	muscle, and any abnormalities. All tissues from the control and 250 ppm cyhalothrin groups and the testes, epididymides, prostate, seminal vesicles, liver, kidney, heart and spleen from the 10 ppm and 50 ppm cyhalothrin groups and any abnormal tissues were examined by light microscopy. In addition, a thin slice of liver tissue from the right median lobe of 6 males and 6 females per group was taken. These liver samples were processed for examination by electron microscopy for evidence of any proliferation of smooth endoplasmic reticulum (SER). The weights of the gonads, spleen, kidneys, adrenals, liver, heart, lungs, brain and pituitary were recorded. Fresh liver samples were taken from the survivors of a designated 10 males and 10 females per group. These were submitted for hepatic aminopyrine-N-demethylase (APDM) activity determination. The rats designated for this assay included those rats from which liver sections had been taken for examination in the electron microscope. * The dose received expressed as mg/kg/day is given in the Results.	X2(iii)
Results:	Most diets contained cyhalothrin concentrations within 8% of the nominal levels. One batch of diet was 26% lower than nominal levels because of an error during the preparation of the premix. This was fed for 9 days until the analyses results were known and the diets replaced. Homogeneity was shown to be within ±7% of the overall mean concentration in the diet. Cyhalothrin was stable in pelleted diet for at least 11 weeks. One moribund and one dead rat, both females from the control group, were found during the study. There were no deaths in the cyhalothrin groups, nor were there any signs of compound-related clinical abnormalities or ocular changes. There was a decrease in bodyweight gain for males fed 250 ppm cyhalothrin. The initial effect was enhanced by a further reduction from about week 8. A very slight reduction in bodyweight gain was also seen in males fed 10 or 50 ppm cyhalothrin but this did not achieve statistical significance except at 50 ppm in week 6. Females fed 250 ppm cyhalothrin showed a marginally lower bodyweight gain than the controls, but this difference was statistically significant only during the first week. Males fed cyhalothrin generally consumed slightly less food than control rats, just achieving statistical significance on a few occasions. In females fed 250 ppm cyhalothrin there was a statistically significant reduction in food consumption during the first week of the study only.	X4 X5 X6

	There was no evidence of any effect on food utilisation in males or females at any dose. Decreased plasma triglyceride levels were seen in males at 50 ppm and 250 ppm cyhalothrin. It was probable that this was a pharmacological effect. Other differences between test and control groups were minor and not dose-related and therefore not of toxicological significance. Mean red blood cell volume of all treated groups was slightly decreased, with compensatory increases in red cell count. These changes were considered not to be of toxicological significance as they resulted in normal haemoglobin levels and haematocrit and therefore the animals were not haematologically compromised. Adaptive changes characterised by elevated hepatic aminopyrine-N-demethylase activity and SER proliferation were seen at 50 ppm and 250 ppm cyhalothrin in males and at 250 ppm in females. No toxicologically significant treatment-related changes were seen at gross post mortem examination or at histopathological examination.	X7
Conclusion:	It was concluded that 250 ppm cyhalothrin produced toxicity as shown by a reduction in bodyweight at 250 ppm, the male rats being more sensitive than the females. It was considered that 50 ppm cyhalothrin (approximately 3 mg cyhalothrin/kg/day) was the no-observed adverse effect level (NOAEL).	

Dose of cyhalothrin Received (mg/kg/day)

Week	**Dietary concentration of cyhalothrin (ppm)**		
	10	50	250
Males			
1	1.16	5.38	21.7
2	1.08	5.31	26.3
6	0.56	2.50	15.7
13	0.29	1.25	6.9
STUDY MEAN (estimated)	**0.61**	**2.80**	**13.67**
Females			
1	1.18	5.71	24.0
2	0.97	4.94	24.6
6	0.45	3.01	12.1
13	0.51	2.66	12.5
STUDY MEAN (estimated)	**0.65**	**3.56**	**15.4**

Values are calculated from nominal dietary concentrations.

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.4.1 / 02	Subchronic toxicity - Oral
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and Methods	[redacted]
Results and discussion	[redacted]

Conclusion	[redacted]d.
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	6.4.1 / 03	Subchronic toxicity - Oral	Official use only
91/414 Annex Point addressed	II 5.3.2	Oral 26 week study	

Title:	Cyhalothrin: Oral toxicity study in Beagle dogs	
Lab Report Number:	No: [redacted]/C/1093	
Authors:	[redacted]	
Test Substance:	cyhalothrin batch numbers [redacted] and [redacted], purity not stated.	X1
Species:	Dog	
Method:	Meets EC B.27 (Directive 87/302/EEC)	
Date of Report:	1981	
Published:	No	
GLP:	Yes	

Material and Methods:	Groups of pure-bred Beagle dogs (6 male and 6 female) of the [redacted] strain were dosed, by capsule, with 0, 1.0, 2.5 or 10 mg cyhalothrin/kg bw/day, dissolved in corn oil for 90 days. The dogs were 25-27 weeks old at the start of treatment. Animals were given 400g dry diet each day. Any food not eaten was weighed. All dogs were checked daily for clinical signs, weighed weekly (and daily for calculations of dose volumes). Food intake was recorded daily. Water consumption was measured before the start of dosing and	X2

	during weeks 1-3, 5-7, 9-11, 13-15, 17-19 and 21-24 of dosing. Ophthalmoscopy was performed before the start of dosing and in weeks 6, 12 and 24. A neurological examination, consisting of testing of reflexes, reactions and responses, was performed on control and high dose animals by a veterinarian in the week prior to start of dosing and in week 6. Blood samples were taken from all animals in the week prior to start of dosing and in weeks 4, 8, 12, 16, 20 and 25. The following parameters were investigated: erythrocyte sedimentation rate, packed cell volume, haemoglobin, red cell count, mean cell volume, mean corpuscular haemoglobin concentration, total white blood cell count, neutrophils, lymphocytes, eosinophils, basophils and monocytes, platelets, prothrombin index, activated partial thromboplastin time. Clinical pathology was also performed; blood urea nitrogen, plasma glucose, total protein, albumin, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, bilirubin, sodium, potassium, chloride, calcium, inorganic phosphorous, cholesterol, creatinine, lactic dehydrogenase, alpha-hydroxy-butyric dehydrogenase, creatinine phosphokinase. Urine samples were collected over a 16-hour period in the week prior to start of dosing and in weeks 8, 16 and 25. Urine was assessed for specific gravity, pH, protein, glucose, ketones, bile pigments, urobiligen and haemoglobin. Urine was also examined microscopically for organisms, cells (epithelial, leucocytes, erythrocytes) and casts. Bone marrow smears were obtained by sternal puncture. All animals were subject to macroscopic necropsy. The following organs were weighed: brain, liver, testes/ovaries, pituitary, kidneys, uterus/prostate, thyroids, lungs, thymus, spleen, adrenals, heart, pancreas. A full list of tissues was taken and examined for all animals.	
Results:	Clinical signs indicative of a disturbance of the nervous system (e.g. unsteadiness, marked lack of coordination, occasionally muscular spasms and convulsions) were seen in animals receiving 10 mg cyhalothrin/kg/day but there was no histopathological evidence of changes in nervous tissue. The signs were mainly seen during week 2, and only on isolated occasions from week 3. Excessive salivation and/or vomiting was seen in occasional dogs receiving 10 mg cyhalothrin/kg/day, particularly in the first week of dosing. There was a dose-related increase in the passage of liquid faeces. This is considered to be a local irritant pharmacological response related to the method of dosing and is of no toxicological significance. No compound-related changes in bodyweight were seen although there were small changes in food (occasional instances of reduced consumption) and increased water consumption at 10 mg/kg bw/day during the first half of the study. There were no deaths, no ocular abnormalities, and no adverse	X4 X5 X6 X7

	findings in a week 6 neurological examination. There were no clinical chemistry or haematology findings, necropsy findings, organ weight changes or histopathology findings that were related to treatment. Group mean liver weight was marginally greater when adjusted for bodyweight, at 10 mg/kg bw/day (442.6 g cf 423.1 for controls, combined sexes data), but the difference was not statistically significant. This was not associated with any histopathological or clinical chemistry changes, and as with rats, may represent a physiological response to increased „workload".	X8
Conclusion:	Administration of cyhalothrin at 10 mg/kg bw/day was associated with increased incidence of vomiting and excessive salivation, particularly in the first weeks of dosing, and occasional clinical observations such as unsteadiness and lack of muscular co-ordination. This was also associated with occasional decreased food intake. There were no adverse effects on bodyweight gain, clinical pathology, gross necropsy findings or histopathology. Water consumption was slightly increased during the first half of the study. Group mean liver weight was marginally greater when adjusted for bodyweight, at 10 mg/kg bw/day, but the difference was not statistically significant. This was not associated with any histopathological or clinical chemistry changes, and as with rats, may represent a physiological response to increased „workload". The no-observed adverse effect level (NOAEL) was 2.5 mg cyhalothrin/kg/day.	

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.4.1 / 03	**Subchronic toxicity - Oral**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and Methods	[redacted]

Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	**6.4.1 / 04**	**Subchronic toxicity - Oral**	**Official use only**
91/414 Annex Point addressed	**II 5.4.1 / 01**	Oral toxicity 52 week study	

Title:	PP321: 1 Year oral dosing study in dogs	
Lab Report Number:	No. [redacted]/P/1316S	
Authors:	[redacted]	X1

Test Substance:	*lambda*-cyhalothrin (also known as PP321), batch [redacted], purity [redacted] w/w,	X2
Species:	Dog	
Method:	Meets EC B.27 (Directive 87/302/EEC)	X3
Date of Report:	1985	
Published:	No	
GLP:	Yes	

Material and Methods:	Groups of pure-bred Beagle dogs (6 male and 6 female) of the [redacted] strain were dosed, by capsule, with at 0, 0.1, 0.5 or 3.5 mg *lambda*-cyhalothrin/kg/day dissolved in corn oil, for 52 weeks. The dogs were 20-26 weeks old at the start of treatment. Animals were given 350g dry diet each day. Any food not eaten was weighed. All dogs were checked daily for clinical signs. They were weighed weekly. They were given a full clinical examination, including ophthalmoscopy, by a veterinarian prior to start of treatment and at 3-monthly intervals. Food intake was recorded daily. Blood samples were taken from all animals in the week prior to start of dosing and in weeks 4, 13, 26, 39 and 52. The following parameters were investigated: haemoglobin, haematocrit, red cell count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, total and differential white blood cell count, platelets, kaolin-cephalin and prothrombin times. Clinical pathology was also performed; blood urea nitrogen, plasma glucose, total protein, albumin, cholesterol, alanine transaminase, aspartate transaminase, alkaline phosphatase, triglycerides, sodium, potassium, calcium, creatinine kinase. Urine samples were collected overnight prior to start of dosing and in weeks 25 and 51. Urine was assessed for blood, protein, glucose, ketones, bile pigments and urobiligen. Urine was also examined microscopically for organisms, cells and casts. Bone marrow smears were taken from the femur at necropsy. All animals were subject to macroscopic necropsy. The following organs were weighed: brain, liver, testes/ovaries, pituitary, kidneys, uterus/prostate, thyroids, lungs, thymus, spleen, adrenals, heart, pancreas. A full list of tissues was taken and examined for all animals.	X3(iv)
Results:	Dose concentrations were all within 10% of nominal. Clinical signs (including ataxia, muscle tremors and convulsions), were seen in animals receiving 3.5 mg *lambda*-cyhalothrin/kg/day but there was no histopathological evidence of changes in nervous tissue. Vomiting and liquid faeces were also seen in dogs in this dose group, predominantly in the first two weeks of the study. The latter is considered to be a local irritant/pharmacological response	X5 X6

	related to the method of dosing. There was one death, a male at 3.5 mg/kg bw/day in week 46, due to adverse clinical effects consistent with pyrethroid toxicity. No compound-related changes in bodyweight were seen although there were small changes in food consumption at 3.5 mg/kg bw/day, which were considered treatment-related. Minor changes in haematology and urinalysis/urine cytology parameters were considered unrelated to treatment. A slight increase in plasma triglycerides, accompanied by a slight decrease in plasma cholesterol, was seen in the top dose group. Other clinical chemistry changes were considered unrelated to treatment. Mean testis weights were marginally lower at 3.5 mg/kg bw/day, principally due to two dogs. There was also a slight increase in liver weight in treated groups which may have represented a mild adaptive response but this was not accompanied by an increase in alkaline phosphatase activity or histopathological changes in the liver and did not achieve statistical significance compared with the control group.	X7 X8 X9
Conclusion:	Administration of cyhalothrin at 3.5 mg/kg bw/day for 52 weeks was associated with increased incidence of vomiting and excessive salivation, particularly in the first weeks of dosing, and occasional clinical observations such as unsteadiness and lack of muscular co-ordination. This was also associated with occasional decreased food intake. There were no adverse effects on bodyweight gain, haematology, urinalysis/urinary cytology, gross necropsy findings or histopathology. A slight increase in plasma triglycerides, accompanied by a slight decrease in plasma cholesterol, was seen in the top dose group. Group mean liver weight was marginally greater when adjusted for bodyweight, at 10 mg/kg bw/day, but the difference was not statistically significant. This was not associated with any histopathological or clinical chemistry changes, and as with rats, may represent a physiological response to increased „workload". The no-observed adverse effect level (NOAEL) was 0.5 mg *lambda*-cyhalothrin/kg/day.	

Reliability Indicator	1
Data Protection Claim	Yes

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.4.1 / 04	**Subchronic toxicity - Oral**
	EVALUATION BY RAPPORTEUR MEMBER STATE

Borttaget: September

Borttaget: 0

Date	February 2007
Materials and Methods	[redacted]
Results and discussion	[redacted]

Borttaget: September

Borttaget: 0

	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.4.2	Subchronic toxicity - Dermal	Official use only
Section 6.4.2 Annex Point IIA 6.4.2	**Subchronic dermal**	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [X]	Other justification [X]	
Detailed justification:	[redacted]	

98/8 Doc IIIA section No. 6.4.2	Subchronic toxicity - Dermal	Official use only
Section 6.4.2 Annex Point IIA 6.4.2	**Subchronic dermal**	Official use only
	[redacted]	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	Evaluation by Competent Authorities
98/8 Doc IIIA Section No. 6.4.2	Subchronic toxicity - Dermal
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Conclusion	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.4.3	Subchronic toxicity - Inhalation	Official use only
Section 6.4.3 Annex Point IIA 6.4.3	**Subchronic inhalation**	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	[redacted]	

98/8 Doc IIIA section No. 6.4.3	Subchronic toxicity - Inhalation	Official use only
Section 6.4.3 Annex Point IIA 6.4.3	**Subchronic inhalation**	Official use only
[redacted]		

	Evaluation by Competent Authorities
98/8 Doc IIIA Section No. 6.4.3	Subchronic toxicity - Inhalation
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Results and discussion	[redacted]

Conclusion Remarks	[redacted]

5. CHRONIC TOXICITY – SEE SECTION 7. CARCINOGENICITY

6. GENOTOXICITY

98/8 Doc IIIA section No.	**6.6.1 / 01**	**In vitro gene mutation study in bacteria**	Official use only
91/414 Annex Point addressed	**II 5.4.1**	In-vitro studies	

Title:	PP321 - An Evaluation in the *Salmonella* Mutagenicity Assay. Central Toxicology Laboratory Report No: CTL/P/1000.	
Lab Report Number:	No. CTL/P/1000	
Authors:	Callander R D	
Test Substance:	Technical grade *lambda*-cyhalothrin (otherwise known as PP321). Purity [redacted]% w/w.	X1
Species:	Not applicable	
Method:	OECD Guideline 471	
Date of Report:	1984	
Published:	No	
GLP:	Yes	

Material and Methods:	This study was conducted to evaluate whether or not the test substance is capable of inducing mutations in various strains of *Salmonella typhimurium*. The study was conducted using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100, using the standard Plate Incorporation assay protocol. Six test concentrations, ranging from 1.6-5000 µg/plate, were tested both in the presence and absence of an auxiliary metabolic activation system (S9-mix) derived from the livers of AROCLOR 1254-induced male Sprague Dawley rats. Three plates were tested per concentration/strain/S9-mix condition. Appropriate positive and solvent controls were included for each strain/S9-mix condition. The compound was assayed in two independent experiments. The incubation period for each experiment was 3 days (at 37°C). The mean number of revertant colonies observed at each dose level tested were compared against the appropriate solvent (negative) control value using a Student's t-test.	X3
Results:	No reproducible, significant increases in revertant colony numbers were observed in any strain, either in the presence or absence of S9. In each case, the positive control chemicals gave the expected responses indicating the sensitivity of the assay and the metabolic competence of the S9-mix.	X4
Conclusion:	Under the conditions of the assay, the test substance gave a negative, ie. non-mutagenic response.	

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.6.1 / 01	**In vitro gene mutation study in bacteria**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and Methods	[redacted]
Results and discussion	[redacted]

Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	**6.6.2 / 01**	**In vitro cytogenicity study in mammalian cells**	**Official use only**
91/414 Annex Point addressed	**II 5.4.1**	In-vitro studies	

Title:	„PP321: A Cytogenetic Study in Human Lymphocytes In Vitro"	
Lab Report Number:	No. [redacted] P/1333	
Authors:	[redacted]	
Test Substance:	Technical grade *lambda*-cyhalothrin (otherwise known as PP321). Purity [redacted]% w/w.	X1
Species:	Not applicable	
Method:	OECD Guideline 473	
Date of Report:	1985	
Published:	No	
GLP:	Yes	

Material and Methods:	This study was conducted to evaluate whether or not the test substance is capable of inducing chromosomal damage *in vitro* in human lymphocytes. The human peripheral blood lymphocyte is a sensitive indicator of *in vitro* chromosomal damage when stimulated to provide large numbers of rapidly dividing cells. The test was carried out using lymphocytes from two donors (one male and one female) in the presence and absence of an auxiliary metabolic activation system (S9-mix). Cultures were initiated in plastic tubes containing RPMI-1640 culture medium supplemented with 10% foetal calf serum, 0.1 mg/ml phytohaemagglutinin, 100 units/ml penicillin and 100 µg/ml streptomycin. The cultures were incubated at 37°C for 44 hours.	

	A range of ten *lambda*-cyhalothrin concentrations of between 1 and 1000 µg/ml were administered to duplicate cultures from both donors, in the absence of S9-mix for a period of four hours. Prior to treatment of cultures treated in the presence of S9-mix, a range of ten *lambda*-cyhalothrin concentrations of between 10 and 1000 µg/ml were pre-incubated with the S9-mix (200 µl of a 1:1 mix of S9 and co-factor) at 37°C for 30 minutes. The cultures were then treated for a period of 3 hours. The solvent control was administered to the cultures at a dosing volume of 1 µl/ml. The positive control, mitomycin C, was administered as a solution in saline at a concentration of 0.5 µg/ml in the absence of S9-mix. The positive control, cyclophosphamide, was administered as a solution in saline at a concentration of 100 µg/ml following pre-incubation with the S9-mix. After treatment, the culture medium was removed by centrifugation and replaced with fresh supplemented RPMI-1640 medium. All cultures were maintained at 37°C for the remainder of the 72 hour growth period. Two hours prior to harvesting at 72 hours, the cultures were treated with colchicine at a final concentration of 10 µg/ml. Seventy-two hours after culture initiation, the cultures were centrifuged, treated with a 0.075M KCl hypotonic solution and were fixed in methanol/acetic acid fixative (3:1 v/v). Slides were prepared for each culture by dropping cell suspension on to clean, dry labeled microscope slides. The slides were air dried, stained in a filtered 10% solution of Giemsa stain in buffered (pH 6.8) double deionised water for 7 minutes, rinsed in water, air-dried and mounted with coverslips in DPX. The mitotic index was determined for cultures treated with *lambda*-cyhalothrin and the relevant solvent and positive control cultures. Duplicate cultures treated with *lambda*-cyhalothrin at concentrations of 100, 500 and 1000 µg/ml were selected for chromosomal aberration analysis along with the solvent control cultures. The highest dose level was limited by the solubility of *lambda*-cyhalothrin in the solvent, DMSO. A single positive control culture was selected for chromosomal aberration analysis for each donor both in the presence and absence of S9-mix. The slides were coded to avoid observer bias and approximately 200 cells in metaphase were analysed for the 3 concentrations of *lambda*-cyhalothrin and the solvent controls, for the incidence of structural chromosomal aberrations. Analysis of the positive control cultures was completed when a sufficient number of cells had been analysed to confirm a positive response.	

Results:	Cultures from both the male and female donors treated at concentrations of 100, 500 and 1000 µg/ml in the presence and absence of S9-mix were selected for chromosomal aberration analysis. Reductions in mitotic activity, compared to the respective solvent control cultures, of 26% for the male donor and 17% for the female donor in the presence of S9-mix and of 42% in the male donor in the absence of S9-mix were noted for cultures treated at 1000 µg/ml. No significant reductions in mitotic activity were seen for the female donor cultures treated in the absence of S9-mix. No statistically or biologically significant increases in the percentage of aberrant cells, compared to the solvent control values, were noted in either the male and female donor cultures treated with *lambda*-cyhalothrin in the presence or absence of S9-mix. The sensitivity of the test system and the metabolic activity of the S9-mix employed, were clearly demonstrated by the marked increases in the frequencies of chromosomal aberrations induced by the positive control agents mitomycin C and cyclophosphamide.	X3 X2(iii)
Conclusion:	In conclusion, under the conditions of this assay, *lambda*-cyhalothrin is considered not to be clastogenic to cultured human lymphocytes in vitro in the presence or absence of metabolic activation.	

Reliability Indicator	1
Data Protection Claim	Yes

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.6.2 / 01	**In vitro cytogenicity study in mammalian cells**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]

Reliability	2
Acceptability	Acceptable
Remarks	The deviations noted are not considered to influence the result.

98/8 Doc IIIA section No.	**6.6.3 / 01**	**In vitro gene mutation study in mammalian cells**	**Official use only**
91/414 Annex Point addressed	**II 5.4.1**	In-vitro studies	

Title:	„PP321: Assessment of Mutagenic Potential using L5178Y MOUSE LYMPHOMA Cells“	
Lab Report Number:	No. [redacted] P/1340	
Authors:	[redacted]	
Test Substance:	*lambda*-cyhalothrin (otherwise known as PP321). Purity [redacted] w/w.	X1
Species:	Mouse	
Method:	OECD Guideline 476	
Date of Report:	1985	
Published:	No	
GLP:	Yes	

Material and Methods:	This study was conducted to evaluate whether or not the test substance is capable of inducing mutations in L5178Y mouse lymphoma cells (at the TK locus). The study was conducted using L5178Y mouse lymphoma cells, screening for forward mutations at the TK locus using trifluorothymidine as the selective agent, both in the presence and absence of a liver S9 preparation derived from AROCLOR 1254-induced male Sprague Dawley rats. Each experiment was conducted in MicrotitreÔ plates, using duplicate cultures per dose point, with an expression period of 2 days. The test substance was tested to a limit concentration of 4000 µg/ml. At concentrations of 1000 µg/ml and above, significant precipitation/agglutination of the test substance was observed in the test medium.	X2 (ii) X2 (iv) X3

Results:	*Lambda*-cyhalothrin demonstrated relatively little cytotoxicity in this assay, even at a nominal concentration of 4000 µg/ml, but at concentrations of 1000 µg/ml and above significant precipitation/agglutination of the test substance was observed in the test medium. No biologically significant increase in mutation frequency was observed either in the presence or absence of auxiliary metabolism (S9). Although some isolated effects were observed both in the presence and absence of S9 at dose levels in excess of the limit of solubility (³ 1000 µg/ml), these were either of limited magnitude, or else were not associated with any increase in the absolute numbers of mutants. These effects are therefore considered not to be due to compound-induced mutational events. The positive control materials responded as expected demonstrating the sensitivity of the assay and the metabolic competence of the S9-mix.	X4
Conclusion:	Under the conditions of this assay, *lambda*-cyhalothrin gave a negative, ie. non-mutagenic response in the L5178Y assay on selection in trifluorothymidine in the presence or absence of S9.	

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA **Section No. 6.6.3 / 01**	**In vitro gene mutation study in mammalian cells**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and Methods	[redacted]

Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	6.6.3/ 02	In vitro gene mutation study in mammalian cells	Official use only
91/414 Annex Point addressed	II 5.4.1	In-vitro studies	

Title:	„*Lambda*-cyhalothrin: Assessment for the Induction of Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures."	
Lab Report Number:	No. [redacted] P/2707	
Authors:	[redacted]	
Test Substance:	Technical grade *lambda*-cyhalothrin (otherwise known as PP321). Purity [redacted]% w/w.	
Species:	Rat	
Method:	Directive 87/302/EEC, part B, p. 64	X2
Date of Report:	1989	
Published:	No	
GLP:	Yes	

Material and Methods:	This study was conducted to evaluate whether or not the test substance is capable of inducing DNA repair (unscheduled DNA synthesis; UDS) in vitro. Hepatocytes were derived from male adult [redacted] (Alpk:APfSD) rats, 238-298 g, supplied by [redacted]. The animals were allowed	

	food (Porton Combined Diet) and tap water ad libitum. The animals were housed at 20-230C with relative humidity of approximately 50%. The room was illuminated on a 12 hour light/12 hour dark cycle. Animals were killed and hepatocytes isolated by a collagenase perfusion technique. Hepatocytes were allowed to attach to coverslips. Three hepatocyte cultures were prepared for each treatment level. Either DMSO (solvent control), or DMSO solutions of *lambda*-cyhalothrin at a range of concentrations from 10^{-2}M to 10^{-9}M of 6-p-dimethylaminophenylazobenzthiazole (6BT, positive control) were added to the cultures in the presence of [3H]thymidine and incubated overnight. Cultures were washed and incubated overnight with medium containing an excess of unlabelled thymidine. Cells were washed, fixed and dipped in photographic emulsion for autoradiography. After 14 days exposure, the emulsion was developed and the cells stained. The preparations were examined and the amount of radiolabel in the nucleus [N] compared with that of the most heavily labelled area of cytoplasm [C]. The value [N-C], the net nuclear grain count is an index of unscheduled DNA synthesis (UDS). Mean [N-C] values were determined for 50 cells/slide, two slides per dose level. To be considered positive, a test treatment should result in a mean net nuclear grain count of +5 or greater with at least 20% of cells scored showing DNA repair, ie. with net nuclear grain counts of +5 or more. This response should be reproduced in an independent experiment.	X3 X4
Results:	Cultures treated with *lambda*-cyhalothrin at concentrations of 10^{-2}, 10^{-3} and 10^{-4}M were not analysed for UDS because of excessive test substance induced cytotoxicity manifest as pyknosis and reduced cell numbers compared with concurrent solvent controls. This was apparent in both experiments. Cultures treated with *lambda*-cyhalothrin at concentrations of 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}M were selected for UDS analysis. None of the *lambda*-cyhalothrin treated hepatocyte cultures scored showed any increase in mean net nuclear grain count nor any significant increase in the number of cells in repair, whilst those derived from the concurrent positive [6-p-dimethylaminophenylazo-benzthiazole] and negative controls gave the expected response.	

Conclusion:	It was concluded therefore that, when examined up to dose levels limited by cytotoxicity, *lambda*-cyhalothrin did not induce UDS in primary cultures of hepatocytes.	

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA Section No. 6.6.3 / 02	**In vitro gene mutation study in mammalian cells**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

98/8 Doc IIIA section No.	**6.6.4 / 01**	**In vivo mutagenicity**	**Official use only**
91/414 Annex Point addressed	**II 5.4.2**	In vivo studies in somatic cells	

Title:	„An Evaluation of PP321 in the Mouse Micronucleus Test"
Lab Report Number:	No. [redacted] P/1090
Authors:	[redacted]
Test Substance:	Technical grade *lambda*-cyhalothrin (otherwise known as PP321). Purity [redacted] % w/w.
Species:	Mouse
Method:	OECD Guideline 474 "Genetic Toxicology: Micronucleus Test"
Date of Report:	1984
Published:	No

GLP:	Yes	

Material and Methods:	This study was conducted to evaluate whether or not the test substance is capable of inducing clastogenic effects in the mouse bone marrow micronucleus test. Groups of 5 male and 5 female C57BL/6J mice (8-12 weeks old) were given a single intraperitoneal dose of either corn oil (vehicle control), cyclophosphamide (positive control, 65 mg//kg) or *lambda*-cyhalothrin at 22 mg/kg (44% median lethal dose determined over a 7 day observation period) or 35 mg/kg (70% median lethal dose determined over a 7 day observation period). Groups of 5 male and 5 female mice were killed by cervical dislocation 24, 48 and 72 hours after dosing and paintbrush smears of the bone marrow from the femur were prepared on clean, labelled microscope slides. The slides were allowed to dry and were stained with polychrome methylene blue and eosin, mounted with coverslips in DPX, coded and analysed blind. Five hundred polychromatic erythrocytes per animal were examined for the presence of micronuclei. Two hundred erythrocytes were also examined to measure the ratio of polychromatic to normochromatic erythrocytes, as evidence of cytotoxicity to the target tissue may be manifest in alterations to the ratio of these cells in the bone marrow. The data were analysed for significant differences from the control group using a one-sided Student's 't'-test.	X2
Results:	No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, over vehicle control values, were seen at either dose level at any of the sampling times investigated. At the 48 hour time point, statistically significant reductions in the percentage of polychromatic erythrocytes, compared to the vehicle control values, were observed in the animals treated with *lambda*-cyhalothrin at 22 and 35 mg/kg. These reductions indicate that *lambda*-cyhalothrin, or one of its metabolites, has exerted a cytotoxic effect on the bone marrow. The positive control material, cyclophosphamide, induced statistically significant and biologically meaningful increases in micronucleated polychromatic erythrocytes, compared to the vehicle control values, thus demonstrating the sensitivity of the test system to a known clastogen.	X3
Conclusion:	It is, therefore, concluded that *lambda*-cyhalothrin, under the conditions of test, is not clastogenic in the mouse bone marrow micronucleus test. Lambda-cyhalothrin gave negative results in all the genetic toxicity in vitro tests but	

	available in vivo data are included for completeness No test to assess possible germ cell effect is required as all genetic toxicity results are negative.	

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA **Section No. 6.6.4 / 01**	**In vivo mutagenicity**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.6.5 **Second in vivo mutagenicity study**	**Official use only**
Section 6.6.5 Annex Point IIA 6.6.5 **Second in vivo mutagenicity study**	**Official use only**
JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ] **Technically not feasible** [ ] **Scientifically unjustified** [X] **Limited exposure** [ ] **Other justification** [ ]	
Detailed justification: [redacted]	

	Evaluation by Competent Authorities
98/8 Doc IIIA Section No. 6.6.5	Second in vivo mutagenicity study
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Conclusion	[redacted]

98/8 Doc IIIA section No. 6.6.6	Test to assess possible germ cell effects	Official use only
Section 6.6.6 Annex Point IIA 6.6.6	**Test to assess possible germ cell effects**	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	[redacted]	

	Evaluation by Competent Authorities
98/8 Doc IIIA Section No. 6.6.6	Test to assess possible germ cell effects
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Conclusion	[redacted]

98/8 Doc IIIA section No. 6.6.7	Further testing if metabolites of concern are formed in mammals	Official use only
Section 6.6.7 Annex Point IIA 6.6.6	**Further testing if metabolites of concern are formed in mammals**	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	[redacted]	

98/8 Doc IIIA section No.	6.6.7	Further testing if metabolites of concern are formed in mammals	Official use only
Section 6.6.7 Annex Point IIA 6.6.6		**Further testing if metabolites of concern are formed in mammals**	Official use only
		[redacted]	

	Evaluation by Competent Authorities
Date	EVALUATION BY RAPPORTEUR MEMBER STATE February 2007
Conclusion	[redacted]
Remarks	

7. CARCINOGENICITY

98/8 Doc IIIA section No.	6.7 / 01	Carcinogenicity study	Official use only
91/414 Annex Point addressed	II 5.5	Long term toxicity and carcinogenicity	

Title:	„CYHALOTHRIN: Two Year Feeding Study in Rats"	
Lab Report Number:	No. [redacted]/P/980	
Authors:	[redacted]	
Test Substance:	Technical grade cyhalothrin. Purity [redacted]% w/w. Batch number [redacted]	X1 X2
Species:	Rat	
Method:	OECD Guideline 453 "Combined Chronic Toxicity/Carcinogenicity Studies"	
Date of Report:	1984	
Published:	No	
GLP:	Yes	

Material and Methods:	The purpose of the study was to assess the chronic toxicity and carcinogenic potential of the test substance in the rat when administered orally (in diet) for a period of up to two	

	years. Technical cyhalothrin was administered in diet to Alpk/AP (now known as the Alpk:APfSD strain) rats. Twenty-one day old rats were delivered to [redacted] over a three-week period with litters of both males and females arriving each week. The study consisted of four groups of animals, 3 test and 1 control, each comprising of 62 rats of each sex. An additional 10 rats per sex per group were included for interim sacrifice after 52 weeks. The study duration was104 weeks. The treated groups received cyhalothrin at dietary concentrations of 10, 50 or 250 ppm*. The control group was treated in an identical manner to the treated groups except that the diet of animals in this group did not contain cyhalothrin. Four rats of each sex were housed per cage and the cages were arranged in single-sex replicates (randomised blocks) each comprising one cage from each group. The animals were housed in one room in a barriered (limited access) rodent facility with a controlled atmosphere, the temperature being within the range of 16-30°C and relative humidity ranging between 33-98%. There were twelve hour alternating periods of light and darkness. During the first fourteen months of the study, diets were pelleted. A change in laboratory policy concerning feeding of rodents meant that after this time powdered diet was presented in food jars. During the course of the study two formulations of diet were used. The later diet was subject to steam expansion which produced a lower microbial content. Small differences in formulation were introduced by the manufacturer to compensate for possible nutrient losses during the steam expansion process. Diets containing cyhalothrin were prepared as required. The maximum duration for which these diets were kept during the study was 45 days. Throughout the course of the study, animals were allowed food and water *ad libitum*. Samples of diet were taken from the first batch of diet and subsequently at approximately four-weekly intervals, from all dose levels including control, for analysis for cyhalothrin content. Samples were also taken from the first batch of diet for serial analyses over a nine week period to confirm the stability of cyhalothrin in pelleted diet. A further series of samples was taken to confirm the homogeneity of the mix. Homogeneity was also assessed by random selection of samples from diet jars when the mix size was increased later in the study, from 25 to 50 kg.	X3

	During the study all animals were examined daily for clinical abnormalities. More detailed examinations were carried out weekly and an additional examination for signs of neurotoxicity was carried out prior to the interim kill.	
	Prior to feeding experimental diets and then weekly thereafter for the next twelve weeks, animals were weighed and food consumption for each cage of rats was recorded. After this period, bodyweights were recorded fortnightly and food consumption measured every fourth week.	X4
	Blood for haematological analyses was collected from a pre-designated ten male and ten female rats per group one day prior to being fed experimental diets, after 4 and 13 weeks and then at 13 week intervals throughout the study. The following parameters were determined: haemoglobin; haematocrit; red cell count; mean cell volume; mean cell haemoglobin; mean cell haemoglobin concentration; total white cell count; differential white cell count; examination of the morphological appearance of red cells, and platelet count. Kaolin-cephalin and prothrombin times were measured for all samples taken at termination (104 weeks). At termination, femoral bone smears were prepared from these animals for haematological and cytological examination.	X5 X6
	Twelve male and twelve female rats (which were different animals from those bled for haematology) were selected to provide blood and urine samples for clinical chemistry. The schedule followed was the same as that as for the haematology analyses. The following were determined: Blood (Plasma): Alkaline phosphatase, alanine transaminase and aspartate transaminase activities; cholesterol; albumin; total protein; urea; glucose, and triglycerides. Urine: Volume; pH; specific gravity; glucose; protein; ketones, and urobilinogen.	X7
	At week 52 and in the week prior to termination the eyes of twenty male and twenty female rats from both the top dose and control group were examined by ophthalmoscope.	X8
	All animals were examined post mortem, and gonads, spleen, heart, kidneys, adrenals, liver, lungs and brain were weighed from animals at the scheduled 53 or 104 week kills. Samples of the following tissues were taken for histopathological examination for neoplastic and non-neoplastic changes: adrenal glands, aorta, bone and marrow (femur), brain, caecum, cervix, colon, duodenum, epididymides, eyes, Harderian glands, heart, ileum, jejunum, kidneys, larynx, liver (left, median and papillary lobes), lungs (inflated), lymph nodes (cervical and mesenteric), mammary gland (females only), mouth, nasal cavity,	

	oesophagus, ovaries, pancreas, parathyroid, pituitary gland, prostate, salivary glands, sciatic nerves, seminal vesicles, skin (flank), spinal cord, spleen, stomach (squamous and glandular), testes, thymus, thyroids, trachea, urinary bladder, uterus, voluntary muscle, and any other tissues observed to be abnormal. * The dose received expressed as mg/kg/day is given in the Summary of Results.	X9
Results:	Analyses showed that dietary concentrations of cyhalothrin and its stability and homogeneity in diet were satisfactory throughout the course of the study. Treatment with cyhalothrin did not increase mortality, nor were there any signs of compound-related clinical abnormalities or ocular changes. There was no evidence of neurotoxicity. The most common clinical findings in the study were the oral and nasal lesions which were found to be caused by dietary fibres penetrating the palate via the periodontal space of the first upper molar tooth. These lesions developed after 64 weeks of the study, were not compound-related, and did not compromise the interpretation of the data. There was a decrease in bodyweight gain for both male and female rats fed 250 ppm cyhalothrin compared to controls. This was statistically significantly different for females throughout the study and in males up until week 84. There was a consistently reduced food consumption in male rats fed 250 ppm cyhalothrin during the first twelve weeks of the study, with a slight reduction seen in females fed this dose. For the remainder of the study food consumption was comparable across the groups. There were some small haematological changes (a minor decrease in haemoglobin and a marginal increase in white cell count) in female rats fed 250 ppm cyhalothrin, but no effects of treatment in male rats at this dose. These changes were considered to be too small to be of haematological significance. There were small changes in blood clinical chemistry (reduced plasma triglyceride levels and alkaline phosphatase activity) of the top dose animals which initially were consistent with a reduced growth rate, however as this trend continued throughout the study it was considered that this was evidence of a toxic effect. No compound-related histopathological effects were seen but a dose of 250 ppm cyhalothrin produced an increase in the	X10 X11 X12 X13 X14 X15

	liver weight of both male and female rats killed at 52-53 weeks. No increase in liver weight was apparent at study termination, supporting the view that this was an adaptive change rather than a toxic effect. The incidence or type of tumours was not increased by cyhalothrin.	X16
Conclusion:	It was concluded that cyhalothrin is not carcinogenic to the rat and that 50 ppm cyhalothrin (equivalent to approximately 2.5 mg cyhalothrin/kg/day) was the no-observed adverse effect level (NOAEL) in this study. The no-observed effect level (NOEL) was 10 ppm (equivalent to approximately 0.5 mg cyhalothrin/kg/day).	X17

DOSE OF CYHALOTHRIN RECEIVED (mg/kg/day)

Week No.	**Dietary concentration of cyhalothrin (ppm)**		
	10	50	250
Males: 1	1.11	5.33	22.75
2	0.90	4.59	21.72
12	0.44	2.30	11.78
24	0.37	1.73	9.72
52	0.36	1.73	9.39
76	0.33	1.70	8.92
102	0.38	2.00	10.35
Overall	**0.47**	**2.31**	**11.75**
Females: 1	1.12	5.38	22.35
2	0.87	4.54	22.75
12	0.53	2.49	13.59
24	0.40	1.90	12.41
52	0.43	2.04	12.62
76	0.46	2.40	11.75
102	0.48	2.44	12.99
Overall	**0.55**	**2.70**	**14.31**

Calculations are based on nominal concentration of cyhalothrin in diet.

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.7 / 01	**Carcinogenicity study**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and Methods	[redacted]

Borttaget: September

Borttaget: 0

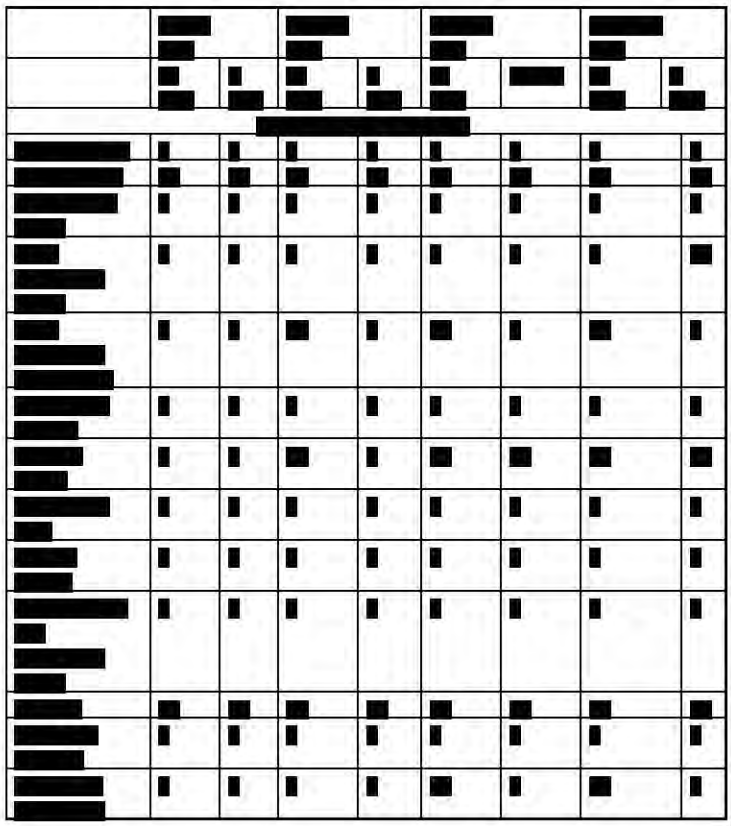
Results and discussion

[redacted]

Borttaget: September

Borttaget: 0

[redacted]



[redacted]

Conclusion	[redacted]).
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	6.7 / 02	Carcinogenicity study	Official use only
91/414 Annex Point addressed	II 5.5	Long term toxicity and carcinogenicity	

Title:	„CYHALOTHRIN: Potential Tumourigenic and Toxic Effects in Prolonged Dietary Administration to Mice"	
Lab Report Number:	No. [redacted]/P/1260	
Authors:	[redacted]	
Test Substance:	Technical cyhalothrin (otherwise known as PP563). Purity [redacted] w/w.	X1
Species:	Mice	
Method:	OECD Guideline 451 "Carcinogenicity Studies"	
Date of Report:	1984	
Published:	No	
GLP:	Yes	

Material and Methods:	The purpose of this study was to characterise the toxic and potential tumorigenic effects of the test substance when administered orally in the diet to mice over a period of up to two years. Technical cyhalothrin was administered in diet to CD-1 mice obtained from [redacted] (age 28±2 days	

	within a 4 g weight range on arrival). There were four groups of animals, 3 test and 1 control, each comprising 64 mice of each sex. From each group, 12 mice per sex were designated for interim kill after 52 weeks. The test groups received diet containing 20, 100 or 500 ppm cyhalothrin*. Each animal was uniquely numbered and identified by earmarking. Four mice of each sex were housed per cage. The animals were housed in a rodent facility with controlled environmental conditions, giving a temperature of approximately 210C and relative humidity of approximately 50%. There were twelve hour alternating periods of light and darkness. Prior to the start of treatment, the stability of the test substance in the diet over 6 weeks and homogeneity in the diet at levels of 20 and 500 ppm cyhalothrin were confirmed. Diets were prepared weekly. During the study all animals were examined daily for clinical abnormalities. All mice were palpated every week to record the appearance and location of any palpable masses. Individual bodyweights were recorded one week before the start of treatment and at weekly intervals thereafter. Food consumption per cage was recorded weekly. Water was monitored daily by visual inspection and measured on a cage basis over a 7 day period in week 48. Blood for haematological analysis was collected from all the animals designated for interim kill during week 49 and from 12 animals per group in week 101. The following parameters were examined: packed cell volume; haemoglobin; red cell count; mean corpuscular haemoglobin concentration; mean cell volume; total white cell count; differential count, and platelet count. Blood for clinical chemistry analysis was collected from all animals designated for interim kill during week 50 and from 12 animals per group in week 102. The following plasma parameters were measured: urea nitrogen; glucose; total protein; albumin; globulin; and alkaline phosphatase, glutamic-pyruvic transaminase, glutamic oxaloacetic transaminase and cholesterol activities. Pooled overnight urine samples were collected from each cage of interim kill animals during week 51 and from 12 animals per group during week 102. Food and water were withheld during the collection period. Volume; pH, and specific gravity were measured and the samples examined	X2 X3

	for the presence of glucose and ketones. All animals were examined post mortem, and the adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen and testes were weighed. A comprehensive selection of tissues was taken for histopathological examination for neoplastic and non-neoplastic changes and included the following: adrenals, bone, brain (medullary, cerebellar and cortical sections), caecum, duodenum, eyes, gall bladder, Harderian gland, head (for nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx and middle ear), heart, ileum, jejunum, kidneys, liver (from at least two lobes), lungs (all lobes and mainstem bronchi), lymph nodes (cervical and mesenteric), mammary gland, mid-colon, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (at least two levels), spleen, sternum (for bone marrow), stomach (glandular and non-glandular), testes, thymus (where present), thyroid (with parathyroid), trachea, urinary bladder and uterus (plus cervix). All nodules, tissue masses and otherwise macroscopically abnormal tissues were routinely preserved, along with samples of adjacent tissue where appropriate. * The achieved dose expressed as mg/kg/day is given in the results.	X4 X5
Results:	The dose of cyhalothrin received (mg/kg/day) was calculated from the food and bodyweight data and the nominal dietary inclusion level. The mean achieved concentrations of cyhalothrin were within 9% of nominal levels, with the exception of one mean value for the 20 ppm which was 21.5% above nominal at week 52. cyhalothrin was stable in the diet for up to 6 weeks and homogeneity was found to be satisfactory. There were no compound-related increases in mortality. The overall percentage mortality <30% at 18 months and by the end of the study was 66% for males and 57% for females. The main clinical signs were an increased incidence of piloerection often associated with hunched posture, in all animals treated with 500 ppm cyhalothrin, with males more affected than females, with similar, but less severe effects in males receiving 100 ppm cyhalothrin. There was a slightly higher incidence of fighting, emaciation, pallor and hyperactivity in all cyhalothrin-treated males compared to the control group. There was reduced weight gain in male mice in the 500 ppm cyhalothrin group, particularly in the initial growth phase but continuing throughout the study. No compound-	X7

	related adverse effects were seen in females at this dose level or in either sex at the lower dose levels. Statistically significantly increased food consumption and reduced food utilization were seen in males receiving 500 ppm cyhalothrin compared to the control group. There was no effect of compound in females at this dose. At week 49, total white cell counts were slightly lower in the 500 ppm group of males. At 101 weeks there was also a slightly lower packed cell volume value for males in this group compared to controls. Slightly reduced plasma glucose values occurred in male and female mice receiving 500 ppm cyhalothrin. Other small differences in blood biochemical parameters showed no consistent effects and were considered to be of no toxicological significance. There were no changes in urine biochemistry which were attributed to treatment with cyhalothrin. Organ weights were not affected by treatment with cyhalothrin. There was an increase in mammary adenocarcinomas in female mice receiving 100 or 500 ppm cyhalothrin compared to the control group, but the incidence was within the range normally found in this strain of mouse, and was therefore not attributed to treatment with cyhalothrin.	X8 X9 X10 X11 X12
Conclusion:	It was concluded that 500 ppm cyhalothrin produced toxicity in both sexes which was more marked in males which had reduced bodyweight gain, clinical signs and slight biochemical changes. Females showed slight clinical signs and biochemical changes only. At 100 ppm cyhalothrin, minimal clinical signs of toxicity were seen in male mice. The no-observed effect level (NOEL) and the no-observed adverse effect level (NOAEL) for both males and females were considered to be 20 ppm cyhalothrin as no effects of compound were seen.	X13

SUMMARY OF RESULTS

Dose of cyhalothrin Received (mg/kg/day)

	Week No.	**Dietary concentration of cyhalothrin (ppm)**		
		20	**100**	**500**
Males:	1	2.77	13.48	60.03
	2	2.53	12.29	59.97

	13	1.98	9.52	53.21
	26	1.79	9.19	51.91
	52	1.72	8.83	55.86
	78	1.61	8.78	47.54
	104	1.82	8.66	48.66
Overall		**1.81**	**9.22**	**53.2**
Females:	1	3.03	14.42	68.87
	2	2.91	13.31	71.21
	13	2.29	11.62	60.09
	26	1.94	10.33	48.48
	52	1.73	9.40	47.41
	78	1.96	10.12	47.55
	104	1.96	11.71	48.07
Overall		**2.03**	**10.58**	**50.7**

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.7 / 02	**Carcinogenicity study**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and Methods	[redacted]

Borttaget: September

Borttaget: 0

Results and discussion

[redacted]

	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

8. REPRODUCTIVE TOXICITY

98/8 Doc IIIA section No.	**6.8.1 / 01**	**Reproductive Toxicity – Teratogenicity test**	**Official use only**
91/414 Annex Point addressed	**II 5.6.2**	Developmental toxicity studies	

Title:	„CYHALOTHRIN: Oral (Gavage) Teratology Study in the Rat"	
Lab Report Number:	No. [redacted]/C/1075	
Authors:	[redacted]	
Test Substance:	Technical cyhalothrin (otherwise known as PP563). Purity [redacted] % (w/w).	X1
Species:	Rat	
Method:	OECD Guideline 414 "Teratogenicity"	
Date of Report:	1981	
Published:	No	

GLP:	Yes	X2

Material and Methods:	The purpose of the study was to assess the potential of the test substance to effect the embryonic and foetal development of the rat when administered during the period of organogenesis. Ninety-six virgin female and thirty sexually mature male CD rats were supplied from a "specific pathogen free" colony. The animals were acclimatised to the laboratory environment for 17 days prior to mating. The animals were kept in a single room throughout the study in which there was a complete air change no less than 15 times each hour. The room was thermostatically maintained at a temperature of 19.5 ± 3.50C and a relative humidity of 65 ± 15%. The temperature was recorded using a maximum/minimum mercury thermometer and the humidity was measured using a whirling hygrometer. Measurements were made twice daily on weekdays and once daily at week-ends. Pelleted diet (modified rat and mouse diet No. 1, (SQC) BP Nutrition (UK) Ltd., Stepfield, Witham, Essex) and mains water in water bottles were available *ad libitum*. Prior to mating the rats were housed in groups of 5 by sex in stainless steel mesh cages and mated females were housed individually in solid floor polypropylene cages. The rats were mated overnight, one male to two females, over an eight day period. Vaginal smears were examined each morning for sperm. The day on which sperm were observed was designated Day 0 of gestation and mating was discontinued. Immediately after mating the female rats were randomly allocated to treatment groups according to bodyweight. These groups of 24, successfully mated females were dosed by gavage with 5, 10 or 15 mg cyhalothrin/kg/day in maize oil on Days 6-15 (inclusive) of gestation. A control group of 24 mated animals was treated in an identical manner except that they received maize oil alone. One dosing formulation was made up for each dose level, 3 days before the start of dosing. Each formulation was divided into aliquots containing sufficient dosing material for each day. Animals were dosed at a rate of 10 ml/kg bodyweight based on the rat's bodyweight on the first day of treatment (Day 6 of gestation). The dose volume was adjusted only in the event of weight loss. The dosing solutions were checked, by chemical analysis, for achieved concentration	X3

	and stability over the period of dosing. As the formulations were solutions, homogeneity was not assessed. All animals were checked on arrival to ensure that they were physically normal externally and were subsequently examined at least once daily for signs of ill-health, toxicity or behavioural change. The bodyweight of each mated female was recorded on Days 0, 6-15, 18 and 20 of gestation and food intake recorded for each mated female from Day 0 on Days 3, 6, 9, 12, 15, 18 and 20. On Day 20 of gestation the rats were killed by cervical dislocation, dissected and examined macroscopically. The ovaries and intact gravid uterus were removed and weighed. The ovaries and uterus were then examined for live fetuses and intra-uterine deaths. Corpora lutea were counted. The foetuses were removed and then killed by intra-cardiac injection. Individual foetuses were weighed, examined, sexed and crown/rump length measured. Two thirds of the foetuses from each litter were dissected and the viscera examined. They were then eviscerated and the skeletons stained with Alizarin Red S and examined for abnormalities and degree of ossification. The remaining foetuses were placed in Bouin's fluid to allow fixation and partial decalcification. They were later transferred to 70% alcohol and sections examined for soft tissue abnormalities.	X4
Results:	Analyses showed that the dose formulations were stable over the period of dosing and that the concentrations achieved were satisfactory. There were no mortalities in any groups. Two animals given 15 mg cyhalothrin/kg/day showed uncoordinated limb movements which were considered to be compound-related. There were no other changes in clinical condition attributable to the compound. The initial effect of dosing was a slight weight loss in all groups including controls. At 15 mg cyhalothrin/kg/day there was an overall statistically significant reduction of bodyweight gain between Days 6 and 20 of gestation. Following the slight initial weight loss, bodyweight gain at 5 and 10 mg cyhalothrin/kg/day was either comparable with or marginally lower than controls with no statistically significant differences, although there were indications of a dose-related trend between Days 6 and 12 of gestation.	X6

	Treatment with cyhalothrin at 15 mg/kg/day was also associated with reduced food intake throughout the study, the difference from controls being statistically significantly different between Days 6 and 12 of gestation. At 5 and 10 mg cyhalothrin/kg/day only slight reductions in food intake compared to controls were apparent during the study (no statistically significant differences). There was no effect of treatment with cyhalothrin on pregnancy incidence. There was no effect on the number, size, weight or sex of the foetuses and no effect on the number of pre-or post-implantation losses. Gravid uterus weights were comparable across all groups. There was no effect of treatment with cyhalothrin on the incidence of major defects, minor external and visceral defects or minor skeletal defects and variants.	
Conclusion:	In conclusion, there was no evidence for teratogenicity or foetotoxicity in the rat at any of the dose levels tested in this study. The no-observed adverse effect level (NOAEL) for maternal toxicity was 10 mg cyhalothrin/kg/day. The no-observed effect level (NOEL) and the NOAEL for developmental toxicity were both in excess of 15 mg cyhalothrin/kg/day since there were no adverse findings.	

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.8.1 / 01	**Reproductive Toxicity – Teratogenicity test**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and Methods	[redacted]
Results and discussion	

Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	**6.8.1 / 02**	**Reproductive Toxicity – Teratogenicity test**	**Official use only**
91/414 Annex Point addressed	**II 5.6.2**	Developmental toxicity studies	

Title:	„CYHALOTHRIN : Oral (Gavage) Teratology Study in the New Zealand White Rabbit."	
Lab Report Number:	No. [redacted]/C/1072	
Authors:	[redacted]	
Test Substance:	Technical cyhalothrin (otherwise known as PP563). Purity [redacted]% (w/w).	
Species:	Rabbit	
Method:	OECD Guideline 414 "Teratogenicity"	
Date of Report:	1981	
Published:	No	
GLP:	Yes	

Material and Methods:	The purpose of the study was to assess the potential of the test substance to affect the embryonic and foetal development of the New Zealand White rabbit when administered during the period of organogenesis. Sexually mature female New Zealand White rabbits were mated with males of the same strain and from the same source after an acclimatisation period of 20 days. There were insufficient suitable females available in this first batch to complete the mating and ten more were delivered at a later date and allowed 8 days acclimatisation prior to mating.	

	The temperature in the animal rooms was within the range 16.5 ± 4.50C and the humidity was within the range 75 ± 11%.The temperature was recorded using a maximum/minimum mercury thermometer and the humidity was measured using a whirling hygrometer. Measurements were made twice daily on weekdays and once daily at week-ends and on public holidays.	X3
	The female rabbits were each mated with 3 different bucks to maximise the chances of successful fertilisation and after coitus had taken place, were injected with chorionic gonadotrophin to ensure ovulation.	X4
	Immediately after mating, the does were randomly allocated to treatment groups. The animals were housed individually in steel cages and allowed free access to filtered mains water via automatic drinking valves. A commercially available pelleted diet (Beta Standard Rabbit Diet, BP Nutrition (UK) Ltd., Stepfield, Witham, Essex) was available to the animals *ad libitum*.	
	Three dose levels were used in this study in addition to the control. Groups of at least 18 mated rabbits received cyhalothrin by gavage at levels of 3, 10 or 30 mg/kg bodyweight daily for 13 consecutive days from Day 6 to 18 of gestation. Another group received the vehicle (corn oil) alone, over the same period and served as the control group. Each animal was dosed at a constant volume based on its bodyweight on the first day of treatment (day 6 of gestation).	X5 X6
	One dosing formulation was made up for each dose level, 3 days before the start of dosing. Each formulation was divided into aliquots containing sufficient dosing material for each day. Samples of each dose level and control were analysed on the day of preparation and on the last day of dosing (28 days after preparation). As the formulations were solutions, homogeneity measurement was not required.	
	All rabbits were checked on arrival to ensure that they were physically normal externally and were subsequently examined at least once daily for signs of ill-health, toxicity or behavioural change.	
	The bodyweight of each mated female rabbit was recorded on Days 0, 6-19, 24 and 28 of gestation and food intake was recorded on gestation Days 0, 3, 6, 9, 12, 15, 18, 21, 24 and 28.	
	On Day 28 of gestation, the does were killed by cervical	

	dislocation, dissected and examined macroscopically. The ovaries and intact gravid uterus were removed and weighed. The ovaries and uterus were then examined for live fetuses and intra-uterine deaths. Corpora lutea were counted. The foetuses were removed and then killed by intra-cardiac injection. Individual foetuses were weighed, examined and crown/rump length measured. They were then skinned, dissected, examined internally for visceral abnormalities, eviscerated and fixed in methanol. The head of each foetus was cut and the brain examined for any macroscopic abnormalities. The carcasses were then stained with Alizarin Red S. The stained foetal skeletons were examined for abnormalities and the degree of ossification of the bones was assessed.	
Results:	Analyses showed that the dose formulations were stable over the period of dosing and that the concentrations achieved were all within 10% of the nominal level. During the study, there were 1, 2, 6 and 2 deaths in the control, 3, 10 and 30 mg cyhalothrin/kg/day groups respectively. Of these, 3 females (one in each of the control, 3 and 10 mg cyhalothrin/kg/day groups) died on day 6 prior to the start of dosing and none of the later deaths was considered to be related to treatment with cyhalothrin. To replace animals that died during the early part of the study, additional mated female rabbits were included (1 in the control group, 1 in the 3 mg/kg group and 4 in the 10 mg cyhalothrin/kg group). No changes in clinical condition attributable to treatment with cyhalothrin were seen in the surviving animals. Treatment with cyhalothrin at 30 mg/kg/day elicited maternal toxicity manifest as initial weight loss. The difference in weight gain from controls was statistically significant between gestation days 6 to 9. Subsequent weight gain during the treatment period was slightly reduced compared to controls. Reduced food intake during the dosing period was also associated with treatment at this dose level. No adverse effects on bodyweight or food consumption were apparent in the 3 or 10 mg cyhalothrin/kg/day groups. Macroscopic examination of the dams revealed no compound-related findings. There was no effect of treatment with cyhalothrin on pregnancy incidence or on gravid uterus weights. At termination, the number of dams with live foetuses *in utero* was 16, 13, 14 and 12 for the control, 3, 10 and 30 mg cyhalothrin/kg/day groups respectively. There was no conclusive evidence of any compound-related effect on any of the litter parameters and no evidence of any compound-related effect on the	X7 X8 X9 X10

	overall incidence of major or minor external, visceral or skeletal defects. Major defects were observed in all groups including controls. The incidence of foetuses with major defects was 5, 1, 3 and 2 foetuses in the control, 3, 10 and 30 mg cyhalothrin/kg/day groups respectively. Neither the type nor the incidence of major defects was considered to be related to treatment with cyhalothrin. Similarly, there was no indication of an effect of treatment with cyhalothrin on either the type or incidence of minor external, visceral or skeletal defects and treatment with cyhalothrin was not associated with an adverse effect on the incidence of skeletal variants.	
Conclusion:	In conclusion, there was no evidence for teratogenicity in this study. Both the no-observed effect level (NOEL) and the no-observed adverse effect level (NOAEL) for maternal toxicity were 10 mg cyhalothrin/kg/day since there were no compound-related effects at this dose level. Since there were no compound-related effects, the NOEL and NOAEL for foetal development were in excess of 30 mg cyhalothrin/kg/day.	X11

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.8.1 / 02	**Reproductive Toxicity – Teratogenicity test**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and Methods	[redacted]

Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	**6.8.2 / 01**	**Two generations reproduction study**	**Official use only**
91/414 Annex Point addressed	**II 5.6.1**	Multi-generation studies	

Title:	„CYHALOTHRIN: Three Generation Reproduction Study in the Rat“	
Lab Report Number:	No. [redacted]/P/906	

Authors:	[redacted]	
Test Substance:	Technical cyhalothrin (otherwise known as PP563). Purity [redacted] w/w.	X1
Species:	Rat	
Method:	OECD Guideline 416	
Date of Report:	1984	
Published:	No	
GLP:	Yes	

Material and Methods:	The purpose of this study was to assess the effects of the continuous feeding of diets containing cyhalothrin on the reproductive performance of three generations of the Alpk/AP (now known as Alpk:APfSD) Wistar derived strain of rat. The fertility of each generation of parental animals and the clinical condition, survival and subsequent growth of their offspring was determined. Groups of 15 male and 30 female (F0 Parents) weanling [redacted] Wistar-derived (now known as the Alpk:ApfSD strain) rats were fed diets containing 0, 10, 30 or 100 ppm cyhalothrin*. All parent animals were individually identified. Test diets were fed continuously throughout the study.	X2
	The temperature of the animal room was generally within the range of 20-250C and humidity between 35% and 65%, with occasional values outside these ranges. Lighting was controlled to give alternate periods of 12 hours light and 12 hours dark. After twelve weeks, the F0 Parents were mated to produce the first (F1A) litter and subsequently re-mated to produce a second (F1B) litter. The breeding programme was repeated with F1 parents selected from the F1B offspring and F2 parents selected from the F2B offspring.	X3
	During the study all rats were observed daily for abnormalities in clinical condition and behaviour. Moribund or dead rats were sent for post mortem examination. During each of the pre-mating periods (12 weeks for the F0 Parents and 11 weeks for the F1 and F2 Parents), a detailed examination of each rat was made weekly to detect any abnormalities in clinical condition or behaviour. Bodyweights of all animals were also	

	recorded weekly (from the day of selection for the F1 and F2 Parents). Food consumption was calculated taking into account the food wastage for each cage. During mating two females were housed with one male. Daily vaginal smear examinations were done to determine when mating occurred (this was then designated Day 1 of gestation). If after ten days there was no evidence of mating, the first male was removed and after three days was replaced by a second male of proven fertility. There was a 3 day period between pairings which ensured that paternity could be correctly ascribed. When there was evidence of pregnancy, i.e. a positive vaginal smear or abdominal enlargement and weight gain, the female was separated from the male and individually housed. At approximately Day 15 of pregnancy the cages housing the females were fitted with solid floors and autoclaved paper bedding material was provided. The females remained in these cages throughout gestation and lactation. Selection of animals for the F1 and F2 generations was done so as to achieve minimal in-breeding. A maximum of two female and one male pup from any one litter was selected (unless the litter consisted of only a single sex, in which case the selection of two males or three females was allowed). Females were not mated with brothers or half-brothers. Mated animals were as close in age as possible. The F0, F1 and F2 parents were subjected to gross post mortem examination at termination of the generation. Testes, epididymides, prostate gland, seminal vesicles and any abnormal tissues from all male rats and the ovaries, uterus, cervix, mammary gland and any abnormal tissue from all female rats were submitted for histopathological examination. In addition, the livers from all control and 100 ppm group animals were also examined. Litters were examined at least once daily and dead or grossly abnormal pups removed for soft tissue examination. A count of all live and still born pups was made within 24 hours of parturition and thereafter at Days 5, 11, 22 and 29 post partum. The sex of the pups was also recorded at these times. Any clinical abnormalities seen in the pups were recorded. Individual pup bodyweights were recorded within 24 hours of birth (Day 1) and at Days 5, 11, 22 and 29 post	X4 X5

	partum for both the A and B litters from each generation. Litters were weaned at 29 days post partum. As pups were not individually identified, data were recorded by sex and litter. The pups selected at Day 35 were weighed to give initial F1 or F2 parent bodyweight values, and thereafter on the same day of each week for the duration of the pre-mating period. On Day 36 post partum, approximately 50% of pups from the A litters were subjected to gross post mortem examination, with abnormal tissues examined histopathologically. Pups over 18 days old which were found dead or moribund were subjected to a full post mortem examination with tissues submitted for histopathological examination and pups found dead up to and including 18 days of age were removed for dissection and free-hand sectioning based on the technique of Wilson J G (1965). After selection of the next generation parents, 5 male and 5 female rats per group from the F1B and F2B litters and 10 male and 10 female rats per group from the F3B litters were subjected to a full post mortem examination with tissues submitted for histopathological examination. Approximately 50% of the remaining rats were subjected to a gross post mortem examination with only abnormal tissues submitted for examination. The remainder was discarded after external examination. * The dose received expressed as mg/kg/day is given in the Summary of Results.	
Results:	Throughout the study, diet analyses showed that most of the diets were within ±10% of nominal concentration (there were four occasions only when the concentration found was not within 10% of nominal - the maximum recorded deviation from nominal was 16.7%). Stability and homogeneity were satisfactory. A single adult male died during the study and two females were found to have imperforate vagina (an occasional congenital abnormality of the Alpk:APfSD strain). All other adult rats from all generations survived the study in good condition. Male rats, of the F0 generation, receiving 100 ppm cyhalothrin had a marginal reduction in weight gain during the first week of the study. There were no effects seen in the other dose groups. In the F1 and F2 generations, males receiving 100 ppm had a lower initial	X6

	starting weight than controls and a marginal reduction in bodyweight gain compared to controls (which sometimes achieved statistical significance). Bodyweight gain was also slightly lower for F1 generation males given 10 ppm cyhalothrin but these differences were not statistically significantly different to control values. No reductions in bodyweight gain were seen in any of the treated females in the F0 generation, compared to controls. In the F1 generation, bodyweight gain was slightly reduced compared to controls at 30 and 100 ppm cyhalothrin, and in the F2 generation bodyweight gain was marginally reduced at 100 ppm compared to controls. As there was an effect on bodyweight at 30 ppm only in the F1 generation, it is considered that this small reduction is of no toxicological significance. There was no evidence of reduced weight gain during pregnancy in the F0 or F1 generations but animals given 100 ppm in the F2 generation did show a very slight reduction in weight gain for both the A and B litters (which only achieved statistical significance for the B litter). Initial bodyweights for both F1 and F2 generations were lower than controls, reflecting the reduced bodyweight gain in the pre-mating period. There were no consistent differences in food consumption between control and treated animals. There was no compound-related effect on indices of male or female fertility, gestation period, number of liveborn or pup survival. There was a small reduction in mean total litter weight of the F2 and F3 generations in rats receiving 100 ppm cyhalothrin, which was attributable to minor decreases in litter size and small reductions in pup weight gain, and which persisted through the lactation period. No effect was seen in litters from rats receiving 30 ppm cyhalothrin. There was no evidence of gross or histopathological changes attributed to treatment with cyhalothrin in either adults or young.	X7 X8 X9 X10 X11
Conclusion:	In conclusion, therefore, rats fed a diet containing 100 ppm cyhalothrin showed minor effects on weight gain throughout the study. There was a small decrease in litter weight and litter weight gain at this dose level. This effect was seen from the second litter of the second generation and became more evident in the third generation. This is considered to be compound-related. A dose of 30 ppm was established as both the no-observed effect level (NOEL) and the no-observed adverse effect level (NOAEL) as no adverse effects were seen at this dose level. No other parameters were	

	affected. There was no evidence of compound-related effects on the nervous system of any rat (adult or young) during the study. Parental toxicity was shown by a slight reduction in bodyweight gain at the top dose of 100 ppm cyhalothrin, particularly in males. No neurological effects were seen in parents or offspring. A reduction in bodyweight gain at 30 ppm cyhalothrin seen in both sexes, but only in the F1 generation, was considered to be of no toxicological significance. Small reductions in total litter weight attributable to small decreases in litter size and pup weight were seen in the F2 and F3 litters in the 100 ppm cyhalothrin group. There were no other effects on reproductive parameters and no compound-related effects at 10 or 30 ppm cyhalothrin. The no-observed adverse effect level (NOAEL) was 30 ppm (approximately 2 mg cyhalothrin/kg/day).	X12

NB.The terms F0 and P are equivalent, both refer to the first parental animals mated.

Doses of cyhalothrin Received (mg/kg/day)

Generation Sex/week Dietary Concentration of cyhalothrin (ppm)	**10**	**30**	**100**
F0			
males/1	1.71	4.7	15.7
males/4	0.89	2.6	9.2
males/12	0.50	1.6	5.3
females/1	1.53	4.5	14.3
females/4	0.79	2.2	8.4
females/12	0.54	1.5	5.2
F1			
males/1	1.50	4.3	12.7
males/4	1.00	2.9	9.7
males/11	0.63	1.8	5.7
females/1	1.30	3.7	13.5
females/4	0.93	2.8	9.0
females/11	0.67	1.9	6.1
F2			
males/1	1.46	4.4	16.1
males/4	0.90	2.8	8.3
males/11	0.54	1.6	5.9
females/1	1.34	3.8	13.8
females/4	0.87	2.7	8.9

females/11	0.57	1.8	6.2

Achieved doses are calculated from nominal concentrations.

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA section No.6.8.2 / 01	**Two generations reproduction study**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and Methods	[redacted]
Results and discussion	[redacted]

[redacted]

Conclusion
Reliability
Acceptability
Remarks

9. NEUROTOXICITY

98/8 Doc IIIA section No.	6.9/ 01	Neurotoxicity study	Official use only
91/414 Annex Point addressed	II 5.7	Delayed neurotoxicity (strictly not a *delayed* neurotoxicity study – more appropriate under 5.8 Other toxicological studies)	

Title:	*Lambda*-cyhalothrin: Acute Neurotoxicity Study in Rats.	
Lab Report Number:	No. [redacted]/P/6151	
Authors:	[redacted]	
Test Substance:	*lambda*-cyhalothrin	X1
Species:	Rat	
Guidelines:	Not specified (not an EU requirement)	
Date of Report:	1999	
Published:	No	
GLP:	Yes	

Material and Methods:	Groups of 10 male and 10 female Alpk:APfSD (Wistar-derived) rats were given single oral doses of 0 (control), 2.5, 10 or 35 mg *lambda*-cyhalothrin/kg bodyweight and were killed 2 weeks later. Samples of each preparation of dosing formulations were analysed prior to the start of dosing to verify the achieved concentrations of *lambda*-cyhalothrin in corn oil. The chemical stability of *lambda*-cyhalothrin in corn oil was determined for all dose levels over a period of up to 15 days. All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, and an assessment of locomotor activity were performed in week -1, and on days 1 (approximately 2 hours after dosing), 8 and 15. Bodyweights and food consumption were measured weekly throughout the study. At the end of the scheduled period, 5 rats/sex/group were deeply anaesthetised, killed by whole body perfusion fixation, subjected to full examination *post-mortem* and submitted for neuropathology. Brain weight, length and width were recorded for these animals. Nervous system tissues from the top dose group and control group were examined microscopically.	X2 X3
Results:	There were no mortalities. Clinical signs were observed at 35 mg/kg at approximately 7 hours and/or 24 hours post-dosing. These signs included decreased activity, ataxia, reduced stability and/or tiptoe gait. Some signs were observed in males and females dosed with 10 mg/kg on day 1 or 2. Recovery from the clinical effects of *lambda*-cyhalothrin was evident by day 4 in all animals.	X4 X5

	Bodyweights were statistically significantly reduced on day 8 in males dosed with 35 mg/kg and food consumption was reduced during week 1 in both sexes given 35 mg/kg. There was a statistically significant reduction in landing foot splay measurements in males and a statistically significant increase in tail flick response in females on day 1. Motor activity was reduced in both males and females dosed with 35 mg/kg on day 1. There was no evidence of any treatment-related effects on brain weight, length or width. There were no macroscopic findings and comprehensive histopathological evaluation revealed no treatment-related changes in the central or peripheral nervous system of animals.	X6 X7
Conclusion:	In conclusion a single oral dose of 35 mg *lambda*-cyhalothrin/kg produced clinical signs of neurotoxicity and minor changes in the functional observational battery on the day of dosing which completely reversed by day 5. There were no pathological changes. The NOEL for neurotoxicity, following a single administration of *lambda*-cyhalothrin in the rat, is 2.5 mg/kg. The NOAEL is considered to be 35 mg *lambda*-cyhalothrin/kg due to the reversibility of the clinical effects.	X8

Intergroup Comparison of Bodyweight (g)

		Dose level of *lambda*-cyhalothrin (mg/kg)			
		0	2.5	10	35
Males	Day 1	206.1	204.4	204.5	198.8
	Day 8	274.0	277.3	271.6	262.7**
	Day 15	315.1	319.3	320.4	308.5

Adjusted means on days 8 and 15
** Statistically significant difference from control group mean, 1% level (Student's t-test, 2-sided)

Group Mean Landing Foot Splay (day 1) for Males

Landing foot splay	Dose level of *lambda*-cyhalothrin (mg/kg)			
	0	2.5	10	35
Day 1	66.7	64.0	61.9	53.0*

* Statistically significant difference from control group mean, 5% level (Student's t-test, 2-sided)

Time To Tail Flick (day 1) for Females (seconds)

Tail Flick	Dose level of *lambda*-cyhalothrin (mg/kg)			
	0	2.5	10	35
Day 1	5.8	5.5	6.9	9.9*

* Statistically significant difference from control group mean, 5% level (Student's t-test, 2-sided)

Intergroup Activity of Motor Activity on Day 1

Minutes	Dose level of ZA1296 (mg/kg)							
	0		20		200		2000	
	male	female	male	Female	male	female	male	female
11-15	48.3	50.9	54.7	55.5	47.2	39.9	42.1	27.0**
16-20	44.6	27.2	48.2	49.5*	40.8	34.5	26.4	36.2
21-25	35.6	38.3	44.6	43.1	34.8	26.7	40.8	16.3*
41-45	37.8	27.9	33.0	32.4	22.8	23.0	14.9*	31.0
46-50	44.7	39.6	30.6	41.9	17.9*	29.3	31.7	36.6
1-50	428.9	398.0	428.0	462.5	350.3	333.1	358.0	311.4

* Statistically significant difference from control group mean, 5% level (Student"s t-test, 2-sided)
** Statistically significant difference from control group mean, 1% level (Student"s t-test, 2-sided)

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
98/8 Doc IIIA section No. 6.9/ 01	**Neurotoxicity study**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

Section A6.9 (02) **Delayed Neurotoxicity**

Annex Point IIA6.9

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (2004). Lambda-cyhalothrin: Developmental Neurotoxicity study in rats. Unpublished laboratory report number RR0969. Report dated 3 November 2004	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Syngenta Crop Protection, Inc	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US EPA Health Effects Test Guideline OPPTS 870.6300, Developmental Neurotoxicity Study, August 1998.	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	*lambda*-cyhalothrin	
3.1.1	Lot/Batch number	Batch [redacted]) Purity [redacted]% w/w	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Dark brown solid	

Section A6.9 (02) Delayed Neurotoxicity

Annex Point IIA6.9

3.1.2.2	Purity	Purity 87.7% w/w	
3.1.2.3	Stability	The test material was used within its stated expiry date and was re-analysed to confirm stability after completion of the in-life phase of the study. A certificate of analysis for each periodic re-analysis is retained in the laboratory archive. Stability of test material in diet as determined as up to 29 days	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Alpk:APfSD (Wistar-derived)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Females (time-mated)	
3.2.5	Age/weight at study initiation	10-12 weeks old, 217 – 296g bodyweight range at time of delivery. Acclimatisation period for dams – six days.	
3.2.6	Number of animals per group	Groups of 30 time-mated Alpk:APfSD (Wistar-derived) rats.	
3.2.7	Control animals	Yes	
3.2.8	Housing	Dams were housed individually in solid plastic cages with wood flake bedding. The temperature range was 19-25°C; humidity was within the range 39-75% RH, 15 air changes per hour were effected and the lighting was artificial on a 12 hour on/off cycle. F1 animals were housed with parent female up to day 29 post partum, after which litters were separated by sex and housed in groups of four/sex/wiremesh cage.	
3.3	**Administration**	Oral by dietary inclusion	
3.3.1	Exposure	Daily administration from day 7 of pregnancy through parturition and lactation to day 23 post partum	
3.3.2	Dose Levels	0, 25, 60 or 150 ppm	
3.3.3	Vehicle	Basal diet	
3.3.4	Controls	Vehicle/basal diet	
3.4	**Examinations**		
3.4.1 and	Clinical examination bodyweight	All rats were examined on arrival to ensure clinical normality. Detailed clinical examinations and body weights were recorded at the same intervals throughout the study (prior to dose administration on day 7; on days 15 and 22 of gestation; on days 1, 5, 8, 12, 15 and 22 post partum and on day of termination). Cageside observations were recorded twice daily. Food consumption was recorded throughout gestation and post partum and calculated as g diet consumed/rat/day.	X2

Section A6.9 (02) Delayed Neurotoxicity

Annex Point IIA6.9

3.4.2	Functional observation battery	On days 10 and 17 of pregnancy, dams were subjected to a functional observation battery of tests outside the cage including assessment of signs of autonomic function e.g. lachrymation, ptosis, papillary function, exophthalmus, salivation, pilo-erection, urination and defecation. Any convulsions, tremors or abnormal behaviour or movements, posture or gait were noted.	
		The F1 offspring were subjected to the same functional observation battery as given to the dams. Examinations were completed on days 5, 12, 22, 36, 46 and 61 post partum.	X3
		In addition, on days 14, 18, 22 and 60, selected offspring were also assessed for effects on motor activity, on days 23 and 61 for response to auditory startle, assessment of learning and memory (Y-shaped swimming maze) on either days 21 or 59, and retested 3 days later.	X4
3.4.3	Litter examinations	Litters were examined after completion of parturition, and on days 1 and 5, sex, weight and clinical condition each pup was recorded. Bodyweights were recorded for F1 animals on days 5, 12, 18, 22, 29, 36, 43, 50 and 57 and at termination on day 63.	X5
3.4.4	Pathology	Yes. At termination, animals were subject to gross necropsy and neuropathological evaluation including morphometry and brain weights. On day 12 post partum and at termination on day 63, ten animals per sex per group were killed by over-exposure to carbon dioxide and the brain weighed after fixation (to prevent damage). On day 63 a further ten rats/sex/group were perfused with formol saline, and various neural tissues taken and preserved, including brain, eye, spinal cord, spinal nerve roots, root ganglia, sciatic and tibial nerves.	X7
3.4.5	Histopathology	Yes	
		Organs: Brains from control and high dose group animals were processed and examined by light microscopy.	X7
3.4.5 remarks	**Further**	Litters were culled to 8 pups on day 5. Offspring were separated from the dam on day 29 post partum, caged in fours by sex and maintained to 63 days of age. The F1 animals were not given test diet after separation from the dam.	X7
			X8

4. RESULTS AND DISCUSSION

Section A6.9 (02) Delayed Neurotoxicity

Annex Point IIA6.9

4.1	Body Weight and food intake	Maternal bodyweight and food intake was lower than controls at 60 and 150 ppm.	X9
4.2	Clinical signs of toxicity - maternal	A number of females were terminated for various reasons prior to scheduled termination – these were randomly spread across control and treated groups and included:	
		failing to litter, death, human termination, total litter loss or insufficient pups (sufficient was deemed to be at least 3 males and 3 females in a litter of at least 7 on day 5)	X10
		The other clinical signs observed were non-specific and commonly encountered in rats and were not considered to be treatment-related.	
4.3	Clinical signs of toxicity - pups	Pup survival and mean pup weight were slightly lower at 150 ppm, but there were no other adverse effects, on duration of gestation, parturition, and numbers and type of macroscopically abnormal offspring.	X11
4.4	Functional observation battery for pups	There were no treatment-related effects on clinical observations, gross neurological and behavioural abnormalities or adverse effects on motor activity, response to auditory startle, assessment of learning and memory as either young pups (age 21-24 days) or	X12
		young adults (age 59-62 days). Day 21 swimming speeds of females at 150 ppm were slightly lower than controls, although this was considered to reflect a difference in swimming performance rather than an effect on learning or memory.	X13
4.5	Pathology	No effects	
4.6	Histopathology	There were no adverse effects on the neuropathological evaluation including morphometry and brain weights.	X14
4.7	Other	Dietary stability and homogeneity for the test material was demonstrated for up to 29 days.	
		Mean maternal intake of test material during gestation was calculated for groups dosed at 25, 60 or 150 ppm to be 2.1, 4.9 or 11.4 mg/kg bw/day. Post partum equivalents were 4.6, 10.7 or 26.3 mg/kg bw/day. (The report assumes that all post partum food was consumed by the dam).	