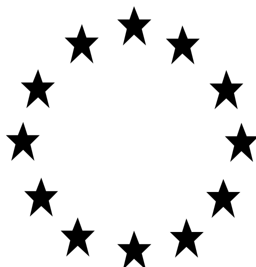
Competent Authority Report

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



Cyphenothrin (PT 18)

CAS-No. 39515-40-7

Sumitomo Chemical (U.K.) PLC

DOCUMENT III-A

Study summaries

Section A6.6

Toxicology section

Rapporteur: Hellas

November, 2017

6.6 Genotoxicity studies

6.6.1 In vitro gene mutation study in bacteria

			Official use only
		1. REFERENCE	
1.1	**Reference**	Reference : A6.6.1/01 Authors : [redacted] Title: Gene Mutation Test of [redacted] in Bacterial System Laboratory : Sumitomo Chemical Co. Unpublished Report no : [redacted] Date : October 21, 1982	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Sumitomo Chemical Co. Japan	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No (but broadly an Ames Test)	
2.2	**GLP**	Yes	x
2.3	**Deviations**	Not applicable	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	None given	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	x
3.2	**Study Type**		
3.2.1	Organism/cell type	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 *Escherichia coli* WP-2 *uvrA*	
3.2.2	Deficiencies / Proficiencies		
3.2.3	Metabolic activation system	PCB-induced Sprague Dawley rat liver S9 reaction mixture The in vitro metabolic activation enzyme system (S9 mix) was prepared according to the method described by Ames	
3.2.4	Positive control	Methyl methanesulfonate, 2-nitrofluorene, 9-aminoacridine, ethylnitronitrosoguanidne without S9 mix. benzo(a)pyrene, 2-aminoanthracene with S9 mix.	

3.3 Administration/ Exposure; Application of test substance

3.3.1 Concentrations: 10, 50, 100, 500, 1000 and 5000 µg/plate

3.3.2 Way of application: The test compound was dissolved in DMS0 and diluted to appropriate concentrations. The test compound solution (100 µl), 0.1 ml indicator cell suspension and 0.5 ml 100 mM Na-phosphate buffer, pH 7.4 (without S9 mix), or 0.5 ml S9 mix were mixed in a small test tube and they were incubated at 37°C for 20 min with shaking (preincubation). Then they were mixed with 2 ml of melted soft agar containing 0.05 mM histidine, 0.05 mM biotin (for *S.typhimurium* or 0.05 mM tryptophan (for *E.coli*) and poured onto a minimal agar plate After 2-day incubation at 37°C, the revertant colonies were counted by an automatic colony counter

3.3.3 Pre-incubation time: 20 min

3.3.4 Other modifications: None

3.4 Examinations

3.4.1 Number of cells evaluated

4. RESULTS AND DISCUSSION

4.1 Genotoxicity

4.4.1 Without metabolic activation: The number of the revertants at each dose level did not increase as compared with those of the corresponding vehicle controls under any test conditions.
Positive controls, methyl methanesulfonate, 2-nitrofluorene, 9-aminoacridine and ethylnitronitrosoguanidne induced significant number of revertants in respective strains.

4.4.2 With metabolic activation: See above
Positive controls, benzo(a)pyrene and 2-aminoanthracene induced significant number of revertants in respective strains.

4.2 Cytotoxicity: Cyphenothrin proved to be non-toxic to the indicator strains.

5. APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods: In an Ames test (1982) using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, and *Escherichia coli* WP-2 *uvrA*, dose levels were selected as 0, 10, 50, 100, 500, 1000 and 5000 µg cyphenothrin/plate. (Cyphenothrin purity 93.6%). The test was performed with metabolic activation provided by PCB-induced Sprague Dawley rat liver S9 and without metabolic activation.

5.2 Results and discussion: Cyphenothrin did not increase the numbers of revertant populations in any strains at any dose level with or without metabolic activation. The positive controls gave the appropriate responses. No evidence of cytotoxicity was reported.

5.3 Conclusion: It can be concluded that cyphenothrin is not mutagenic under the conditions in this study.

5.3.1 Reliability: 2

5.3.2 Deficiencies: Not conducted to a recognized OECD guideline

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November, 2017
Guidelines and quality assurance	Point 2.2: No official GLP certificate is provided.
Materials and methods	The applicant's version is acceptable. Point 3.1.2.3: The stability of the test item was not mentioned in the study
Results and discussion	The applicant's version is acceptable.
Conclusion	The applicant's version is acceptable.
Reliability	2
Acceptability	The study is considered acceptable.
Remarks	No further remaks.

6.6.2 In vitro cytogenicity study in mammalian cells

			Official use only
		1. REFERENCE	
1.1	**Reference**	A6.6.2/01 Authors : [redacted] Title: Mutagenicity test on Gokilaht in an *in vitro* cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells Laboratory : [redacted] Unpublished Report no : [redacted] Date : April 13, 1989	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Sumitomo Chemical Co. Japan	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	Yes	
2.3	**Deviations**	Not applicable	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Clear yellow viscous liquid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	
3.2	**Study Type**		
3.2.1	Organism/cell type	Chinese hamster ovary (CHO) cells	
3.2.2	Deficiencies / Proficiencies	-	
3.2.3	Metabolic activation system	PCB-induced Sprague Dawley rat liver S9 reaction mixture	
3.2.4	Positive control	The positive control agents which were used in the assays were mitomycin C (MMC) for the nonactivation series and cyclophosphamide (CP) in the metabolic activation series.	
3.3	**Administration/ Exposure; Application of test substance**		

3.3.1	Concentrations	The range finding assay was conducted at concentrations ranging from 0.0133 to 400 µg/ml (up to solubility limit in the culture medium), 1) to determine the dose range to be used in the chromosomal aberrations assay and 2) to determine the optimal times of harvest for analyzing primarily metaphase cells in the chromosomal aberrations. The cultures were incubated for 25-26 hours with 5-bromo-2'-deoxyuridine (BrdUrd). Without metabolic activation: A precipitate was visible at 133 and 400 µg/ml. Visible mitotic cells measured as indicator of cytotoxicity were not found at 40.0 µg/ml or more. A reduction in visible mitotic cells was observed at 13.3 µg/ml. Cell cycle kinetics were evaluated up to 13.3 µg/ml, severe cell cycle delay was observed at 13.3 µg/ml. Based on these findings, the testing concentration range from 7.56 to 40.0 µg/ml with 20 hour harvest (17.25 hours chemical treatment) was set for the chromosomal aberrations assay. Following concentrations were analyzed. 12.6, 20.0, 30.0, 40.0 µg/ml (without metabolic activation – 20 hour harvest) With metabolic activation: A precipitate was visible at 133 and 400 µg/ml. A reduction in visible mitotic cells and a cell cycle delay were observed at the top concentration of 400 µg/ml. Cell cycle kinetics were evaluated up to 400 µg/ml, severe cell cycle delay was observed at 400 µg/ml. Based on these findings, the testing concentrations ranging from 100 to 400 µg/ml with 10, 20 and 30 hour harvests (2 hours chemical treatment) were used for the chromosomal aberrations assay. Following concentrations were analyzed. 150, 200 µg/ml (with metabolic activation – 10 hour harvest) 150, 200, 300, 400 µg/ml (with metabolic activation – 20 hour harvest) 200, 300, 400 µg/ml (with metabolic activation – 30 hour harvest)
3.3.2	Way of application	McCoy's 5a medium (DMSO as solvent)
3.3.3	Pre-cultivation time	1 day
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	One hundred cells from each culture for test chemical, negative control and solvent control. Twenty-five cells from positive control culture.
		4. RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	Without metabolic activation	No significant increase in chromosomally aberrant cells was observed at the concentrations analyzed. The sensitivity of the cell culture for induction of chromosomal aberrations is shown by the increased frequency of aberrations in the cells exposed to the positive control agent. The test article is considered negative for inducing chromosomal aberrations under nonactivation conditions.
4.1.2	With metabolic activation	No significant increase in chromosomally aberrant cells was observed at the does analysed with any of the three harvests(10, 20 and 30 hours). The successful activation of the metabolic system is illustrated by the increased incidence of chromosomally aberrant cells in the cultures induced with cyclophosphamide the positive control agent. The test article is considered negative for inducing chromosomal aberrations under conditions of metabolic activation.

4.2	**Cytotoxicity**	Yes, observed all conditions.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Duplicates cultures were used at each dose level for each trial. Single cultures were used for the negative, solvent control and at each of two doses of the positive control. In the non-activated assay, a 20 hour harvest (17.25 hours chemical treatment) was conducted. In the activation assay, 10, 20, 30 hour harvests (2 hours chemical treatment) were conducted. Non-activated assay Cultures were initiated by seeding approximately 1.2 x10-6 cells per 75cm² flask into 10ml of complete McCoy's 5a medium. One day after cell culture initiation, the CHO cells to be used in the non-activation trial were treated with the test article at predetermined doses for 17.25 hours. The cultures were then washed and complete McCoy's 5a medium containing Colcemid® was placed back onto the cells. 2.5 hours later the cells were harvested and placed on slides for analysis. The slides were stained with Giemsa solution. Activated assay Cultures were initiated by seeding approximately 1.0x10-6 cells (30 hour assay), 1.2 x 10-6 cells (20 hour assay) and 1.5x10-6 cells (10 hour assay) per 75cm² flask into a 10ml of complete McCoy's 5a medium. One day after culture intiation, the cultures were incubated at 37°C for 2 hours in the presence of the test article and the S9 reaction mixture in McCoy's 5a medium without FSC. After the 2 hours exposure period, the cultures were then washed and complete McCoy's 5 a medium was placed back onto the cells. The cells were then incubated for an additional 7.75 (10 hour assay), 17.75 (20 hour assay) and 27.75 (30 hour assay) hours with 0.1 ug/ml Colcemid present during the last 2.5 hours of incubation. The metaphase cells were prepared for cytogenetic analysis. Statistical analysis employed the Fisher's Exact Test with an adjustment for multiple comparisons to compare the percentage of cells with aberrations in each treatment group with the results from the pooled solvent and negative controls (the solvent and negative controls were statistically evaluated for similarity prior to the pooled evaluation). Test article significance was established where $p \leq 0.01$.	x
5.2	**Results and discussion**	No significant increase in chromosomally aberrant cells was observed at any of the concentrations analysed. The positive controls gave the appropriate response.	
5.3	**Conclusion**	The test substance is considered negative for inducing chromosomal aberrations in Chinese hamster ovary cells under both non-activation and activation conditions.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	This study is not reported as being done to a recognized guideline	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November, 2017
Guidelines and quality assurance	Point 2.2: No official certificate for GLP is provided.
Materials and methods	The applicant's version is acceptable. Point 5.1: According to the OECD guideline No.473 (adopted July 1997) both in the presence and absence of S9 metabolic activation, cells should be exposed to the test substance for 3-6 hours and sampled at a time equivalent to 1.5 normal cell cycle length (approximately 20 hours) after the beginning of treatment.
Results and discussion	The applicant's version is acceptable.
Conclusion	The applicant's version is acceptable.
Reliability	2
Acceptability	The study is considered acceptable.
Remarks	No further remarks.

6.6.2 In vitro cytogenicity study in mammalian cells

			Official use only
		1. REFERENCE	
1.1	**Reference**	A6.6.1/02 Authors : [redacted] Title: - *In vitro* sister chromatid exchanges test of [redacted] in CHO-K1 cells Laboratory : Sumitomo Chemical Co. Unpublished Report no : [redacted] Date : October 11, 1983	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Sumitomo Chemical Co. Japan	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	Yes	x
2.3	**Deviations**	Not applicable	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	None given	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	x
3.2	**Study Type**		
3.2.1	Organism/cell type	Chinese hamster ovary (CHO-K1) cells	
3.2.2	Deficiencies / Proficiencies		
3.2.3	Metabolic activation system	PCB-induced Sprague Dawley rat liver S9 reaction mixture	
3.2.4	Positive control	Mitomycin C and cyclophosphamide.	
3.3	**Administration/ Exposure; Application of test substance**		
3.3.1	Concentrations	first experiment : 0.375, 3.75, 37.5 and 375 µg/ml second experiment at 3.75, 11.25, 37.5, and 112.5 µg/ml	
3.3.2	Way of application	The cells were treated with the chemical as follows; 1) without S9 mix 0.05 ml chemical solution and 5 ml medium without serum were	

added to a dish.

2) with S9 mix

0.05 ml chemical solution, 0.5 ml S9 mix and 4.5 ml medium without serum were added to a dish.

After 2 hr-treatment at 37°C, the cells were washed and cultured in the complete medium supplemented with 10 µM 5-bromodeoxy- uridine for 40 hr. Colcemid (0.lµg/ml) was added to them 2 hr before termination.

The cells were collected by trypsinization with 0.25 % trypsin in phosphate buffed saline pH 7.4 and centrifuged, then were treated with 75 m KC1 for 20 min at room temperature, fixed with methanol:acetic acid (3:1) for 3 times, and spread onto a clean slide glass. The differential staining was performed by FPG method described by Perry and Wolff

The slides were stained with Hoechst 33258 (2 µg/ml in distilled water) for 20 min and rinsed with distilled water.

The preparations were mounted in McIlvaine buffer (pH 8.0) with coverslips and exposed with 15 W UV—light at 15 cm distance on a hot plate (56 °C) for 1.5 hr. Then, removing the coverslips, the slides were incubated in 2 x SSC (0.3 M KC1, 0.03 M sodium citrate) at 60 °C for 1.5 hr and stained with 3 % Giemsa in phosphate buffer pH 6.8 for 20 min

All slides were coded and analyzed in a blind study

In the-first experiment, CHO-K1 cells were treated with 0.375, 3.75, 37.5 and 375 µg/ml of cyphenothrin for 2 hr in the presence and absence of S9 mix and the incidence of SCE was determined. To determine the toxicity of cyphenothrin the cells were counted 48 hr after the treatment.

In the second experiment, 112.5 µg/ml was selected as the maximum concentration from the result of the first experiment. The incidence of SCE was examined at the concentration of 3.75, 11.25, 37.5, and 112.5 µg/ml of cyphenothrin both with and without S9 mix.

Statistical analysis for the incidence of SCE was performed with t-test.

3.3.3 Pre-cultivation: 24 hours

3.3.4 Other modifications: none

3.4 Examinations

3.4.1 Number of cells evaluated: 50 (25 for the positive controls)

4. RESULTS AND DISCUSSION

4.1 Genotoxicity

4.4.1	Without/without metabolic activation	In the first experiment no increase of SCE was observed at 0.375, 3.75 and 37.5 µg/ml cyphenothrin with and without S9 mix. Cyphenothrin indicated high toxicity to the cells at 375 µg/ml both with and without S9 mix, so the SCE frequency could not be determined. In the second experiment no significant increase of SCE was observed by any treatment of cyphenothrin. Mitomycin C and cyclophosphamide, positive controls, induced significant increase of SCE in both the first and second experiments. From the above data, it is concluded that cyphenothrin does not induce any SCE in CHO-K1 cells under the test conditions.
4.2	**Cytotoxicity**	Cyphenothrin showed high toxicity to the cells at 375 µg/ml both with and without S9 mix.
		5. APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In an *in vitro* sister chromatid exchange test using Chinese hamster ovary cells (CHO-K1), concentrations of cyphenothrin (93.6%) were conducted at dose levels of 0.375, 3.75, 37.5 and 375 µg/ml (first experiment) and 3.75, 11.25, 37.5, and 112.5 µg/ml cyphenothrin (second experiment). The test systems were provided without and with metabolic activation (PCB-induced Sprague-Dawley rat liver S9). Cells were treated with the solutions containing cyphenothrin for 2 hours.
5.2	**Results and discussion**	In the first experiment, no increase in sister chromatid exchange was observed at 0.375, 3.75, or 37.5µg/mL cyphenothrin with or without metabolic activation. The concentration of 375 µg/mL was highly cytotoxic, so the frequency of the sister chromatid exchange could not be determined. In the second experiment, no significant increase in sister chromatid exchange was observed at any concentration with or without metabolic activation. No cytotoxic effects were observed. The positive controls gave the appropriate responses.
5.3	**Conclusion**	It can be concluded that cyphenothrin is not mutagenic under the conditions in this study.
5.3.1	Reliability	2
5.3.2	Deficiencies	This study is not reported as being done to a recognized guideline.

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November, 2017
Guidelines and quality assurance	Point 2.2: No official certificate for GLP is provided.
Materials and methods	The applicant's version is acceptable. Point 3.1.2.3: The stability of the test item was not mentioned in the study.
Results and Discussion	The applicant's version is acceptable.
Conclusion	The applicant's version is acceptable.
Reliability	2
Acceptability	The study is considered acceptable.
Remarks	No further remarks.

6.6.3 In vitro gene mutation assay in mammalian cells

			Official use only
		1. REFERENCE	
1.1	**Reference**	A6.6.3/01 Authors : [redacted] Title: In vitro gene mutation test of [redacted] in V79 Chinese hamster cells in culture Laboratory : Sumitomo Chemical Co. Unpublished Report no : [redacted] Date : April 3, 1989	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Sumitomo Chemical Co. Japan	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	Yes	x
2.3	**Deviations**	Not applicable	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	None given	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	x
3.2	**Study Type**		
3.2.1	Organism/cell type	V79 Chinese hamster cells in culture at the HGPRT locus	
3.2.2	Deficiencies / Proficiencies	Induction of 6-thioguanine resistant mutants can be detected	
3.2.3	Metabolic activation system	PCB-induced Sprague Dawley rat liver S9 reaction mixture	
3.2.4	Positive control	Ethyl methylsulfonate (EMS, 200µg/ml) without S9 mix and 9,10-dimethyl-1,2-benzanthracene (DMBA, 5µg/ml) with S9 mix	
3.3	**Administration/ Exposure; Application of test substance**		

3.3.1	Concentrations	In the preliminary cytotoxicity test V79 cells were treated with cyphenothrin at the concentrations of 0.3, 1, 3, 10, 30, 100 and 300 µg/ml. Both in the presence and absence of S9 mix, cytotoxicity was not observed at concentations from 0.3 to 10 µg/ml. However weak cytotoxicity was observed at concentrations above 30 µg/ml and relative survival ratios at 100 µg/ml were 62% and 51% (with and without metabolic activation respectively). Precipitations were onserved at 100 and 300 µg/ml. Therefore, the 100 µg/ml (the limit of solubility) was chosen as the top dose for the gene mutation test. The gene mutation test of cyphenothrin was performed at the concentrations of 3, 10, 30 and 100 µg/m both with and without activation.
3.3.2	Way of application	Incubated for 5 hours at 37°C in the presence of the test substance. After the treatment, the cells were washed and cultured in fresh medium. One and four days after treatment with chemical the cell populations were subcultured to be maintained in an exponential growth phase. Seven days after treatment with chemicals the cells were harvested by trypsinization and number of cells were counted using Coulter counter. The mutation frequency and the plating efficiency were examined according to the following procedures. The cells were inoculated at the rate of 3 x 10^5 cells/dish to ten of 100 mm-diameter dishes coutaining the medium and 10 µg/ml of 6-thioguanine. A portion of the cell suspension was diluted and inoculated at the rate of 100 cells/dish to five of 60 mm-diameter dishes containing the medium. After the cultivation for six days (plating efficiency) and for seven days (mutation frequency), colonies of V79 cells were fixed in a mixture of methanol and acetic acid, stained with 3% Giemsa solution and counted manually. The plating efficiency and the mutation frequency were determined.
3.3.3	Pre-cultivation	One day
3.3.4	Other modifications	The gene mutation test (without metabolic activation) gave inconsisten results, therefore an additional experiment was performed.

3.4 Examinations

3.4.1	Number of cells evaluated	

4. RESULTS AND DISCUSSION

4.1 Genotoxicity

4.4.1	Without metabolic activation	In one of the experiments without S9 mix, the mutation frequency was over threefold that of the vehicle control at a concentration of 10 µg/ml only. However there was no dose-dependent increase in the mutation frequencies of the treated groups. The slight increase of mutation frequency at the dose of 10 µg/ml was notreproduced in the remaining two experiments so it is considered that this was not treatment related but was due to normal assay variation. The positive control, EMS, induced marked increase in the mutation frequencies.
4.4.2	With metabolic activation	No increased mutation frequencies were seen at any concentration. The positive control, DMBA, induced marked increase in the mutation frequencies.

4.2	**Cytotoxicity**	In the preliminary cytotoxicity test, weak cytotoxicity was observed at concentrations above 30 µg/mL and relative survival ratios at 100 µg/mL were 62% and 51% (with and without metabolic activation respectively). Precipitations were onserved at 100 and 300 µg/mL Therefore, the 100 µg/mL (the limit of solubility) was chosen as the top dose for the gene mutation test.
		5. APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Cyphenothrin (94.3%) was examined for mutagenic activity by assaying the induction of 6-thioguanine resistant mutants of V79 Chinese hamster cells in culture in the presence and absence of S9 mix. The cells were treated with cyphenothrin for 5 hours at doses of 3, 10, 30 and 100 µg/m, both with and without activation. The experiment was conducted three times without metabolic activation and twice with metabolic activation. The chemical is judged to be mutagenic when all the following requirement are fulfilled. 1) The mutation frequencies of the chemical-treated groups are higher than threefold of that of corresponding vehicle control. 2) The mutation frequencies of the chemical-treated groups are higher than the highest historical control data. 3) The mutation frequency increase dose-dependently. To examine the dose-dependense, linear regression analysis is performed. 4) These increase of mutation frequency are reproduced.
5.2	**Results and discussion**	In the absence of S9 mix the mutation frequency of cyphenothrin - treated group was over three times that of the vehicle control only at 10µg/ml in one of the three experiments. However, there was no reproducibility or dose-dependency in the increase of mutation frequency. From these findings, it is considered that the slight increase of mutation frequency at the dose of 10 µg/ml in one experiment is not due to an effect of cyphenothrin, but due to normal assay variation. In the presence of S9 mix cyphenothrin did not induce any increases in the mutation frequency as compared with vehicle controls. The positive controls – ethyl methansulfonate (without S9 mix) and 9, 10-dimethyl-1,2-benzanthracene (with S9 mix) gave the appropriate responses.
5.3	**Conclusion**	It can be concluded that cyphenothrin is not mutagenic under the conditions in this study.
5.3.1	Reliability	2
5.3.2	Deficiencies	This study is not reported as having been done to a recognized guideline.

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November, 2017
Guidelines and quality assurance	Point 2.2: No official certificate for GLP is provided.
Materials and methods	The applicant's version is acceptable. Point 3.1.2.3: The stability of the test item was not mentioned in the study.
Results and Discussion	The applicant's version is acceptable.
Conclusion	The applicant's version is acceptable.
Reliability	2
Acceptability	The study is considered acceptable.
Remarks	No further remarks.

6.6.4 *In vivo* mutagenicity study

			Official use only
		1. REFERENCE	
1.1	**Reference**	A6.6.4/01 Authors : [redacted] Title: micronucleus Test of [redacted] Laboratory : Sumitomo Chemical Co. Unpublished Report no : [redacted] Date : October 11, 1983	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Sumitomo Chemical Co. Japan	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	Yes	x
2.3	**Deviations**	Not applicable	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	None given	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	Stable	x
3.1.2.4	Maximum tolerable dose	800 mg/kg In the preliminary acute toxicity test of cyphenothrin, 1/6, 1/6 and 6/6 animals were died within 3 days after intraperitoneal administration of 1000, 2000 and 3000 mg/kg, respectively. From the results of the preliminary acute toxicity test, the highest dose of 800 mg/kg was selected.	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	ICR	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male	x
3.2.5	Age/weight at study initiation	7 – 8 weeks old 30 – 38 g	
3.2.6	Number of animals per group	6	
3.2.7	Controls animals	Yes	

3.3	**Administration/ Exposure**		
3.3.1	Number of applications	1	
3.3.2	Interval between applications	Not applicable	
3.3.3	Postexposure period	24 hours (dose response study) 24, 48, 72 hours (time-course study)	
3.3.4	Type	Intraperitoneal	
3.3.5	Concentration	To give doses of 200, 400 and 800 mg/kg (dose response study) 800mg/kg (time-course study)	
3.3.6	Vehicle	Corn oil	
3.3.7	Dose applied	10 ml/kg	
3.3.8	Substance used as Positive Control	Mitomycin C was dissolved in saline and injected at 2 mg/kg	
3.3.159	Controls	Vehicle control group – corn oil	
3.4	**Examinations**		
3.4.1	Clinical signs	No	
3.4.2	Tissue	Bone Marrow (femur)	
3.5	**Further remarks**		
		4. RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Not applicable	
4.2	**Haematology / Tissue examination**	Normochromatic erythrocytes (NCE) : polychromatic erythrocytes (PCE) ratio was not affected by treatment.	
4.3	**Genotoxicity**	In both the dose response and time-course studies, no significant increase in micronucleated cells was observed in any cyphenothrin treated groups as compared with the vehicle control. The positive control induced the appropriate response. It is concluded that cypenothrin does not induce any micronuclei in bone marrow erythrocytes of mice under the test conditions.	x
4.4	**Other**		

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In the preliminary acute toxicity test of cyphenothrin, 1/6, 1/6 and 6/6 animals were died within 3 days after intraperitoneal administration of 1000, 2000 and 3000 mg/kg, respectively. From the results of the preliminary acute toxicity test, the highest dose of 800 mg/kg was selected. In a micronucleus study, male ICR mice were dosed intraperitoneally once with 200, 400 or 800 mg/kg cyphenothrin (93.6%) using corn oil as the vehicle. Bone marrow smears were prepared after 24h to evaluate each dose response. In addition, the time-response effect was tested using bone marrow smears prepared 24, 48 and 72 h after treated once at 800 mg/kg. The smear slides were fixed with methanol for 5 min and stained with 5% Giemsa in phosphase buffer (pH 6.8) for 30 min. The slides were coded and analyzed in a blind study. The incidence of micronucleated cells in 1000 whole erythrocytes and 1000 polychromatic erythrocytes were investigated per animal. The ratio of PCEs to whole erythrocytes was also examined. Statistic analysis for the incidence of micronucleated cells was performed according to Kastenbaum and Bowman. T-test was used for analysis of the ratio of PCEs to whole erythrocytes.	x
5.2	**Results and discussion**	The NCE:PCE ratio was not affected by treatment, indicating either that the test material possibly did not reach bone marrow erythrocytes or that it was not cytotoxic. However, tissue distribution data suggest that it would reach the bone marrow. In both dose-response and time-response tests no significant increases in micronucleated cells were observed. The positive control group gave the appropriate response.	
5.3	**Conclusion**	It can be concluded that cypenothrin does not induce any micronuclei in bone marrow erythrocytes of mice under the test conditions in this study.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	This study is not reported as having been done to a recognized guideline.	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November, 2017
Guidelines and quality assurance	Point 2.2: No official certificate for GLP is provided.
Materials and methods	The applicant's version is acceptable. Point 3.1.2.3: The stability of the test item was not mentioned in the study Point 3.2.4: No justification for testing a single sex is provided. Point 5.1: At least 2000 polychromatic cells per animal should be observed for the incidence of micronuclei.
Results and discussion	The applicant's version is acceptable. Point 4.3: No genotoxicity was observed. No clinical signs for the animals were reported.
Conclusion	The applicant's version is acceptable.
Reliability	2
Acceptability	The study is considered acceptable.
Remarks	No further remarks.

6.6.5 In vivo mutagenicity test for DNA damage

	Justification for non-submission of data		**Official use only**
Other existing data [ ]	**Technically not feasible [ ]**	**Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**		
Detailed justification:	As all the preceeding studies are negative further testing is not required.		
Undertaking of intended data submission [ ]			
	EVALUATION BY COMPETENT AUTHORITIES		
	EVALUATION BY RAPPORTEUR MEMBER STATE		
Date	November, 2017		
Evaluation of applicant's justification	The applicant's justification is acceptable.		
Conclusion	The applicant's justification is acceptable.		
Remarks	No further remarks.		

6.6.6 In vivo mutagenicity test re germ cell effects

	Justification for non-submission of data		**Official use only**
Other existing data [ ]	**Technically not feasible [ ]**	**Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**		
Detailed justification:	As all the preceeding studies are negative further testing is not required.		
Undertaking of intended data submission [ ]			
	EVALUATION BY COMPETENT AUTHORITIES		
	EVALUATION BY RAPPORTEUR MEMBER STATE		
Date	November, 2017		
Evaluation of applicant's justification	The applicant's justification is acceptable.		
Conclusion	The applicant's justification is acceptable.		
Remarks	No further remarks.		