± GSD (geometric standard deviation) [µm]	
3.3.4.3	Type or preparation of particles	Test article was injected into a specially-prepared glass atomizer and sprayed under compressed air. The mists generated were transported into the exposure chamber after removal of larger sized particles while passing through two bottles connected in series. The flow rate of the exhausted air was adjusted by means of a vacuum pump in order to keep a constant pressure.	
3.3.4.4	Type of exposure	Whole body	
3.3.4.5	Vehicle	Deodorized kerosene	
3.3.4.6	Concentration in vehicle	1.0%, 3.0%, and 7%, in solution (deodorized kerosene) to achieve 15.1, 54.0, and 152.0 mg/m^3, respectively.	
3.3.4.7	Duration of exposure	4 h	
3.3.4.8	Controls	Control animals were exposed to only deodorized kerosene using an exposure equivalent to the test substance exposures. Negative control animals were assumed to be exposed to ambient air, but not clearly stated in the report.	
3.4	**Examinations**		
3.4.1	Observations		

mg/m^3	15.1*	54.0	152.0
Mean MMAD (Microns):	ND	0.92	0.8
Mean GSD:	NA	1.73	1.68
N:	-	8	8

*: aerodynamic diameter of particles could not be measured because number of particles in the exposure chamber were insufficient. However, the mist generator used for the low-doses was the same type as generator used for the high-dose. Therefore, it is believed that there was no significant difference in the particle size of mist particles generated at any dose in this subacute inhalation study.

SECTION A6.3.3/01 Annex Point IIA6.3		REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION
3.4.1.1	Clinical signs	Yes Prior to exposure – 0, 0.5, 1, 2, 3, and 4 hours of exposure and 1 hour post exposure (or until signs of toxicity were no longer present), daily.
3.4.1.2	Mortality	Yes Prior to exposure – 0, 0.5, 1, 2, 3, and 4 hours of exposure and 1 hour post exposure (or until signs of toxicity were no longer present), daily.
3.4.2	Body weight	Yes Individual body weights were taken twice a week at intervals of 3 or 4 days.
3.4.3	Food consumption	Yes Food consumption was recorded for 2 consecutive days per week for the entire study.
3.4.4	Water consumption	Yes Water consumption was recorded for 2 consecutive days per week for the entire study.
3.4.5	Ophthalmoscopic examination	Yes Ophthalmological examinations were made on study days 27 and 28, of both eyes of surviving animals.
3.4.6	Haematology	Yes Blood was collected from all animals after the final exposure following an overnight fast. Blood was collected from anesthetized animals from the abdominal aorta.
3.4.7	Clinical Chemistry	Yes Blood was collected from all animals after the final exposure following an overnight fast. Serum was separated from the remaining blood sample after the hematology and analyzed on a Hitachi Automatic Blood Chemistry Analyzer Type 705.
3.4.8	Urinalysis	Yes Fresh urine samples were collected on individual animals on day 28 of the study
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes organs: liver, kidneys, adrenals, testes, ovaries, pituitary, thyroid, spleen, brain, lungs, and heart.
3.5.2	Gross and histopathology	Yes all dose groups had the following organs examined: eye, spinal cord, sciatic nerve, nasal cavity, trachea, bone marrow, mandibulary lymph node, mesenteric lymph node, tongue, esophagus, stomach, small intestine, large intestine, salivary gland, pancreas, urinary bladder, epididymus, uterus, prostate, preptuial gland, skin, liver, kidneys, adrenals, testes, ovaries, pituitary, thyroid, spleen, brain, lungs, thymus and heart.
3.5.3	Other examinations	
3.5.4	Statistics	The mean values of the dose groups were statistically analysed for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the vehicle control group by a student's t-test on the body weight and body weights gain, food consumption, water

SECTION A6.3.3/01 REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION
Annex Point IIA6.3

		consumption, hematology, biochemistry, organ weight and its ratio to body weight. Urinarysis was analyzed by a Student's t-test. Histopathology evaluations were analyzed by a CHI-SQUARE test.	
3.6	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Decrease activity was observed in the treated animals as well as the vehicle control animals. Irregular respiration and slight nose discharge (1-4 rats observed during the latter stage of exposure period) in both sexes at the 15.1 mg/m^3 dose group. Hyperpenea, slight salivation, slight lacrimation, slight nose discharge and slight urinary incontinence were observed in both sexes at the 54.0 mg/m3 level and above with the incidence of these observations being increased in the 152 mg/m^3 dosed group. These effects were observed daily throughout the study, but disappeared within one hour after exposure. No cumulative effects were observed.	
4.1.2	Mortality	No deaths were observed at any dose throughout the entire study.	
4.2	Body weight gain	Male animals were observed to have a slight decrease in bodyweight gain on the final day of the study at the 15.1 mg/m^3 dosed group. A decrease in bodyweight and bodyweight gain was observed in male animals treated with 54.0 mg/m^3 and above from day 6 to day 29. There were no differences in body weights for female animals at any dose. The mild nature of the decrease in body weight gain, the transient nature of the effect and the lack of any dose dependency indicate these findings were of no toxicological significance in the 15.1 mg/m^3 treatment group.	X
4.3	Food and water consumption and compound intake	No wide differences were noted in any of the treated animals when compared to the vehicle control group at any dose tested for food or water consumption.	
4.4	Ophthalmoscope examination	One male animal in the 152 mg/m^3 dosed group was observed with a slight opacity of the lens. It was not considered to be test article related.	
4.5	Blood analysis		
4.5.1	Haematology	A higher mean corpuscular volume was observed in male animals exposed at the 152 mg/m^3 level when compared to vehicle control animals. However, the value was within normal physiological range for rats of this strain and age.	X
4.5.2	Clinical chemistry	Lower calcium concentrations, total proteins, blood urea nitrogen, cholesterol, cholinesterase activity, and higher glutamic oxalacetic transaminase activity were observed for mostly males exposed to 152 mg/m^3 level but also infrequently for males at 54.0 mg/m^3 level and females. These findings were extremely slight and failed to demonstrate any dose dependency so the findings were not considered to be of any toxicological significance.	X
4.5.3	Urinalysis	No remarkable changes were observed in either male or female animals at any dose tested.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	When compared to vehicle control animals a lower absolute weight was observed for the liver (males – 54 and 152 mg/m3), and higher lung weights (females – 152 mg/m3) and higher relative brain, pituitary, adrenal (male – 54 and 152 mg/m3 groups) and lungs (male – 152 mg/m3 group, and females at 15.1 and 152 mg/m3 dose groups) were	X

SECTION A6.3.3/01 REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION
Annex Point IIA6.3

		observed. Only the relative lungs and adrenals demonstrated dose dependency for male rats. However, when taking into account the changes in body weight gain and no accompanying histological lesions these effects were considered of little to no toxicological significance.	
4.6.2	Gross and histopathology	No findings were considered to be test article related. Changes observed were considered to be consistent with those common findings for the rat strain and age.	X
4.7	**Other**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	This study was conducted according to US EPA FIFRA guideline 82-4, equivalent to EC B.29 Subacute Inhalation Toxicity (28 days), OECD 412. Animals were divided into 5 groups with a vehicle control, negative control, low dose, intermediate, and high dosed groups. Ten males and ten females per treatment group were exposed to test article for 4 hours per day for 29 consecutive days. A deviation from the guideline was that the animals were exposed for 4 hours daily instead of for 6 hours per day.	
5.2	**Results and discussion**	Clinical signs observed during the 29 days of exposures included irregular respiration and slight nose discharge in both sexes in the 15.1 mg/m^3 dose group. Hyperpenea, slight salivation, slight lacrimation, slight nose discharge and slight urinary incontinence were observed in both sexes at the 54.0 mg/m^3 level and above with the incidence of these observations increased in the 152 mg/m^3 dosed group. These effects were observed daily throughout the study, but disappeared within one hour after exposure. No cumulative effects observed. A slight decrease in bodyweight and bodyweight gain in male animals was observed at the 54 and 152 mg/m^3 exposure levels; this was observed over days 6 through 29 of treatment. No changes from controls were noted for any of the animals at any dose tested for food and water consumption, urinalysis, ophthalmology, hematology, clinical chemistry, organ weights, and gross- and histopathological evaluations.	
5.3	**Conclusion**	The no-observable-adverse-effect level (NOAEL) is determined to be lower than 15.1 mg/m^3 based on an increased incidence and frequency of clinical symptoms.	
5.3.1	LO(A)EL	LO(A)EL: 15.1 mg/m^3	
5.3.2	NO(A)EL	NO(A)EL: lower than 15.1 mg/m^3	
5.3.3	Other	-	
5.3.4	Reliability	2	
5.3.5	Deficiencies	No	

Clinical Findings: Vehicle Control Animals

		Clinical Signs	Day 1	Day 7	Day 14	Day 29	Comments
Vehicle Control	Male/Female	No. of animals examined	10/10	10/10	10/10	10/10	
		Decr Activity	10/10	10/10	10/10	10/10	
		Irreg Resp.	0/0	0/0	0/0	0/0	Day 18 –

							2 males and 1 female
		Nasal Discharg	0/0	0/0	0/0	0/0	Days 17,19 noted in males and day 17 in females

Clinical Findings: 15.1 mg/m^3 Treated Animals

		Clinical Signs	Day 1	Day 7	Day 14	Day 29	Comments
15.1 mg/m3	Male/Female	No. of animals examined	10/10	10/10	10/10	10/10	
		Decr Activity	10/10	10/10	10/10	10/10	
		Irreg Resp.	10/10	10/10	10/10	10/10	
		Nasal Discharg	0/0	0/0	0/0	0/0	Days 17,18, 26, 27. & 28 noted in males and females

Clinical Findings: 54 mg/m^3 Treated Animals

		Clinical Signs	Day 1	Day 7	Day 14	Day 29	Comments
54 mg/m3	Male/Female	No. of animals examined	10/10	10/10	10/10	10/10	
		Decr Activity	10/10	10/10	10/10	10/10	
		Hyperpnea	10/10	10/10	5/4	10/10	
		Irreg Resp.	10/10	10/10	10/10	10/10	
		Nasal Discharg	3/3	0/1	3/4	0/1	
		Lacrimation	0/1	3/1	0/1	0/0	
		Salivation	1/3	0/0	0/0	0/0	
		Urinary incontinence	0/2	0/0	0/0	0/0	Observed in females on days 1 – 3

Clinical Findings: 152 mg/m^3 Treated Animals

		Clinical Signs	Day 1	Day 7	Day 14	Day 29	Comments
152 mg/m3	Male/Female	No. of animals examined	10/10	10/10	10/10	10/10	
		Decr Activity	10/10	10/10	10/10	10/10	
		Hyperpnea	10/10	10/10	4/5	10/10	
		Irreg Resp.	10/10	10/10	10/10	10/10	
		Nasal Discharg	10/10	7/6	3/7	4/6	
		Lacrimation	2/2	0/4	1/0	0/0	
		Salivation	9/6	3/5	0/2	1/2	
		Urinary incontinence	1/1	0/0	0/0	0/0	

Body weight and Body weight gain

Sex	Treatment Group	No of Rats	Body Weight, Grams (SD) Day 0	Day 13	Day 28	Days 0-28 Bodyweight Gain, Grams	
	Vehicle Control	10	144 (8.96)	174 (13.2)	203 (15.6)	59.3 (8.44)	
	Negative	10	142	183	213	**70.7**	

	Control		(8.62)	(12.2)	(17.5)	**(11.8)***	
Females	15.1 mg/m^3	10	141 (6.31)	173 (7.92)	199 (12.0)	58.6 (13.3)	
	54 mg/m^3	10	140 (9.53)	169 (8.54)	192 (10.7)	52.0 (7.96)	
	152 mg/m^3	10	141 (7.20)	174 (8.28)	200 (9.26)	59.0 (7.01)	
Males	Vehicle Control	10	187 (10.4)	260 (12.7)	333 (21.1)	146 (18.1)	
	Negative Control	10	185 (9.60)	271 (13.6)	343 (16.5)	157 (11.2)	
	15.1 mg/m3	10	187 (8.99)	249 (13.0)	**312 (21.9)***	**124 (21.0)***	
	54 mg/m3	10	187 (9.42)	**239 (20.6)***	**301 (29.4)***	**115 (25.5)****	
	152 mg/m3	10	187 (4.56)	**243 (12.7)****	**308 (22.6)***	**120 (23.0)***	

	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November, 2017
Materials and Methods	Point 3.1.2.3: Stability is declared by the applicant. However, there is no relevant information in the original study report. Point 3.2.5: According to the original study report, on day of ramdomisation the animals were slightly younger (approx. 5 weeks old) that indicated in the OECD GD 412 (7-9 weeks). This change in study protocol is considered of minor toxicological significance and does not compromise the reliability of the study. Point 3.3.2: The animals were exposed whole body, 4-hours per day instead of 6-hours per day as proposed in the OECD GD 412. However, since the exposure was for 7-days per week, instead of 5 days per week proposed by OECD, it may overall be concluded that the change in study protocol is not expected to have an effect in the overall quality of the study (see Point 2.3). **Point 3.3.4.1:** A conversion of the doses tested in mg/m^3 to mg/kg b.w./day is performed as indicated in Table R.8-2 of the relevant REACH Guidance document [1]: $NOAEL_{oral} = \frac{\text{Breathing rate} \times NOAEC_{inhalation}}{ABS_{oral} / ABS_{inhalation}}$ where, - ABS_{oral} = 26% (see Doc. IIA and Assessment report) - The breathing rate of 0.8 L/min/kg bw in rats was adjusted for the 4-hour duration of daily exposure in the study, as follows: 0.8 L/min/kg bw x (4h/day x 60 min/h) = 192 L/kg bw/day.

mg/L	0.015	0.054	0.152
mg/kg b.w./day	11.1	39.9	112.3

This conversion is necessary for comparison of the study doses and outcome with the oral studies.

Results and discussion

Point 4.2: Although significant decrease in bodyweight (↓6.3%) and bodyweight gain (↓15%) was observed in males of the 15.1 mg/m^3 dose group, this was not considered to be adverse at this dose level. This is because the effect was not confirmed in the following study performed according to the same protocol (Kohda *et al.*, 1984; Doc IIIA 6.3.3/2), where there was no effect on body weight up to 20.2 mg/m^3 (highest dose).

Point 4.5.1: The statistically significant increase in MCV at 152 mg/m^3 is of questionable toxicological significance.

Table 4.5.1-1 MCV changes in rats after 4-week inhalation exposure to cyphenothrin

	Sex	Group				
		Vehicle control	**Negative control**	**15.1 mg/m^3**	**54.0 mg/m^3**	**152 mg/m^3**
MCV (μ^3)	Males	53.3	52.2 *	52.9	53.3	54.5 *
	Females	55.3	55.1	55.7	53.8 *	54.0

[1] ECHA, Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health, Version: 2.1 November 2012

MCV: Mean cell volume, * : P < 0.05,

Point 4.5.2: Changes in clinical chemistry parameters are included in tables 4.5.2-1 a & b, for clarity.

Table 4.5.2-1a Changes in clinical chemistry parameters in male rats after 4-weeks inhalation exposure with cyphenothrin

Clinical chemistry parameter	Group				
	Vehicle control	Negative control	15.1 mg/m^3	54.0 mg/m^3	152 mg/m^3
Na (meq/l)	148	141*	139	140	140
K (meq/l)	4.6	4.71	4.63	4.36 *	4.53
Ca (mg/dl)	10.3	10.3	10.4	10.2	10.1 *
Total protein (g/dl)	6.10	6.06	6.01	5.86 #	5.88 *
Albumin (g/dl)	2.93	2.74 #	2.99	2.78 #	2.86
Glucose (mg/dl)	136	156 #	123 *	131	126
BUN (mg/dl)	18.6	17.0	17.4 *	17.8	17.0 *
Bilirubin (mg/dl)	0.14	0.14	0.13	0.14	0.11
Cholesterol (mg/dl)	51.2	50.0	47.1	54.4	42.2 *
AP (I.U./l)	453	468	468	450	421
GOT (I.U./l)	93.2	107	104	92.7	104 *
GPT (I.U./l)	42.0	45.3	39.8	38.7	36.8
CHE (I.U./l)	698	696	694	699	688

BUN: Blood Urea Nitrogen, AP: Alkaline phosphatase, GOT: glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase, CHE: Cholinesterase
* : P < 0.05, # : P < 0.01

Table 4.5.2-1b Changes in clinical chemistry parameters in female rats after 4-weeks inhalation exposure with cyphenothrin

Clinical chemistry parameter	Group				
	Vehicle control	Negative control	15.1 mg/m^3	54.0 mg/m^3	152 mg/m^3
Na (meq/l)	140	140	140	140	140
K (meq/l)	4.03	4.60 #	4.14	4.26	4.29
Ca (mg/dl)	10.7	10.5	10.5	10.7	10.4 #
Total protein (g/dl)	5.57	6.26 #	6.42	6.44	6.39
Albumin (g/dl)	3.24	2.92 #	3.10	3.14	3.09 *
Glucose (mg/dl)	113	120	118	116	121
BUN (mg/dl)	21.5	16.5 #	21.4	17.9 *	19.9
Bilirubin (mg/dl)	0.14	0.11	0.13	0.11	0.12
Cholesterol (mg/dl)	63.9	60.6	65.0	68.7	68.1
AP (I.U./l)	262	294	290	301 *	286
GOT (I.U./l)	105	102	94.4	109	91.2
GPT (I.U./l)	28.8	32.5 *	27.8	29.5	28.9
CHE (I.U./l)	2577	3471	2406	1670 #	1884 *

BUN: Blood Urea Nitrogen, AP: Alkaline phosphatase, GOT: glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase, CHE: Cholinesterase
* : P < 0.05, # : P < 0.01

Point 4.6.1: Changes in organ weights are included in tables 4.6.1-1 a & b, for clarity.

Table 4.6.1-1a Changes in organ weights in male rats after 4-weeks inhalation exposure with cyphenothrin

Organ weight		Group				
		Vehicle control	Negative control	15.1 mg/m³	54.0 mg/m³	152 mg/m³
Final body weight (g)		333	343	312 *	301 *	308 *
Brain	Abs (g)	2.02	1.97	1.97	1.98	1.99
	Rel.	0.64	0.58	0.64	0.66 *	0.65 *
Lungs	Abs (g)	1.37	1.34	1.33	1.31	1.37
	Rel.	0.41	0.39	0.43	0.44	0.45 *
Heart	Abs (g)	1.06	1.05	0.98	0.91 #	1.01
	Rel.	0.32	0.31	0.31	0.30 #	0.33
Spleen	Abs (g)	0.63	0.66	0.59	0.57	0.58
	Rel.	0.19	0.19	0.19	0.19	0.19
Liver	Abs (g)	10.7	10.2	9.80	9.22 *	9.40 *
	Rel.	3.20	2.99 *	3.15	3.05	3.05
Kidney	Abs (g)	2.31	2.21	2.19	2.18	2.20
	Rel.	0.69	0.64 *	0.70	0.72	0.71
Testis	Abs (g)	3.34	3.30	3.31	3.19	3.08
	Rel.	1.00	0.96	1.07	1.06 *	1.01
Pituitary	Abs (g)	9.20	10.1	10.6	12.6 *	10.0
	Rel.	2.76	2.94	3.39	3.86 #	3.25 *
Thyroid	Abs (g)	16.7	15.2	15.4	15.3	15.7
	Rel.	5.02	4.43	4.93	5.13	5.11
Adrenal	Abs (g)	53.1	51.5	55.3	55.2	59.3
	Rel.	16.0	15.1	17.7	18.4 *	19.3 #

Abs.: Absolute, Rel. : ratio of organ weight to 100g body weight
* : P < 0.05, # : P < 0.01

Table 4.6.1-1b Changes in organ weights in female rats after 4-weeks inhalation exposure with cyphenothrin

Organ weight		Group				
		Vehicle control	Negative control	15.1 mg/m³	54.0 mg/m³	152 mg/m³
Final body weight (g)		203	213	199	192	200
Brain	Abs (g)	1.89	1.94	1.88	1.88	1.88
	Rel.	0.94	0.91	0.95	0.94	0.94
Lungs	Abs (g)	1.03	1.03	1.12	1.05	1.15 *
	Rel.	0.51	0.49	0.56 *	0.55	0.58 *
Heart	Abs (g)	0.71	0.76	0.65	0.67	0.69
	Rel.	0.35	0.36	0.32	0.35	0.35
Spleen	Abs (g)	0.48	0.51	0.45	0.46	0.46
	Rel.	0.23	0.24	0.22	0.24	0.23
Liver	Abs (g)	6.14	5.67	6.15	5.87	6.31
	Rel.	3.02	2.66 #	3.09	3.05	3.15
Kidney	Abs (g)	1.51	1.57	1.50	1.48	1.52
	Rel.	0.74	0.74	0.75	0.77	0.76
Ovaries	Abs (mg)	61.4	60.1	56.4	55.9	59.3
	Rel.	30.1	28.1	28.3	29.1	29.6
Pituitary	Abs (g)	11.6	13.7	11.2	12.7	11.3
	Rel.	5.73	6.42	5.62	6.60	5.62
Thyroid	Abs (g)	11.1	12.7	11.0	13.5	12.1
	Rel.	5.48	5.99	5.52	7.04 *	6.04
Adrenal	Abs (g)	65.3	68.5	65.8	74.2 *	67.3
	Rel.	32.1	32.2	33.0	38.6 #	33.6

Abs.: Absolute, Rel. : ratio of organ weight to 100g body weight
* : P < 0.05, # : P < 0.01

Point 4.6.2: Slight increase in the incidence of lymphocytic infiltration was observed in males and females of the top dose group. Further, slight increase in the incidence of epididymidal and testicular atrophy with decrease in spermatogenesis was observed in one male of the 152 mg/m³ dose group. Due to

low incidence the finding was not attributed to cyphenothrin administration. However, it should be noted that individual data for organ histopathology, and spermatogenesis data were not included in the original study report.

Table 4.6.2-1 Summary of histopathological changes observed in male and female rats after 4-weeks inhalation exposure with cyphenothrin

Organ	Finding	Group				
		Vehicle control	**Negative control**	**15.1 mg/m³**	**54.0 mg/m³**	**152 mg/m³**
Males						
Liver	Cytoplasmic vacuolation	2 (1.0)	10 (1.0)	2 (1.0)	1 (1.0)	3 (1.0)
	Lymphocytic infiltration	1 (1.0)	3 (1.0)	3 (1.0)	0	1 (1.0)
Epididymis	Atrophy	0	0	0	0	1 (2.0)
Testis	Atrophy of seminiferous tubules	0	0	0	0	1 (3.0)
Females						
Liver	Cytoplasmic vacuolation	2 (1.0)	10 (1.1)	1 (1.0)	1 (1.0)	3 (1.0)
	Lymphocytic infiltration	0	1 (1.0)	0	0	2 (1.5)

(..) Values in brackets indicate the severity grade of the finding

Conclusion NOAEL systemic toxicity < 15.1 mg/m³ (~ 11.1 mg/kg bw/day), based on irregular respiration in both sexes in the 15.1 mg/m³ dose group.

NOAEL local toxicity < 15.1 mg/m³ (~ 11.1 mg/kg bw/day), based slight nose discharge in males in the 15.1 mg/m³ dose group.

Reliability 2

Acceptability Acceptable

Remarks Deviations from the study protocol are described in Points 3.2.5 and 3.3.2, above, and are not considered to have a major impact on study reliability.

The original study report submitted did not include individual data for organ histopathology and spermatogenesis data.

SECTION A6.3.3/02 REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION
Annex Point IIA6.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	**Reference: A6.3.3/02**	
		[redacted] (1984) Subacute Inhalation Toxicity of [redacted] in Rats. Laboratory of Biochemistry and Toxicology, Takarazuka Research Center, Sumitomo Chemical Co., LTD., Hyogo, Japan. December 20, 1984.	
1.2	**Data protection**	Yes	
1.2.1	Data owner		
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection		
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Methods used comparable to guidelines US EPA, Health Effects Test Guideline 82-4, Subdivision F and Equivalent to EC Guideline B.29: OECD Guideline for Testing of Chemicals, Number 412 Subacute Inhalation Toxicity: 28-Day Study (2009)	
2.2	**GLP**	Yes, Study was validated to indicate compliance with GLPs	
2.3	**Deviations**	Yes, exposures were 4 hours per day instead of 6 hours per day.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification		
3.1.2.1	Description	Brownish yellow viscous oily liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rats	
3.2.2	Strain	Sprague Dawley	
3.2.3	Source	Shizuoka Laboratory Animal Center – Shizuoka, Japan	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	approximately 6 weeks	X
3.2.6	Number of animals per group	20 animals per group with 10 animals per sex per group	
3.2.7	Control animals	Yes, vehicle control animals were exposed to deodorized kerosene and negative control animals were assumed to be exposed to ambient air.	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	28 days	

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SECTION A6.3.3/02 **Annex Point IIA6.3**		**REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION**	
3.3.2	Frequency of exposure	Daily 4 hour exposures for 28 days	X
3.3.3	Postexposure period	no	
3.3.4	**Inhalation**		
3.3.4.1	Concentrations	Nominal concentration 13.1 and 26.2 [mg/m³] Analytical concentration 7.76 and 20.2 [mg/m³]	X
3.3.4.2	Particle size	The distribution of aerodynamic diameter of mist particles in the exposure chamber was tried to measure using Microscopic Sedimentation Analyzer (SA-MID, Shimadzu Corporation). However, particle size of the test material could not be determined because the concentration of particles in each chamber was below the minimum detection limit of the above apparatus. However, the mist generator used for the low-doses was the same type as generator used for the high-dose in the previous study (Subacute Inhalation Toxicity of S-2703 Forte in Rats (1983)). Therefore, it is believed that there was no significant difference in the particle size of mist particles generated at any dose in the subacute inhalation studies.	
3.3.4.3	Type or preparation of particles	Test article was injected into a specially-prepared glass atomizer and sprayed under compressed air. The mists generated were transported into the exposure chamber after removal of larger sized particles while passing through two bottles connected in series. The flow rate of the exhausted air was adjusted by means of a vacuum pump in order to keep a constant pressure.	
3.3.4.4	Type of exposure	Whole body	
3.3.4.5	Vehicle	Deodorized kerosene	
3.3.4.6	Concentration in vehicle	[redacted] in solution (deodorized kerosene) to achieve 7.76 and 20.2 mg/m3, respectively.	
3.3.4.7	Duration of exposure	4 h	
3.3.4.8	Controls	Control animals were exposed to only deodorized kerosene using an exposure regimen equivalent to the test substance exposures. Negative control animals were assumed to be exposed to ambient air, but not clearly stated in the report.	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes Prior to exposure – 0, 0.5, 1, 2, 3, and 4 hours of exposure and hourly until toxic signs disappeared after exposure (up to 4hr).	
3.4.1.2	Mortality	Yes Prior to exposure – 0, 0.5, 1, 2, 3, and 4 hours of exposure and hourly until toxic signs disappeared after exposure (up to 4hr).	
3.4.2	Body weight	Yes Individual body weights were taken twice a week at intervals of 3 or 4 days.	
3.4.3	Food consumption	Yes Food consumption was recorded for 2 consecutive days per week for the entire study.	

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SECTION A6.3.3/02 **Annex Point IIA6.3**		**REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION**
3.4.4	Water consumption	Yes Water consumption was recorded for 2 consecutive days per week for the entire study.
3.4.5	Ophthalmoscopic examination	Yes Ophthalmological examinations were made on the day of the final exposure, of both eyes of surviving animals.
3.4.6	Haematology	Yes Blood was collected from all animals after the final exposure following an overnight fast. Blood was collected from anesthetized animals from the abdominal aorta.
3.4.7	Clinical Chemistry	Yes Blood was collected from all animals after the final exposure following an overnight fast. Serum was separated from the remaining blood sample after the hematology and analysed on a SMAC.
3.4.8	Urinalysis	Yes Fresh urine samples were collected on individual animals on day 27 of the study
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes organs: liver, kidneys, adrenals, testes, ovaries, pituitary, thyroid, spleen, brain, lungs, and heart.
3.5.2	Gross pathology	Yes all dose groups had the following organs examined: eye, spinal cord, sciatic nerve, nasal cavity, trachea, bone marrow, mandibulary lymph node, mesenteric lymph node, tongue, esophagus, stomach, small intestine, large intestine, salivary gland, pancreas, Urinary bladder, epididymus, uterus, prostate, preptuial gland, skin, liver, kidneys, adrenals, testes, ovaries, pituitary, thyroid, spleen, brain, lungs, and heart.
3.5.3	Other examinations	None Histopathological examination was not performed.
3.5.4	Statistics	The mean values of the dose groups were statistically analysed for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the vehicle control group by a student's t-test on the body weight and body weights gain, food consumption, water consumption, hematology, biochemistry, organ weight and its ratio to body weight. Urinalysis was analyzed by U-test (Mann-Whitney).
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Decrease activity with the same degree as the vehicle control group and irregular respiration were observed in the 20.2 mg/m3 group. Slight nose discharge and slight urinary in continence were also observed sporadically. These effects were disappeared within one hour after exposure. No cumulative effects were observed. No toxic signs were observed in 7.76mg/m3 exposure group.
4.1.2	Mortality	No deaths were observed at any dose throughout the entire study.
4.2	**Body weight gain**	In both sexes, there was no differnece in body weight between the

SECTION A6.3.3/02 Annex Point IIA6.3		REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION	
		vechicle control group and any dose group.	
4.3	**Food and water consumption and compound intake**	In both sexes, there was no differnece in food consumption between the vechicle control group and any dose group. In male, there was no remarkable differecne in water consumption between vehicle control group and any dose group. In females, each dose group gave higher water consumotion at the 4th week, compared with the vehicvle control group. However, this chage was considered due to the fact that water consumption of the vehicle control group at the 4th weeek was lower than that at the each weeks.	
4.4	**Ophthalmoscope examination**	No remarkable changes attributable to the exposure to S-2703 Forte were observed in any dose group.	
4.5	**Blood analysis**		
4.5.1	Haematology	A higher thrombocyte count was observed in 20.2mg/m3 females. However, this change was very slight in extent and within the physiological range observed in our laboratory with this strain at the same age. Therefore, this change was considered not to be toxicologically significant.	
4.5.2	Clinical chemistry	Lower potassium concentration in 20.2 mg/m3 males and lower glutamic oxalacetic transaminase (GOT) activity in 20.2 mg/m3 females were observed. However, this change was very slight in extent and within the physiological range observed in our laboratory with this strain at the same age. Therefore, this change was considered not to be toxicologically significant.	
4.5.3	Urinalysis	No remarkable changes were observed in either male or female animals at any dose tested.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	A lower absolute brain weight (males – 7.76 and 20.2 mg/m3), higher absolute and relative lung weights and thyroid weights (female – 7.76 and 20.2 mg/m3 groups), lower relative kidney weight (males – 20.2mg/m3) and higher relative kidney weight (female – 20.2mg/m3) were observed. Among the above changes, absolute and relative thyroid weight in females showed a trend of dose - dependency. However, these changes were very slight in extent and within the physiological range. Therefore, they were considered not to be toxicologically significant.	X
4.6.2	Gross pathology	No findings were considered to be test article related. Changes observed were considered to be consistent with those common findings for the rat strain and age.	
4.7	**Other**	None Histopathological examination was not conducted in this study because there were no significant histopathological changes in the previous study which concentrations were higher than the concentrations in this study (Section A6.3.3/1).	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and**	This study was conducted according to US EPA FIFRA guideline 82-4, equivalent to EC B.29 Subacute Inhalation Toxicity (28 days), OECD	

SECTION A6.3.3/02 REPEATED DOSE TOXICITY – 28-DAY, RAT, INHALATION
Annex Point IIA6.3

	methods	412. It was reported that the no-affect level of S-2703 Forte in a subacute inhalation toxicity study in rats was found to be slightly lower than 15.1mg/m3 for both sexes in previous study (Subacute Inhalation Toxicity of S-2703 Forte in Rats (1983)). Therefore, this study was conducted to examine the no-effect level of S-2703 Forte for subacute inhalation toxicity in rats. Animals were divided into 4 groups with a vehicle control, negative control, low and high dosed groups. Ten males and ten females per treatment group were exposed to test article for 4 hours per day for 28 consecutive days. A deviation from the guideline was that the animals were exposed for 4 hours daily instead of for 6 hours per day.
5.2	**Results and discussion**	Clinical signs observed during the 28 days of exposures included decrease activity (identical to vehicle control animals), irregular respiration, slight nose discharge and urinary incontinence, and they were observed in both sexes in the 20.2 mg/m3 dose group. Comparing above findings with toxic signs observed in rats which were exposed to 15.1mg/m3 S-2703 Forte in the previous study, there was no distinct difference in toxic signs between 20.2mg/m3 and 15.1mg/m3, except urinary incontinence being observed in this study. Urinary incontinence was considered to be attributable to S-2703 Forte because this toxic sign was observed at higher exposure level of S-2703 Fore (54 mg/m3 and more). Although some sight changes were observed in hematology, biochemistry and organ weight in 20.2 mg/m3, those changes did not show a reproducibility of the changes observed at higher exposure level (152mg/m3 S-2703 Forte) in the previous study. No changes from controls were noted for any of the animals at any dose tested for body weight, food and water consumption, urinalysis, ophthalmology, hematology, clinical chemistry, organ weights, and gross pathological evaluations.
5.3	**Conclusion**	The no-observable-adverse-effect level (NOAEL) is 7.76 mg/m3 based on an increased incidence and frequency of clinical symptoms and a slight decrease in bodyweight and bodyweight gain observed in male animals at 20.2 mg/m3.
5.3.1	LO(A)EL	LO(A)EL: 20.2 mg/m3
5.3.2	NO(A)EL	NO(A)EL: 7.76 mg/m3
5.3.3	Other	-
5.3.4	Reliability	2
5.3.5	Deficiencies	No

Clinical Findings: Vehicle Control Animals

		Clinical Signs	Day 1	Day 7	Day 14	Day 28	Comments
Vehicle Control	Male/Female	No. of animals examined	10/10	10/10	10/10	10/10	
		Decr Activity	0/0	0/0	3/4	7/7	

Clinical Findings: 7.76 mg/m3 Treated Animals

		Clinical Signs	Day 1	Day 7	Day 14	Day 28	Comments
7.76 mg/m3	Male/Female	No. of animals examined	10/10	10/10	10/10	10/10	
		Decr Activity	0/0	0/0	3/3	7/6	

Clinical Findings: 20.2 mg/m3 Treated Animals

		Clinical Signs	Day 1	Day 7	Day 14	Day 28	Comments
20.2 mg/m3	Male/Female	No. of animals examined	10/10	10/10	10/10	10/10	
		Decr Activity	2/2	6/7	5/6	8/6	
		Irreg Resp.	4/2	6/5	6/4	4/3	
		Nasal Discharg	4/1	0/0	0/0	4/0	
		Urinary incontinence	0/0	0/1	0/0	0/0	

Evaluation by Competent Authorities

USE SEPARATE "EVALUATION BOXES" TO PROVIDE TRANSPARENCY AS TO THE COMMENTS AND VIEWS SUBMITTED

Evaluation by Rapporteur Member State

Date

November, 2017

Materials and Methods

Point 3.1.2.3: Stability is declared by the applicant. However, there is no relevant information in the original study report.

Point 3.2.5: According to the original study report, on day of ramdomisation the animals were slightly younger (approx. 5 weeks old) that indicated in the OECD GD 412 (7-9 weeks). This change in study protocol is considered of minor toxicological significance and does not compromise the reliability of the study.

Point 3.3.2: The animals were exposed whole body, 4-hours per day instead of 6-hours per day as proposed in the OECD GD 412. However, since the exposure was for 7-days per week, instead of 5 days per week proposed by OECD, it may overall be concluded that the change in study protocol is not expected to have an effect in the overall quality of the study (see Point 2.3).

Point 3.3.4.1: A conversion of the doses tested in mg/m^3 to mg/kg b.w./day is performed as indicated in Table R.8-2 of the relevant REACH Guidance document [2]:

$$\text{NOAELoral} = \frac{\text{Breathing rate x NOAEC}_{\text{inhalation}}}{\text{ABS}_{\text{oral}} / \text{ABS}_{\text{inhalation}}}$$

where,

- ABS_{oral} = 26% (see Doc. IIA and Assessment report)
- The breathing rate of 0.8 L/min/kg bw in rats was adjusted for the 4-hour duration of daily exposure in the study, as follows:
  0.8 L/min/kg bw x (4h/day x 60 min/h) = 192 L/kg bw/day.

mg/L	0.00776	0.0202
mg/kg b.w./day	5.73	14.92

This conversion is necessary for comparison of the study doses and outcome with the oral studies.

Only two doses were considered instead of at least 3 recommended by the OECD test guideline. This study may be acceptable as supplementary to the previous study by Kohda *et al.*, 1983 (Doc IIIA 6.3.3/1).

Results and discussion

Point 4.2: Body weight data are included to assist evaluation of the finding:

Table 4.2-1 Body weight and Body weight gain data in male and female rats after 4-weeks inhalation exposure with cyphenothrin

Sex	Treatment Group	No of Rats	Body Weight, Grams (SD)			Days 0-28 Bodyweight Gain, Grams
			Day 0	Day 14	Day 27	
Males	Vehicle Control	10	197 (8.17)	273 (17.0)	323 (23.8)	125 (18.8)
	Negative Control	10	199 (5.05)	279 (11.7)	338 (13.7)	138 (11.4)
	7.76 mg/m^3	10	198 (7.32)	272 (10.5)	319 (15.9)	121 (14.9)
	20.2 mg/m^3	10	199 (7.23)	271 (12.3)	322 (16.8)	122 (12.1)

[2] ECHA, Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health, Version: 2.1 November 2012

Females	Vehicle Control	10	135 (4.53)	174 (5.92)	197 (8.82)	62.1 (7.57)
	Negative Control	10	136 (5.38)	181 (7.67)*	207 (12.0)*	71.3 (8.41)*
	7.76 mg/m^3	10	135 (5.24)	177 (10.7)	197 (12.0)	62,1 (10.1)
	20.2 mg/m^3	10	133 (6.14)	171 (10.2)	195 (15.0)	62.5 (11.8)

Point 4.6.1: Changes in organ weights are included in tables 4.6.1-1 a & b, for clarity.

Table 4.6.1-1a Changes in organ weights in male rats after 4-weeks inhalation exposure with cyphenothrin

Organ weight		Group			
		Vehicle control	**Negative control**	**7.76 mg/m^3**	**20.2 mg/m^3**
Final body weight (g)		323	338	319	322
Brain	Abs (g)	2.01	1.98	1.94 #	1.96 *
	Rel.	0.63	0.59	0.61	0.61
Lungs	Abs (g)	1.22	1.27	1.23	1.24
	Rel.	0.38	0.37	0.39	0.39
Liver	Abs (g)	9.43	9.48	9.20	8.94
	Rel.	2.91	2.80	2.89	2.78
Testis	Abs (g)	3.22	3.25	3.16	3.13
	Rel.	1.00	0.96	0.99	0.97
Pituitary	Abs (mg)	10.4	11.5 *	10.2	10.9
	Rel.	3.22	3.43	3.22	3.37
Thyroid	Abs (mg)	14.2	15.3	14.5	14.4
	Rel.	4.41	4.54	4.56	4.45
Adrenal	Abs (mg)	52.3	54.3	55.2	57.0
	Rel.	16.3	16.1	17.3	17.7

Abs.: Absolute, Rel. : ratio of organ weight to 100g body weight
* : P < 0.05, # : P < 0.01

Table 4.6.1-1b Changes in organ weights in female rats after 4-weeks inhalation exposure with cyphenothrin

Organ weight		Group			
		Vehicle control	**Negative control**	**7.76 mg/m^3**	**20.2 mg/m^3**
Final body weight (g)		197	207 *	197	195
Brain	Abs (g)	1.82	1.86	1.86	1.84
	Rel.	0.92	0.90	0.94	0.95
Lungs	Abs (g)	0.89	0.96 *	**0.98 #**	**0.97 ***
	Rel.	0.45	0.46	**0.50 #**	**0.50 #**
Liver	Abs (g)	5.30	5.45	5.53	5.51
	Rel.	2.68	2.63	2.81	2.82
Ovaries	Abs (mg)	61.0	66.7	71.4 *	66.6
	Rel.	31.0	32.1	36.6 *	34.1
Pituitary	Abs (mg)	12.9	14.0	13.2	13.6
	Rel.	6.54	6.74	6.66	6.96
Thyroid	Abs (mg)	10.7	12.2	**13.5 #**	**13.6 #**
	Rel.	5.42	5.89	**6.89 ***	**7.00 #**
Adrenal	Abs (mg)	61.0	61.7	65.5	66.6
	Rel.	31.0	29.8	33.3	34.3

Abs.: Absolute, Rel. : ratio of organ weight to 100g body weight
* : P < 0.05, # : P < 0.01

A statistically significant dose-related increase in the absolute and relative thyroid weight was observed in females treated with cyphenothrin.
A statistically significant increase in the absolute and relative lung weight was observed in females treated with cyphenothrin.
Changes in organ weights in females were not accompanied by relevant gross pathology

	findings and were not observed in males. Their toxicological relevance is questionable. It is noted that histopathological examination was not conducted in this study because there were no significant histopathological changes in the previous study which concentrations were higher than the concentrations in this study (Section A6.3.3/1).
Conclusion	NOAEL systemic toxicity = 7.76 mg/m^3 (~ 5.73 mg/kg bw/day), based on irregular respiration in both sexes and urinary incontinence in females at 20.2 mg/m^3. NOAEL local toxicity = 7.76 mg/m^3 (~ 5.73 mg/kg bw/day), based on slight nose discharge in both sexes in the 20.2 mg/m^3 dose group Based on adverse effects in a rat 29-day inhalation toxicity study, i.e. irregular respiration and/or sporadic nose discharge (males only) observed from the dose of 0.015 mg cyphenothrin/L air (equiv. 15.1 mg/m3, whole body exposure, 4 h/day, 7 days/week), STOT-RE 1 (inhalation, mist) is proposed. It is noted that according to CLP, Category 1 classification of mists is applicable when significant toxic effects are observed in a rat 90-day repeated-dose study at ≤ 0.02 mg/L/6h/day. Although the repeated dose inhalation toxicity study in rats with cyphenothrin was a 29-day study, the eCA considers the guidance value still relevant, since it represents a worst-case (90-day inhalation exposure would lead to effects at same or lower doses).
Reliability	2
Acceptability	Acceptable as supplementary to the study by Kohda *et al*., 1983 (Doc IIIA 6.3.3/1)
Remarks	Deviations from the study protocol are described in Points 3.2.5 and 3.3.2, above, and are not considered to have a major impact on study reliability. Further, only two doses were considered instead of at least 3 recommended by the OECD test guideline. Histopathological examination was not conducted in this study. The study is considered as supplementary to the previous 28-day inhalation toxicity study.

6.4 Subchronic toxicity

6.4.1 Subchronic oral toxicity test

			Official use only
		1. REFERENCE	
1.1	**Reference**	Reference : A6.4.1/01 Authors : [redacted] Title: [redacted]: Thirteen week range finding toxicity study in rats Laboratory : Life Sciences Research Ltd, UK Unpublished Report no : [redacted] Date : September 1985	
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 existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes – US -EPA	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No	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]	X
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	5 weeks 90 - 94g (male) and 82-85g (female)	X
3.2.6	Number of animals per group	10/sex/dose	
3.2.7	Control animals	Yes: 10/sex/dose	
3.3	**Administration/ Exposure**	Oral	

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3.3.1	Duration of treatment	13 weeks	
3.3.2	Frequency of exposure	daily	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	Dietary	
3.3.4.2	Concentration	0, 100, 300, 1000 or 2000 ppm *ad libitum*	X
3.3.4.3	Vehicle	None	
3.3.4.4	Concentration in vehicle	Not applicable	
3.3.4.5	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes Rats were inspected twice daily for evidence of reaction to treatment or ill-health. In addition, all animals were handled and palpated once weekly throughout.	
3.4.1.2	Mortality	Yes Each cage was inspected for the presence of dead or moribund animals twice daily.	
3.4.2	Body weight	Yes Each animal was weighed on the day after arrival, on the day treatment commenced (Day 0), after 3, 7, 10 and 14 days, and at weekly intervals thereafter.	
3.4.3	Food consumption	Yes The weight of food consumed by each rat was recorded weekly, over consecutive seven day periods.	
3.4.4	Water consumption	Yes Water consumption was assessed daily by visual inspection of the water bottles at approximately 10.00 hours. Accurate measurements were performed during Weeks 1, 6 and 11 of treatment, when the water consumption of all surviving animals was measured over five day periods.	
3.4.5	Opthalmoscopic examination	Yes Before commencement of treatment both eyes of all rats assigned to the study were examined using a Fison binocular indirect ophthalmoscope, after instillation of 0.5% tropicamide. Animals with non-resolving lesions were replaced. After 12 weeks of treatment all survivng animals were similarly examined.	
3.4.6	Haematology	Yes Number of animals: All animals (100) Time points : After 12/13 weeks of treatment. The following parameters were examined: Packed cell, Haemoglobin concentration, Erythrocyte count (RBC), Leucocyte count (WBC) ((total), Leucocyte count (WBC) (differential), Platelet count	

3.4.7	Clinical chemistry	Yes Number of animals: All animals (100) Time points : After 12/13 weeks of treatment. The following parameters were examined: Glucose concentration , Alkaline phosphatase, Alanine amino-transferase activity (ALT), Aspartate amino-transferase activity, Creatine phosphokinase activity (CPK), Lactate dehydrogenase activity (total) (LDH-L), Total protein concentration, Electrophoretic protein fractions, Sodium (Na) and Potassium (K) concentrations, Chloride concentration (Cl), Calcium concentration (Ca), Phosphorus (inorganic) concentration (P), Urea concentration, Creatinine concentration, Total Bilirubin concentration and Total Cholesterol concentration	
3.4.8	Urinalysis	Yes Number of animals: All animals (100) Time points : After 12/13 weeks of treatment. The following parameters were examined: Appearance, Specific gravity (SG), Glucose, Protein, Ketones, Blood, Bilirubin, Urobilin, Total reducing substances (IRS),	X
		Microscopy — the sediment from centrifugation at approximately 3000 rpm for ten minutes was examined for epithelial cells (E), polymorphonuclear leucocytes(P), red blood cells (R), spermatozoa (5), crystals (C) or other abnormalities (A).	X
3.5	**Sacrifice and pathology**		
3.5.1	Organ weights	Yes adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus, thyroid (with parathyroid)	
3.5.2	Gross and histopathology	Yes High and intermediate dose groups Organs: adrenals, aorta arch, brain, caecum, colon, duodenum, femoral bone and marrow, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes, oesophagus, ovaries, pituitary, rectum, salivary glands, sciatic nerve, spleen, stomach, testes, thyroid with parathyroids, trachea, urinary bladder, uterus/cervix	
3.5.3	Other examinations		
3.5.4	Statistics	Intergroup differences in bodyweight gain and blood composition were assessed by a series of Student' t-tests using pooled within treatment error variance. A least significant difference was calculated at the 99.9%, 99% and 95% confidence levels. Intergroup differences in organ weights, expressed in absolute terms or relative to bodyweight were assessed by the test of Dunnett	
3.6	**Further remarks**		
		4. RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Severe body tremors and irritability were noted amongst rats receiving 2000 ppm during the early weeks of the study; irritability was also observed amongst rats receiving 1000 ppm. Lateral displacement of hindlimbs, piloerection, facial and urinogenital staining and tail lesions were common features amongst rats receiving 2000 ppm.	

4.1.2	Mortality	All male rats receiving 2000 ppm died or were killed on humane grounds during the first six weeks of treatment. Four female rats receiving 2000 ppm died or were killed on humane grounds. One male rat receiving 300 ppm died at blood sampling during Week 13.	
4.2	**Body weight gain**	Rats receiving 2000 ppm gained less weight than their controls.	
4.3	**Food consumption and compound intake**	Food consumption of male rats receiving 2000 ppm was lower than their controls. Females receiving 1000 or 2000 ppm were similarly affected in the first week. Overall food conversion efficiency was lower amongst female rats receiving 2000 ppm than their controls Water consumption was unaffected by treatment with S-2703F.	
4.4	**Ophthalmoscopic examination**	The incidences of ophthalmic findings were unaffected by treatment with cyphenothrin	
4.5	**Blood analysis**		
4.5.1	Haematology	When compared with the controls, the lymphocyte count of males receiving 100, 300 or 1000 ppm was lower, but that of females receiving 2000 ppm was higher. These conflicting trends render the toxicological significance equivocal	X
4.5.2	Clinical chemistry	Plasma cholesterol levels were slightly lower than those of the controls among male rats receiving 300 or 1000 ppm and among females receiving 1000 or 2000 ppm. Slightly higher urea concentrations than control values were evident in males and females receiving 1000 ppm, and females receiving 2000 ppm. Female rats receiving 2000 ppm also displayed slightly higher plasma alkaline phosphatase, alanine and aspartate amino—transferase activities and plasma phosphorus levels and slightly lower glucose and calcium levels than those of controls. Albumin was higher in males receiving 300 or 1000 ppm.	
4.5.3	Urinalysis	The chemical and cellular composition of the urine was unaffected by treatment.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Organ weight analysis revealed higher adrenal glands weights and bodyweight-relative liver weights in females receiving 2000 ppm than in the controls.	
4.6.2	Gross and histopathology	Treatment with cyphenothrin was without influence on the distribution of macroscopic entities observed. Microscopic examination revealed, among rats receiving 2000 ppm, treatment related changes in the liver of males. In addition there were a number of findings present in rats of both sexes given the highest dosage particularly in the tail, the majority of which were ascribed to trauma.	
4.7	**Other**		
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Groups of 10 male and 10 female F-344 rats received cyphenothrin (95.4%) in the diet at concentrations of 100, 300, 1000 or 2000 ppm for thirteen weeks. A similarly constituted group received diet containing no S-2703F and served to generate contemporaneous control data.	

5.2	**Results and discussion**	Severe body tremors and irritability were noted amongst rats receiving 2000 ppm during the early weeks of the study; irritability was also observed amongst rats receiving 1000 ppm. Lateral displacement of hindlimbs, piloerection, facial and urinogenital staining and tail lesions were common features amongst rats receiving 2000 ppm. All male rats receiving 2000 ppm died or were killed on humane grounds during the first six weeks of treatment. Four female rats receiving 2000 ppm died or were killed on humane grounds. One male rat receiving 300 ppm died at blood sampling during Week 13. Rats receiving 2000 ppm gained less weight than their controls. Food consumption of male rats receiving 2000 ppm was lower than their controls. Females receiving 1000 or 2000 ppm were similarly affected in the first week. Overall food conversion efficiency was lower amongst female rats receiving 2000 ppm than their controls. Water consumption was unaffected by treatment with cyphenothrin. The incidences of ophthalmic findings were unaffected by treatment with cyphenothrin When compared with the controls, the lymphocyte count of males receiving 100, 300 or 1000 ppm was lower, but that of females receiving 2000 ppm was higher. These conflicting trends render the toxicological significance equivocal. Plasma cholesterol levels were slightly lower than those of the controls among male rats receiving 300 or 1000 ppm and among females receiving 1000 or 2000 ppm. Slightly higher urea concentrations than control values were evident in males and females receiving 1000 ppm, and females receiving 2000 ppm. Female rats receiving 2000 ppm also displayed slightly higher plasma alkaline phosphatase, alanine and aspartate amino—transferase activities and plasma phosphorus levels and slightly lower glucose and calcium levels than those of controls. The chemical and cellular composition of the urine was unaffected by treatment. Organ weight analysis revealed higher adrenal gland weights and bodyweight-relative liver weights in females receiving 2000 ppm than in the controls. Treatment with cyphenothrin was without influence on the distribution of macroscopic entities observed. Microscopic examination revealed, amongh rats receiving 2000 ppm, treatment related changes in the liver of males. In addition, there were a number of findings present in rats of both sexes given the highest doage particularly in the tail, the majority of which were ascribed to trauma.	X
5.3	**Conclusion**		
5.3.1	LO(A)EL	300 ppm (clinical chemistry changes in males)	
5.3.2	NO(A)EL	100 ppm (7.6 mg kg^{-1} per day),	
5.3.3	Other		
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

Table A6.4.1-1 Group distribution of rats with significant observations recorded at in vivo examination on one or more occasions

	Control		100ppm		300ppm		1000ppm		2000ppm	
	m	f	m	f	m	f	m	f	m	f
Colonic convulsion	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10
Tremors	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10	10/10	10/10
Irritable	0/10	0/10	0/10	0/10	0/10	0/10	10/10	8/10	10/10	10/10
Hindlimbs laterally displaced	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10	7/10
Facial staining	0/10	2/10	0/10	0/10	0/10	1/10	1/10	2/10	10/10	10/10
Urinogenital staining	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	5/10	7/10
Piloerection	0/10	0/10	0/10	0/10	0/10	0/10	610	3/10	6/10	9/10
Salivation	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	3/10
Tail lesion	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	4/10	5/10

Table A6.4.1-2 Mean Body weight gain(g)

	Control		100ppm		300ppm		1000ppm		2000ppm	
	m	f	m	f	m	f	m	f	m	f
Day 0 -91	210	106	202	104	211	108	208	101	!	80*

* - p<0.001;
!All Group 5 male animals were killed or died by the end of week 6

Table A6.4.1-3 Achieved dosage – group mean values (mg/kg/day)

Week number	Control		100ppm		300ppm		1000ppm		2000ppm	
	m	f	m	f	m	f	m	f	m	f
1	0	0	13.3	14.1	40.0	40.5	132.5	128.9	266.3	284.7
2	0	0	11.1	12.7	33.8	37.3	111.4	118.2	285.5	298.1
3	0	0	9.8	11.1	30.1	32.3	99.4	107.4	267.4	282.8
4	0	0	8.7	10.7	25.8	29.6	89.0	94.6	237.4	240.0
5	0	0	7.7	8.9	23.3	27.3	78.8	88.4	227.6	223.2
6	0	0	7.2	8.5	22.2	25.5	70.3	80.2	!	216.5
7	0	0	6.5	8.2	20.0	24.1	65.0	79.4		201.0
8	0	0	6.1	7.8	18.9	23.8	61.3	78.5		188.8
9	0	0	6.0	7.6	18.2	23.1	59.5	73.1		181.2
10	0	0	6.0	7.7	18.0	24.0	59.1	75.9		174.6
11	0	0	5.8	7.4	17.6	22.8	56.7	72.7		167.4
12	0	0	5.5	7.2	17.0	22.2	55.1	73.9		166.1
13	0	0	5.0	7.1	15.0	21.3	49.6	71.9		167.7

Table A6.4.1-4 Haematology – group mean values after 12/13 weeks

Parameter		Control		100ppm		300ppm		1000ppm		2000ppm	
		m	f	m	f	m	f	m	f	m	f
PCV (%)		51 1	51 1	51 1	51 1	51 1	50 1	50[a] 1	51 1	!	50 1
Hb (g%)		16.2 0.3	16.3 0.3	16.1 0.3	16.2 0.4	16.1 0.3	16.1 0.3	16.0 0.3	16.1 0.3	!	16.1 0.3
RCB mil/cmm		9.42 0.13	8.80 0.10	9.5 0.14	8.76 0.16	9.49 0.18	8.71 0.18	9.53 0.18	8.73 0.18	!	8.70 0.15
MCHC (%)		32 1	32 0	31 1	32 1	32 0	32 1	32 1	31 1	!	32 1
MCV (cμ)		54 1	58 1	54 1	58 1	54[a] 1	58 0	53[c] 1	59 1	!	58 0
MCH (pg)		17 0	19 1	17 0	18 1	17 0	19 1	17[a] 0	18 0	!	19 1
WBC 1000/ cmm	Total	7.4 0.5	4.1 0.3	6.8[a] 0.7	4.5a 0.4	6.6[b] 0.5	4.2 0.3	6.8[a] 0.7	4.3 0.4	!	5.7[c] 0.5
	N	0.9 0.2	0.5 0.2	1.0 0.3	0.6 0.3	1.0 0.3	0.7 0.2	0.9 0.3	0.5 0.1	!	06 0.2
	L	6.5 0.6	3.5 0.3	5.7a 0.8	3.9[a] 0.4	5.6[b] 0.5	3.5 0.4	5.8[a] 0.6	3.7 0.4	!	5.0[c] 0.5
	E	0.1 0.1	0.1 0.1	0.1 0.1	0.0 0.1	0.1 0.1	0.0 0.0	0.1 0.1	0.1 0.1	!	0.1 0.1
	B	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	!	0.0 0.0
	M	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	!	0.0 0.0
	Normoblasts /100 WBC	0 0	1 1	0 0	2 2	0 0	2 2	1 1	1 1	!	0 0
	Platelets 1000/cmm	262 9	265 7	257 11	256 13	264 13	252[a] 14	258 7	253[a] 25	!	256 10

figure beneath is Standard deviation
[a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

EVALUATION BY COMPETENT AUTHORITIES

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

November, 2017

Materials and methods

Point 3.1.2.2: According to the submitted study, the purity of the tested material is 95.6% and not 95.4% as it is stated in this report.

Point 3.2.5: There is an inconsistency regarding animal weight at the beginning of the study. According to the submitted study, the weight range of males and females is 78-101g and 71-92g, respectively.

Point 3.3.4.2: The applicant's version is generally acceptable. However, 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 A6.4.1-5: Average amount of ingested cyphenothrin for 90 days

Dose level (ppm)	Compound ingested (mg/kg bw/day)	
	Males	Females
0	0	0
100	7.6	9.2
300	23.1	27.2
1000	76.0	87.9
2000	256.8	214.8

Point 3.4.8: Regarding Urina nalysis, measurements of urine volume, pH and nitrite were also performed but are not stated in this report. Moreover, there was a typographical error in Microscopy: the correct term is "spermatozoa (S)" instead of "spermatozoa (5)".

Results and discussion

Points 4.5.1 & 5.2: At the top dose (2000 ppm) both sexes had mortalities, therefore, this dose exceeds the Maximum Tolerated Dose (MTD) for cyphenothrin in rats and data derived from these animals (such as WBC count) should not be taken into account in the evaluation of toxicological trends. As a consequence, the RMS does not agree with the applicant's comment: "*When compared with the controls, the lymphocyte count of males receiving 100, 300 or 1000 ppm was lower, but that of females receiving 2000 ppm was higher. These conflicting trends render the toxicological significance equivocal.*". The higher lymphocyte count in the female top dose group may be either linked 1) to the decreased number of females (n=6) compared to the proposed by the B.26 testing method (n=10), due to mortalities, or 2) to the very stressful condition in which these animals were in at that time. No effect on lymphocyte count was noted in females of the other dose groups.
With regard to the statistically significant decrease in lymphocyte counts in males of the 100, 300 and 1000 ppm dose groups, the RMS notes that since the changes were not dose-related, the toxicological significance of the finding is not clear. Moreover no other treatment-related effects on both haematology and the immune system were noted at doses up to 1000 ppm cyphenothrin.

Point 5.2: The RMS considers that a table depicting clinical chemistry changes should be included in the report, as follows:

Table A6.4.1-6: Clinical chemistry – group mean values after 12/13 weeks

Parameter	Sex	0	100 ppm	300 ppm	1000 ppm	2000 ppm
CPK (IU/L)	M	71	69	57 [a]	55 [b]	-
	F	106	88	93	80 [a]	105
Total cholesterol (mg %)	M	61	61	55 [a]	54 [b]	-
	F	88	83 [a]	83	80 [b]	70 [c]
A/G ratio	M	0.8	0.9	0.9 [a]	1.0 [c]	-
	F	0.9	0.8	0.9	0.8	0.8
Total protein (g %)	M	7.5	7.5	7.8 [c]	7.7 [c]	-
	F	7.3	7.4	7.3	7.3	7.1 [b]

a: significantly different from controls, p<0.05
b: significantly different from controls, p<0.01
c: significantly different from controls, p<0.001

Conclusion

LOAEL= 300 ppm (equivalent to 23.1 mg/kg bw/day), based on clinical chemistry changes, i.e. decreased total cholesterol levels (males; females at 1000 ppm), increased albumin, A/G ratio and total protein (males).

NOAEL= 100 ppm (equivalent to 7.6 mg/kg bw/day)

Other conclusions:

- Clinical signs consistent with synthetic pyrethroid toxicity (i.e. tremors, irritability, facial staining and piloerection) were evident at doses of ≥ 1000 ppm cyphenothrin.
- The RMS considers that the top dose of 2000 ppm exceeds the maximum tolerated dose (MTD) for both sexes as evidenced by a high mortality rate (40% in females and 100% in males).
- In general, males seemed to be more severely affected than females.

Reliability

2

Acceptability

Acceptable.

Remarks

Table A6.4.1-3: There are some inconstistencies regarding achieved dosage values at week 10 for the female group of 100 ppm and at week 13 for both groups of 300 ppm. According to the submitted study (p.35) the values should read 7.7, 15.0 and 21.3 instead of 6.6, 16.0 and 21.2, respectively.

Table A6.4.1-4: There are some inconstistencies regarding haematology according to the submitted study. The MCV values of female group of 1000 ppm should read 59.1 instead of 59 0. Total WBC of male groups of 300 ppm and 1000 ppm should read 0.5 and 0.7 instead of 0.7 and 0.5, respectively.

Point 2.1: It is noted that the appropriate experimental protocol is the OECD 408, equivalent to the EU testing method B.26.

Point 2.3:
1. In Haematology, there was no measurement of blood clotting potential.
2. In Organ weights, there was no weightening of epididymides and uterus.

6.4.1/02: 90-day repeated dose toxicity (oral)

			Official use only
		1. REFERENCE	
1.1	**Reference**	Reference : A6.4.1/02 Authors : [redacted] Title: Subacute (13week) oral toxicity study of [redacted] in beagles Laboratory : Shin Nippon Biomedical Laboratories Ltd, Japan Unpublished Report no : [redacted] Date : July 29, 1987	
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 existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No (No suitable OECD Guideline)	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	Viscous yellow liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	dog	
3.2.2	Strain	beagle	
3.2.3	Source	Shin Nippon Biomedical Laboratories Ltd, Japan	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	6-8 months 6.7 - 8.7 kg (male) and 6.1 - 8.5 kg kg (female)	
3.2.6	Number of animals per group	4	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	13 weeks	
3.3.2	Frequency of exposure	daily	
3.3.3	Postexposure period	None	

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3.3.4	**Oral**	
3.3.4.1	Type	Gelatine capsule
3.3.4.2	Concentration	1, 3, 10 and 30 mg/kg/day
3.3.4.3	Vehicle	None
3.3.4.4	Concentration in vehicle	Not applicable
3.3.4.5	Controls	Empty capsule
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes All animals Behavior, nutritional state, fur brightness, color of palpebral and oral mucosa, swelling of palpable lymph nodes, feces and urine excretion, and response to external noise (whistle) and light stimuli (pen-light) were examined once daily during quarantine period and 3 times daily during the administration period.
3.4.1.2	Mortality	Yes All animals 3 times daily during the administration period.
3.4.2	Body weight	Yes Each animal was weighed weekly.
3.4.3	Food consumption	Yes Food intake was calculated daily from the amount supplied and that left over
3.4.4	Water consumption	Yes Water intake was calculated weekly from the amount supplied and that left over.
3.4.5	Opthalmoscopic examination	Yes Eyes were examined daily for gross changes. One week before and 13 weeks after administration of the test article conjunctiva, iris, cornea, and pupillary reflex were examined.
3.4.6	Haematology	Yes Number of animals: All animals Time points : one week before, and 4, 8, and 13 weeks after the initiation of treatment The following parameters were examined: numbers of red and white blood cells, platelets, hematocrit and hemoglobin concentrations, calculations of MCV, MCIH, and MCHC; number of reticulocyte, leucocyte differential In the examination made 13 weeks after the start of administration of the test article, prothrombin time and activated partial thromboplastin time were measured

3.4.7	Clinical chemistry	Yes Number of animals: All animals Time points : one week before, and 4, 8, and 13 weeks after the initiation of treatment The following parameters were examined: Serum GOT, ALP, LDF, LAP, CPK, total bilirubin, total protein, total cholesterol, triglycerides, phospholipid, glucose, BUN, creatinine, inorganic phosphorous, calcium . Serum Na, K and Cl and serum protein fractions	X
3.4.8	Urinalysis	Yes Number of animals: All animals Time points : one week before, and 6 and 13 weeks after the start of administration of the test article: The following parameters were examined: Volume, specific gravity, colour, pH, protein, glucose, ketone bodies, bilirubin, occult blood and urobilinogen. Sediments after centrifugation.	
3.5	**Sacrifice and pathology**		
3.5.1	Organ weights	Yes The pituitary, thyroids (L and R), adrenals (L and R), testes (L and R), ovaries (L and R) , thymus, submaxillary glands (L and R) , spleen, brain (with cerebellum) , heart, lung, liver, kidneys (L and R) , epididymides (L and It) prostate and uterus	
3.5.2	Gross and histopathology	Yes Gross pathological examination The day after the last administration of the test article all animals that had been fasted for 20 hours were killed by exsanguination under anesthesia with sodium pentobarbital for gross observation of organs and tissues. Histopathological examination (light microscopy) The following organs and tissues of all of the control and dosed animals were examined: heart, aorta, spleen, thymus, bone and bone marrow (femur and sternum) , submaxillary and mesenteric lymph nodes, trachea, bronchus, lung, tongue, esophagus, stomach (fundus and pylorus) , small intestine (duodenum, jejunum and ileuin) , large intestine (cecum, colon and rectum) , pancreas, liver, gall bladder, kidney, urinary bladder, testis, epididymis, prostate, ovary, uterus, vagina, pituitary, thyroid, parathyroid, adrenal, cerebrum, cerebellum, brain stem, spinal cord, sciatic nerve, eye with optic nerve, skeletal muscle (in. quadriceps femoris) , skin, mammary gland , and submaxillary gland.	
3.5.3	Other examinations		
3.5.4	Statistics	The results on clinical signs, food and water intake, body weight, urinalysis, hematology, serum biochemistry, organ weights (absolute and relative weights)., and gross and histopathological examinations were analyzed statistically by Pitman's test. The results on the urinary sediment, and clinical signs such as vomiting and tremor which are not measurable were analyzed by Fisher's test. All the data were analyzed by Jonckheer's test for dose response relationship.	
3.6	**Further remarks**		

4. RESULTS AND DISCUSSION

4.1	**Observations**	
4.1.1	Clinical signs	Vomiting was observed at 10 mg/kg and higher, and tremor and paleness or intense redness of the mucous membrane was observed at 30 mg/kg. Vomiting occurred once during the first half of the administration period in one male and one female at 10 mg/kg, and one to four times during the whole administration period in all animals except one at 30 mg/kg: the vomitus was mainly meal mass of 40 to 550 g. Systemic tremor appeared one to seven times during the administration period in three males and one female the tremor appeared about three hours after the administration and disappeared about six hours after. The conjunctiva and the oral mucous membrane became slightly pale between 43 to 58 days in two males and the same mucous membranes were found slightly red from the day 45 to the end of administration in two males and two females. Abnormal signs were not observed at 3 or 1 mg/kg.
4.1.2	Mortality	Death did not occur.
4.2	**Body weight gain**	No abnormal change in the body weight gain was present in any of the experimental animals.
4.3	**Food consumption and compound intake**	All the animals, except one, consumed the food completely. A decrease in food consumption was noted in one male only on day 2. Change in food efficiency was not found in any group. No abnormality in water consumption was noted in any of the animals of any group.
4.4	**Ophthalmoscopic examination**	No abnormality was found in any of the items examined in any animal of each group.
4.5	**Blood analysis**	
4.5.1	Haematology	No abnormality was found in any of the examinations made at various intervals. Although a dose response relationship or significant changes were observed in some items such as leucocyte differentiation, the individual values were within the standard deviation range and these changes were considered to be incidental.
4.5.2	Clinical chemistry	A slight decrease in LDH and a slight increase in total bilirubin in dose related manner were noted in the examinations made at 8 and 13 weeks, respectively, at 30 mg/kg. In addition, statistically significant changes were noted in some items of the biochemical analysis. However, these changes were considered to be incidental because the individual values were within the standard deviation range, or no dose response relationship was present in these changes.
4.5.3	Urinalysis	No abnormality was found in any of the items examined in any animal of each group.
4.6	**Sacrifice and pathology**	

4.6.1	Organ weights	No abnormal changes were found in the absolute or relative organ weights in any animal of each group.
4.6.2	Gross and histopathology	No gross abnormality was found in any of the organs or tissues in any animal of each group. No dose-related changes were found in each group. The following incidental changes were observed: moderate and slight granulomatous changes in the lung, respectively, in one male at 3 mg/kg and in another male at 10 mg/kg; very slight calcification in the kidney in one male and very slight cell infiltration in another male and also one female at 30 mg/kg; slight granulomatous change in the liver in one male at 10 mg/kg; and marked hematopoetic depression in the femoral bone marrow in one female of the control and one male at 3 mg/kg.
4.7	**Other**	-

5. APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Male and female beagle dogs were administered cyphenothrin in gelatin capsules at doses of 0, 1, 3, 10 and 30 mg/kg daily for 13 consecutive weeks.
5.2	**Results and discussion**	There were no deaths during the study. Overt signs of toxicity included vomiting at 10 mg/kg/day and above, and tremors and paleness or intense redness of the mucous membranes at 30 mg/kg/day. There were no treatment-related effects on food or water consumption, bodyweight gains or during ophthalmoscopic investigations. Urinalysis and haematology investigations revealed no treatment-related findings. Clinical chemistry investigations revealed reduced LDH activities and increased bilirubin levels in males at 30 mg/kg/day at weeks 8 and 13 respectively. There were no treatment-related organ weight changes and no abnormal gross necropsy findings. Histopathology investigations included staining of nervous tissue (cerebrum, cerebellum, brain stem, spinal cord and sciatic nerve) with haematoxylin and eosin, oil red O and Klüver-Barrera stains. They revealed no treatment-related findings.
5.3	**Conclusion**	
5.3.1	LO(A)EL	10 mg/kg/day based on overt signs of toxicity (vomiting)
5.3.2	NO(A)EL	3 mg/kg/day
5.3.3	Other	
5.3.4	Reliability	1
5.3.5	Deficiencies	None

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

November, 2017

Materials and methods

Point 3.4.7: Regarding Clinical chemistry, measurement of GPT was also performed but not stated in this report. Moreover, there was a typographical error: the correct term is "LDH" instead of "LDF".

Results and discussion

The applicant's version is generally acceptable.

However, the RMS considers that a table depicting the treatment-related clinical signs should be included in the report for clarity purposes, as follows:

6.4.1/02-1: Summary of clinical signs

Parameter	Sex	Dose (mg/kg bw/day)				
		0	1	3	10	30
Vomiting	M	0	0	0	1 (1)	4 (1-4)
	F	0	0	0	1 (1)	3 (1-2)
Tremors	M	0	0	0	0	3 (1-7)
	F	0	0	0	0	1 (2)
Paleness*	M	0	0	0	0	2
	F	0	0	0	0	0
Redish mucosa	M	0	0	0	0	2
	F	0	0	0	0	2

*palenes was observed in the conjunctiva & oral mucous membranes
Data are given as Number of animals (frequency of finding)

Conclusion

LOAEL = 10 mg/Kg bw/day, based on clinical signs consistent with synthetic pyrethroid toxicity (vomiting)

NOAEL = 3 mg/Kg bw/day

Other conclusions:

- Clinical signs consistent with synthetic pyrethroid toxicity (vomiting, tremors and paleness or intense redness of the mucous membranes) were more prominent at the top dose of 30 mg/Kg bw/day.
- The RMS notes that the LOAEL derived from this study is consistent with the one derived from the preliminary 4-week oral study in dogs (see Section A6.3.1/02)

Reliability

2

Acceptability

Acceptable

Remarks

Point 2.1: The RMS considers that the OECD Guideline 409 (equivalent to the EU testing method B.27) is the suitable experimental protocol for a 90-day oral toxicity study in non-rodents (Beagle dogs, in this case).

Point 2.3:
No significant deviations from the OECD Guideline 409.

6.4.3 Subchronic inhalation toxicity test

	Justification for non-submission of data	Official use only
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Subchronic oral dog and rat studies are presented. As the compound is not acutely toxic by the inhalation route, a subchonic inhalation study should not be necessary.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	November, 2017	
Evaluation of applicant's justification	In Doc IIC as submitted by the applicant it is mentioned that: "*The aerosol will produce a droplet aerosol size median diameter of approximately 40µg, with less than 4 % of the droplets below 10µg in diameter, and a discharge rate of approximately 1.6 g of preparation per second (Unpublished, 1997)*". It is noted that the units should be µm and not µg. Considering the above statetement as valid, and taking into account that cyphenothrin is not a volatile substance (vapour pressure < 10^{-2} Pa, 25 ^{0}C), a subchronic study by the inhalation route is not required. However, during the commenting period, the evaluation of the LC_{50} from acute inhalation was revised and classification as Acute Tox. 4 with H332 (Harmful if inhaled) was proposed. Following this conclusion, repeated-dose inhalation toxicity studies with cyphenothrin were requested by the eCA. The applicant provided sub-acute inhalation toxicity studies. These data are considered sufficient to address repeated dose inhalation toxicity.	
Conclusion	Waiving of a 90-day inhalation toxicity study in rats is considered acceptable. Since there are reliable 29-day inhalation studies available, further testing would result in unnecessary animal suffering.	
Remarks	No further remarks.	