# 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 A6.8

Toxicology section

Rapporteur: Hellas

November 2017

## 6.8 Reproductive toxicity

### 6.8.1 Teratogenicity test

| | | 1. REFERENCE | Official use only |
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| **1.1** | **Reference** | A6.8.1/01<br>Authors : [redacted]<br>Title: Teratology study in rats treated subcutaneously with [redacted]<br>Laboratory : [redacted]<br>Unpublished<br>Report no : [redacted]<br>Date : April 25, 1984 | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | Sumitomo | |
| 1.2.2 | Companies with letter of access | None | |
| 1.2.3 | Criteria for data protection | Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry into Annex I | |
| | | **2. GUIDELINES AND QUALITY ASSURANCE** | |
| **2.1** | **Guideline study** | Yes<br>Study was according to the Notifications No. 529 of the Evaluation and Registration and Biologics and Antibiotics Division, Pharmaceutical Affairs Bureau, the Ministry of Health and Welfare in Japan, entitled "Animal experiments concerning the effects of drugs on re production", and dated March 31, 1975. | **X** |
| **2.2** | **GLP** | Yes | **X** |
| **2.3** | **Deviations** | Not applicable | **X** |
| | | **3. MATERIALS AND METHODS** | |
| **3.1** | **Test material** | As described in Section 2 | |
| 3.1.1 | Lot/Batch number | [redacted] | |
| 3.1.2 | Specification | As described in Section 2 | |
| 3.1.2.1 | Description | Brown, transparent and viscous liquid | |
| 3.1.2.2 | Purity | [redacted] | |
| 3.1.2.3 | Stability | Stable | |
| **3.2** | **Test Animals** | | |
| 3.2.1 | Species | Rat | |
| 3.2.2 | Strain | Crj: CD (SD) | |
| 3.2.3 | Source | [redacted] | |
| 3.2.4 | Sex | Male and female | |
| 3.2.5 | Age/weight at study initiation | 12 weeks<br>216 to 309 g.(females) | |
| 3.2.6 | Number of animals per group | 24/sex/dose | X |
| 3.2.7 | Control animals | Yes: 24 males and 24 females | X |

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| 3.2.8 | Mating period | Overnight | |
| **3.3** | **Administration/ Exposure** | | |
| 3.3.1 | Duration of exposure | Days 7 to 17 of gestation | |
| 3.3.2 | Postexposure period | Not relevant | **X** |
| 3.3.3 | Type | Subcutaneous | |
| 3.3.4 | Vehicle | Corn oil | |
| 3.3.5 | Concentration in vehicle | Concentrations of the solution were adjusted to give 2 ml/kg of administration volume for each group.<br>To give dosage groups of 0, 50, 150 or 500 mg/kg/bw/day | |
| 3.3.6 | Total volume applied | 2 ml/kg | |
| 3.3.7 | Controls | Vehicle only | |
| **3.4** | **Examinations** | | |
| 3.4.1 | Body weight | Yes<br>Dams were weighed on day 0 and day 4 of gestation and everyday from day 7 to day 20 of gestation and on days 0, 4, 7, 11, 14, 17 and 21 after delivery. | |
| 3.4.2 | Food consumption | On days 4, 9, 11, 14, 17 and 20 of gestation and days 2, 4, 7, 11, 14, 17 and 21 after delivery, 48-hr food consumptions were measured from two days before the indicated days and 24-hr consumption for each animal was calculated | |
| 3.4.3 | Clinical signs | After beginning of administration, dams were observed at a certain time everyday for toxic symptoms, abnormal behavior and mortality. When animals died, they were immediately subjected to autopsy. | |
| 3.4.4 | Examination of uterine content | Animals, in which Cesarean section was expected on day<br>20 of gestation, were killed by cervical dislocation and were immediately subjected to laparotomy for macroscopic observation of the main organs. The ovaries and uterus were removed to examine number of corpora lutea, number of implantation, number of live fetuses and number of resorbed or dead fetuses and its classification (resorbed embryo, placental remnant, early macerated fetus, late macerated fetus and dead fetus).<br>The heart, lungs, liver, kidneys, spleen, ovaries and placenta of dams were removed, weighed | |
| 3.4.5 | Examination of foetuses | | |
| 3.4.5.1 | General | The live fetuses were examined for the sex, body weight and external malformations including those in the oral cavity. | |
| 3.4.5.2 | Skeletal | Half of live fetuses from each litter were fixed in 70 % alcohol solution and the skeleton of chosen specimens were prepared with alizarin red S staining according to Dawson's method and skeletal abnormalities and variations and degree of ossification were examined with a stereoscopic microscope. | |
| 3.4.5.3 | Soft tissues | The remaining half of the live fetuses from each litter were fixed in Bouin's solution and according to the freehand razor method of Wilson) visceral abnormality was examined in the head, thorax and abdomen. | |

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| 3.4.6 | Observation of dams at delivery | Dams which were not killed at the terminal of gestation were subjected to delivery to observe signs during delivery and calculate the duration of gestation. Thereafter, dams were made to rear newborns for 21 days and their rearing manner was observed. On day 21 after delivery, all animals were killed by cervical dislocation to observe macroscopically the main organs and to examine number of implantation sites. Further, the heart, lungs, liver, kidneys, spleen and ovaries of dams were removed, weighed. Based on body weight on day 21 after delivery, relative organ weights were calculated. |
| 3.4.7 | Observation of newborns $F_1$ pups | After number of live newborns, number of still births, external malformations including those in the oral cavity and sex were examined, live newborns were weighed. Thereafter, body weight was measured twice a week until day 21 after birth and once a week from day 21 to day 70 after birth. After weighed on day 4 after birth, the size of each litter was adjusted by eliminating extra newborns by random selection to yield 8 animals (4 males and 4 females) per litter. However, when one sex did not amount to 4 animals, one or more animals of another sex were culled to yield a total of 8 animals. The extra newborns were killed and subject to macroscopic observation of the main organs and carcass.<br>On day 21 after birth, 3 males and 3 females - were selected at random from each litter. On the same day (day 21 after birth) one male and one female from each litter were killed by ether anesthesia and after macroscopic observation of the main organs, the brain, heart, lungs, liver, kidneys, adrenals, spleen, testes and ovaries were removed and weighed. When a litter-size was not enough to supply 3 males and 3 females, autopsy was not performed.<br><br>With respect to growth and development of newborns, pinna detachment was examined on day 4 after birth, appearance of abdominal hair on days 7 and 11, eruption of lower incisor on days 11 and 14, separation of eyelid on days 14 and 17, descent of testis on days 21 and 28, and opening of vagina on days 35 and 42 after birth.<br><br>Based on the number of surviving offspring during the post natal period the delivery, viability, lactation and growth after weaning indices were calculated |
| 3.4.8 | Behavioral and functional tests of $F_1$ pups | On day 21 after birth, one male and one female from each litter were observed for spontaneous activity by the revolving wheel method and for neuromuscular activity by the inclined plane method and rotor rod method.<br>On the same day pupillary reflex and Preyer's reflex were observed<br><br>One male and one female from each litter were subjected to an open field test to examine emotion at age of 8 weeks, water T maze test to examine learning ability at age of 9 weeks and shuttle box conditioned avoidance response test to examine acquisition of conditioned avoidance response at age of 10 weeks. |
| 3.4.8 | Reproductive performance test of $F_1$ pups | Reproductive performance test of F1<br>Two males and two females from each litter were selected from F1 animals on attaining 10 weeks of age for mating in the same administration group. Mating between members of the same litter was avoided.<br><br>Where no mating was confirmed in a pair the animals were swopped with other animals in the same dosage group and the new pair were allowed to mate for up to 28 days<br>When numbers of males and females were different in a particular |

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| | | group mating was done between surplus animals and non-treated animals of the same strain to observe the reproductive performance.<br><br>The age of newly purchased males and females was 10 weeks old at the beginning of mating. Results of non-reated males and females were separately treated from those of treated females but the same regime of observation and tests were conducted. In the females in which copulation was confirmed, the date was noted as day 0 of gestation. For these animals, the number of days required for confirmed copulation, mating index of males and females and fertility index were calculated. Body weight during pregnancy was measured on days 0, 4, 7, 11, 14, 17 and 20 of gestation.<br>Dams were individually housed in each plastic cage with bedding after day 20 of gestation to observe delivery and were allowed to rear newborns until day 4 after delivery and body weight was measured on days 0 and 4 after delivery. At delivery, numbers of live born and still born offsprings were counted. Live born offsprings were observed with re spect to external malformations including those in the oral cavity, weight and sex.<br>On day 4 after birth, number of surviving offspring was counted and body weight was measured. | |
| **3.5** | **Further remarks** | Test substances in reproductive studies in Europe are normally administered via the oral route. However, in Japan for where these studies were originally conducted, if usage means that human exposure is most likely through an inhalation or dermal route, then reproduction studies by the subcutaneous dose should be carried out. In other cases, oral administration would normally be performed.<br><br>Although the subcutaneous toxicity of cyphenothrin is low metabolism studies, with both oral and subcutaneous administration, show no significant differences in the concentraiton in blood distribution pattern, excretion rate and pattern or metabolic pathway.<br><br>As human exposure to cyphenothrin products is mostly through the inhalation and dermal routes it is considered justifiable to use a subcutaneous study | **X** |
| | | **4. RESULTS AND DISCUSSION** | |
| **4.1** | **Maternal toxic effects** | In the 500 mg/kg - treated group, body weight gain was suppressed during the period of administration. Also in this group there were increases in weights of the heart and kidney at Cesarean section and of the spleen at weaning and one animal died 3 days after delivery. Except these, there were no abnormal findings on general condition, macropathological examination and observation during delivery and lactation periods. | |
| **4.2** | **Teratogenic / embryotoxic effects** | No abnormality was detected in Cesarean section findings (litter data) and in external, visceral and skeletal findings of fetuses, showing neither embryonic lethal toxicity, nor fetal growth retardation nor teratogenic action of the test compound. | |
| **4.3** | **Growth of $F_1$ offspring** | In the 500 mg/kg - treated group, there was a very slight decline in viability index on 4th day after birth but no effect of the test compound was observed in body weight, lactation index, growth index after weaning, differentiation, behavior and learning ability. | |

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| **4.3** | **Reproductive performance of $F_1$ offspring** | No abnormality was demonstrated in mating performance, fertility index, general behavior during pregnancy, delivery and lactation periods and $F_0$ offspring, showing no effect of the test compound on reproductive performance of $F_1$ off spring. |

## 5. APPLICANT'S SUMMARY AND CONCLUSION

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| **5.1** | **Materials and methods** | Sprague-Dawley rats were administered 0, 50, 150 or 500 mg/kg/day cyphenothrin ([redacted] in corn oil subcutaneously during days 7 to 17 of gestation to examine effects on dams, fetuses and offspring of the next two generations (F1 and F2). |
| **5.2** | **Results and discussion** | The majority of animals in all treatment groups, including controls, retained oily liquid in the back and lumbar regions at caesarean section. A smaller proportion of dams examined at weaning retained oily liquid in the back and lumbar regions. Two dams from the top dose group died during the study. Body weight gain was decreased in the 500 mg/kg/day group during the treatment period (days 7 to 17 of gestation). Food consumption was reduced in dams treated at 50 and 500 mg/kg/day on day 9 of gestation, but overall food consumption was not significantly affected by treatment.<br><br>At caesarean section of two thirds of the dams of each group, there were found to be no treatment-related effects on the number of implantations, numbers of dead or resorbed fetuses, sex ratio, numbers and weights of live fetuses or placental weights. There were no treatment-related external malformations or visceral and skeletal variations.<br><br>The remaining one-third of dams were allowed to progress to delivery. During delivery and lactation of F1 offspring, there were no treatment-related effects on the numbers of implantations, stillborn or live-born offspring, sex ratio or body weights of both sexes. In addition, no external malformations were observed. There was a slight decrease in the viability index of the offspring of dams treated at the top dose level between days 0-4 post-partum. However, the mean number of live offspring was highest in the top dose group at day 0 after birth, and slightly more losses would be expected from the larger litters.<br><br>There were no treatment-related effects on bodyweights and lactation indices of F1 offspring during the weaning period. There were no treatment-related developmental effects on F1 offspring, and no treatment-related effects on organ weights at weaning.<br>In behavioural and functional tests performed on F1 offspring, there were no biologically significant effects noted. Tests included a revolving wheel test for spontaneous activity, an inclined plane test and a rotor rod test for neuromuscular activity, pupillary and Preyer's reflex tests, an open field test, a water T maze test and a shuttle box conditioned avoidance response test. There were no treatment-related macropathological findings.<br><br>Reproductive performance of F1 offspring was assessed and no treatment-related effects were noted. There were no treatment-related effects on the gestation period, implantations, numbers of still born., numbers of live born, body weights, sex ratio, and external malformations of F2 offspring. Macropathological examination of mated F1 males and females revealed no treatment-related changes. There was no evidence of fetotoxicity or teratogenicity at any dose |

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| | | level. The no observed adverse effect level (NOAEL) for maternal toxicity was 150 mg/kg/day based on the decrease in body weight gain in F0 dams from days 7 to 17 of gestation and the deaths of two dams treated at the 500 mg/kg/day dose level.<br><br>The NOEL effect for offspring was >500 mg/kg/day |
| **5.3** | **Conclusion** | |
| 5.3.1 | LO(A)EL maternal toxic effects | 500 mg/kg/day |
| 5.3.2 | NO(A)EL maternal toxic effects | 150 mg/kg/day |
| 5.3.3 | LO(A)EL embryotoxic / teratogenic effects | No adverse effects seen in this study |
| 5.3.4 | NO(A)EL embryotoxic / teratogenic effects | ≥ 500 mg/kg/day |
| 5.3.5 | Reliability | 1 |
| 5.3.6 | Deficiencies | No |

**Table A6.8.1-1 Table for Teratogenic Effects – Maternal Effects**

| Parameter | Control data | | Low dose | Medium dose | High dose | Dose response +/- |
|---|---|---|---|---|---|---|
| | Historical | Study | | | | |
| **Body weight gain g (day 7 – 17)** | | 60.5 | 57.0 | 62.5 | 53.6* | |
| **Necropsy findings in dams dead before end of test** | | | | | | |
| **Organ weights (g)** | | | | | | |
| **Heart** | | 0.91 | 0.93 | 0.94 | 0.97* | |
| **Kidney (R)** | | 1.00 | 1.04 | 1.07 | 1.09* | |

* $p<0.05$

**EVALUATION BY COMPETENT AUTHORITIES**

**EVALUATION BY RAPPORTEUR MEMBER STATE**

**Date** November, 2017

**Materials and methods**

Point 3.1.2.3: There is no information on the stability of the test substance in the study report. Nevertheless, fresh solution of the test substance was prepared daily and thus stability may be assumed.
Points 3.2.6 & 7: The applicant's information is incorrect. The number of females with confirmed copulation that were used in the study was 38 per group. Approximately 2/3 of the females that mated successfully were subjected to cesarean section at the end of the gestation period, whereas the rest were left to deliver their offspring and were terminated at weaning as indicated in the following table:

Table 6.8.1-1: Groups and number of animals

| Group | No. of females with confirmed copulation | No. of sterile females | No. of females with cesaerean section | No. of females with delivery |
|---|---|---|---|---|
| Control | 38 | 2 | 24 | 12 |
| 50 mg/kg bw/day | 38 | 5 | 21 | 12 |
| 150 mg/kg bw/day | 38 | 1 | 25 | 12 |
| 500 mg/kg bw/day | 38 | 4 [a] | 21 | 13 [b] |

a – one animal died after administration of 8 doses
b – one animal died 3 days after delivery

Point 3.3.2: The test substance was administered on days 7-17 *post coitum*.
The post exposure period was from days 17-20 of gestation for all dams and their foetuses.
Moreover, for the dams that were left to deliver and their offspring ($F_1$) the post-exposure period was extended up to the end of lactation (day 21 after delivery), when all dams were sacrificed.
Post-weaning, the $F_1$ pups were either killed immediately (1 animal/sex/group), or subjected to behavioural and functional tests and autopsied at the age of 11 to 12 weeks (1 animal/sex/group), or assessed for their reproductive performance after reaching the age of 10 weeks (2 animals/sex/ group). The $F_1$ dams that mated were allowed to breed their newborns until day 4 after delivery, when both dams and offspring were sacrificed.
(See also sections 3.4.6-8 of the applicant's version)

**Conclusion**

The subcutaneous $NOAEL_{maternal}$ = 150 mg/kg b.w./day proposed by the applicant is agreed, based on decreased body weight gain, increased absolute weight of the heart, spleen and kidneys, as well as mortality observed at the top dose of 500 mg/kg b.w./day.
The subcutaneous $NOAEL_{developmental}$ > 500 mg/kg b.w./day for developmental effects proposed by the applicant is also agreed, since no foetotoxic, teratogenic and/or behavioural changes were noted.

**Reliability** 2

**Acceptability** Acceptable.
The amount aministered subcutaneously is considered to be 100% available to systemic circulation despite the fact that it might be slowly absorbed.

**Remarks**

Points 2.1: The RMS considers that the OECD Guideline 414 (equivalent to the EU testing method B.31) is the suitable experimental protocol for a prenatal developmental toxicity study.

Point 2.2: There is no GLP statement in the study.

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| | Point 2.3: There is no information on the stability of the test substance in the report. Nevertheless, fresh solution of the test substance was prepared daily and thus stability may be assumed.<br>Point 3.5: The subcutaneous administration is not considered equivalent to the dermal administration. The amount administered subcutaneously is considered to be 100% available to systemic circulation despite the fact that it might be slowly absorbed. On the contrary, the dermal absorption degree of cyphenothrin formulated as a 1% dilution in ethanol has been established to be 2.4%. |

| | | | Official use only |
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| | | **1. REFERENCE** | |
| **1.1** | **Reference** | A6.8.1/02<br>Authors : [redacted]<br>Title: Teratology study of [redacted] in the rabbit (second study)<br>Laboratory : Bozo Research Centre, Japan<br>Unpublished<br>Report no : [redacted]<br>Date : October 29, 1984 | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | Sumitomo | |
| 1.2.2 | Companies with letter of access | None | |
| 1.2.3 | Criteria for data protection | Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry into Annex I | |
| | | **2. GUIDELINES AND QUALITY ASSURANCE** | |
| **2.1** | **Guideline study** | Yes<br>Study was according to the Notifications No. 529 of the Evaluation and Registration and Biologics and Antibiotics Division, Pharmaceutical Affairs Bureau, the Ministry of Health and Welfare in Japan, entitled "Animal experiments concerning the effects of drugs on re production", and dated March 31, 1975. | X |
| **2.2** | **GLP** | Yes | X |
| **2.3** | **Deviations** | Not applicable | X |
| | | **3. MATERIALS AND METHODS** | |
| **3.1** | **Test material** | As described in Section 2 | |
| 3.1.1 | Lot/Batch number | [redacted] | |
| 3.1.2 | Specification | As described in Section 2 | |
| 3.1.2.1 | Description | a brown viscous liquid | |
| 3.1.2.2 | Purity | [redacted] | |
| 3.1.2.3 | Stability | Stable | X |
| **3.2** | **Test Animals** | | |
| 3.2.1 | Species | Rabbit | |
| 3.2.2 | Strain | New Zealand White rabbits | |
| 3.2.3 | Source | [redacted] | |
| 3.2.4 | Sex | Male and female | |
| 3.2.5 | Age/weight at study initiation | 6 Months | |
| 3.2.6 | Number of animals per group | 15/sex/dose | |
| 3.2.7 | Control animals | Yes: 15/sex/dose | |
| 3.2.8 | Mating period | Until copulation was confirmed twice | |
| **3.3** | **Administration/ Exposure** | | |

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| 3.3.1 | Duration of exposure | Days 6 – 18 gestation | X |
| 3.3.2 | Post exposure period | | X |
| 3.3.3 | Type | Subcutaneous | |
| 3.3.4 | Vehicle | Corn oil | |
| 3.3.5 | Concentration in vehicle | 50, 25 and 10% | |
| 3.3.6 | Total volume applied | 0.5ml/kg | |
| 3.3.7 | Controls | Vehicle only | |
| **3.4** | **Examinations** | | |
| 3.4.1 | Body weight | Yes<br>measured on day 0 of gestation and every day from day 6 until day 28 | |
| 3.4.2 | Food consumption | Yes<br>measured as a 24-hr value from the previous day on days 6, 10 14, 17, 21, 24 and 28 of gestation. | |
| 3.4.3 | Clinical signs | Yes<br>Clinical signs including mortality and signs of re-absorption or premature delivery of each animal were observed before and 1 and 4 hours after administration and then once daily during the periods administration and of non-administration. | |
| 3.4.4 | Examination of uterine content | On day 28 of gestation, every animal was killed by intravenous air injection and immediately thereafter thoracotomy and laparatorny were carried out for macroscopic examination of the organs. After the ovaries and -uterus were removed, the numbers of corpus lutea, implantations, and live and dead or resorbed fetuses (early: implantation sites, placental remnants, and macerated fetuses with un-clearly differenciated extremities visible at termination late: macerated fetuses with clearly differenciated extremities and dead fetuses visible at termination) were determined and at the same time the placenta was weighed. Subsequently, the heart, lung, liver, kidney, spleen and ovary were weighed. | |
| 3.4.5 | Examination of foetuses | | |
| 3.4.5.1 | General | After measurement of body weight, live fetuses were examined for external and oral malformations and were opened, and the sex was determined. The contents of the abdominal and thoracic cavities were examined in situ. thoracic and abdominal organs were collected and fixed in phosphate buffered 10% formalin solution. | |
| 3.4.5.2 | Skeletal | After the skin and subcutaneous adipose were removed, the eviscerated fetuses were fixed with 95% alcohol, cleared and stained with alizarin red S according to the Dawson's method to permit examination for skeletal abnormality, skeletal variation and the degree of ossification | |
| 3.4.5.3 | Soft tissues | The heart and kidneys were examined by the macro— dissection method | |

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|---|---|---|---|
| **3.5** | **Further remarks** | Test substances in reproductive studies in Europe are normally administered via the oral route. However, in Japan for where these studies were originally conducted, if usage means that human exposure is most likely through an inhalation or dermal route, then reproduction studies by the subcutaneous dose should be carried out. In other cases, oral administration would normally be performed.<br><br>Although the subcutaneous toxicity of cyphenothrin is low metabolism studies, with both oral and subcutaneous administration, show no significant differences in the concentraiton in blood distribution pattern, excretion rate and pattern or metabolic pathway.<br><br>As human exposure to cyphenothrin products is mostly through the inhalation and dermal routes it is considered justifiable to use a subcutaneous study | **X** |
| | | **4. RESULTS AND DISCUSSION** | |
| **4.1** | **Maternal toxic effects** | No compound-related abnormal clinical signs nor death were observed during the study.<br><br>A depressing tendency of maternal mean body weight were noticed in the 125 and 250mg/kg groups.<br><br>No compound related effects were observed in maternal macropathological findings at cesarean section and mean organ weight. | |
| **4.2** | **Teratogenic / embryotoxic effects** | No effects of the test compound administered were noted in the mean numbers of implantations and corpora lutea, placental weight, the mean numbers of dead and live fetuses and sex ratio. On mean fetal weights and the progress of fetal ossification as well, no effects of the test substance administered were evident.<br><br>No external and visceral abnormalities were observed in any group fetuses. A low incidence of skeletal abnormalities was noted and appeared unrelated to administration.<br><br>On the basis of these results, it is concluded that cyphenothrin had neither lethal nor teratogenic effect on embryo/fetus in this study. | |
| | | **5. APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **Materials and methods** | In a teratogenicity study (1984), New Zealand White rabbits were administered 0, 50 or 125 mg/kg cyphenothrin (95.7%) in corn oil subcutaneously on days 6 to 18 of gestation and another group were subcutaneously administered 250 mg/kg/day from day 6 to 10 of gestation. There were 15 pregnant dams per dose level. | |
| **5.2** | **Results and discussion** | There were no deaths, but re-absorption occurred in one female dosed at 50 mg/kg/day on day 25 of gestation and in two given 125 mg/kg/day on days 22 and 24. No re-absorbtions were seen at 250 mg/kg/day<br>Body weight gain was slightly reduced from day 6 to 10 of gestation and significantly reduced from day 11 to 28 at 125 250 mg/kg/day (7%-7.5%). Dams treated at 250 mg/kg/day had similarly reduced body weight gain from day 6, but statistical significance was reached at day 10 to 28 (-6%). Food consumption was slightly decreased in dams treated at 125 mg mg/kg/day from day 6 to 27 of gestation and | |

slightly decreased in dams treated at the top dose level from day 6 to 21, although measurements at days 24 and 27 showed increased food consumption over controls.

Gross pathological examination of dams at autopsy revealed retention of yellowish oily liquid in the top two dose levels, yellowish abscess in 2/13 at 125 mg/kg/day and a dose-dependent increase in viscosity at the injection area or in the abdomen/ pectus. Absolute organs weights were not significantly different between control and treatment groups.

Caesarean section of all dams revealed no treatment-related effects on numbers of implantations, live fetuses, sex ratio, body weights of male and female fetuses and placental weights and no external malformations. Early and late fetal deaths were increased in treatment groups as illustrated in the table Table A6.8.1.2-1.

Due to the lack of dose-dependency in early and late deaths, effects on fetal deaths were not considered to be treatment-related. Visceral and skeletal examination of fetuses revealed no treatment-related findings.

There was no evidence of fetotoxicity and teratogenicity at the top dose levels – NOEL > 250 mg/kg/day.

The NOAEL for maternal toxicity was 50 mg/kg/day based on reduced body weight gain and food consumption in dams treated at 125 and 250 mg/kg/day

**5.3 Conclusion**

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| 5.3.1 | LO(A)EL maternal toxic effects | 125 mg/kg/day |
| 5.3.2 | NO(A)EL maternal toxic effects | 50 mg/kg/day |
| 5.3.3 | LO(A)EL embryotoxic / teratogenic effects | None observed |
| 5.3.4 | NO(A)EL embryotoxic / teratogenic effects | > 250 mg/kg/day |
| 5.3.5 | Reliability | 1 |
| 5.3.6 | Deficiencies | No |

**Table A6.8.1-2 Early and late fetal deaths**

| | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | **0** | **50** | **125** | **250** |
| No. of fetal deaths | | | | |
| Early dead fetuses | 1 | 5 | 2 | 6 |
| Late dead fetuses | 1 | 5 | 6 | 2 |
| Total | 2 | 10 | 8 | 8 |
| No of fetal deaths/ No of implantations (%) | 1.52 | 8.33 | 6.15 | 5.80 |

| EVALUATION BY COMPETENT AUTHORITIES | |
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| | |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | November, 2017 |
| **Materials and methods** | Point 3.1.2.3: There is no information on the stability of the test substance in the report. Nevertheless, fresh solution of the test substance was prepared daily prior to administration and thus stability may be assumed.<br>Point 3.3.1: The duration of exposure was not the same for all groups. For the control, 50 and 125 mg/kg b.w./day groups cyphenothrin was administered during days 6-18 of gestation, whereas for the 250 mg/kg b.w./day group the test substance was only administered during days 6-10 of gestation. The RMS considers that the exposure period of the high dose group does not cover the organogenesis period as indicated by the OECD Guideline 414 (equivalent to the EU testing method B.31). Therefore toxicological assessment of the top dose animals is not considered to be reliable.<br>Point 3.3.2: The post exposure period is from days 19-28 of gestation for the control, 50 and 125 mg/kg b.w./day groups and from days 11-28 of gestation for the 250 mg/kg b.w./day group. |
| **Conclusion** | The subcutaneous $NOAEL_{maternal}$ = 50 mg/kg b.w./day proposed by the applicant is agreed, based on decreased body weight gain and food consumption at 125 and 250 mg/kg b.w./day.<br>The subcutaneous $NOAEL_{developmental}$ = 125 mg/kg b.w./day is proposed, since exposure of fetuses throughtout the organogenesis period was not achieved for top dose group animals (250 mg/kg b.w./day). |
| **Reliability** | 3 (only two doses may be considered as reliable for evaluation, since the top dose group was under-exposed) |
| **Acceptability** | Acceptable as a supplementary study to a previous teratology study in rabbits, conducted by [redacted] This previous teratology study was submitted after an RMS request and has not been evaluated by the applicant. The RMS evaluation is presented in Doc. IIA of the CAR. |
| **Remarks** | Points 2.1: The RMS considers that the OECD Guideline 414 (equivalent to the EU testing method B.31) is the suitable experimental protocol for a prenatal developmental toxicity study.<br>Point 2.2: There is no GLP statement in the study.<br>Point 2.3:<br>- There is no information on the stability of the test substance in the report. Nevertheless, fresh solution of the test substance was prepared daily prior to administration and thus stability may be assumed.<br>- For the 250 mg/kg b.w./day group the test substance was only administered during days 6-10 of gestation. The RMS considers that this exposure period does not cover the organogenesis period as indicated by the OECD Guideline 414 (equivalent to the EU testing method B.31). Therefore toxicological assessment of the top dose animals is not considered to be reliable.<br>Point 3.5: The subcutaneous administration is not considered equivalent to the dermal administration. The amount aministered subcutaneously is considered to be 100% available to systemic circulation despite the fact that it might be slowly absorbed. On the contrary, the dermal absorption degree of cyphenothrin formulated as a 1% dilution in ethanol has been established to be 2.4%.. |

### 6.8.2 Two generation reproduction study

| | | 1. REFERENCE | Official use only |
|---|---|---|---|
| **1.1** | **Reference** | A6.8.2/01<br>Authors : [redacted]<br>Title: [redacted]: Effects upon reproductive performance of rats treated continuously throughout two successive generations<br>Laboratory : [redacted]<br>Unpublished<br>Report no : [redacted]<br>Date : September 19,1990 | |
| **1.2** | **Data protection** | Yes | |
| 1.2.1 | Data owner | Sumitomo | |
| 1.2.2 | Companies with letter of access | None | |
| 1.2.3 | Criteria for data protection | Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry into Annex I | |
| | | **2. GUIDELINES AND QUALITY ASSURANCE** | |
| **2.1** | **Guideline study** | Yes<br>US EPA | **X** |
| **2.2** | **GLP** | Yes | |
| **2.3** | **Deviations** | No | |
| | | **3. MATERIALS AND METHODS** | |
| **3.1** | **Test material** | As described in Section 2 | |
| 3.1.1 | Lot/Batch number | [redacted] | |
| 3.1.2 | Specification | As described in Section 2 | |
| 3.1.2.1 | Description | Yellowish/orange viscous liquid | |
| 3.1.2.2 | Purity | [redacted] | **X** |
| 3.1.2.3 | Stability | Stable | |
| **3.2** | **Test Animals** | | |
| 3.2.1 | Species | Rat | |
| 3.2.2 | Strain | Sprague Dawley CD | |
| 3.2.3 | Source | [redacted] | |
| 3.2.4 | Sex | Male and female | |
| 3.2.5 | Age/weight at study initiation | 8-9 weeks<br>282 – 324g (males)<br>202 – 241g (females) | |
| 3.2.6 | Number per group | 24/sex/day | **X** |
| 3.2.7 | Mating | | |
| 3.2.8 | Duration of mating | Up to 21 days | |
| 3.2.9 | Deviations from standard protocol | No | |
| 3.2.10 | Control animals | Yes: 24 per sex | **X** |

| | | | |
|---|---|---|---|
| **3.3** | **Administration/ Exposure** | **Oral** | |
| 3.3.1 | Animal assignment to dosage groups | Random | |
| 3.3.2 | Duration of exposure before mating | 71 days | |
| 3.3.3 | Duration of exposure in general P, F1, F2 males, females | $F_0$: the males were treated continuously until killed after successful $F_1$ littering and the females were treated continuously until killed after weaning of the $F_1$ litters.<br>The $F_1$ generation, selected from $F_1$a litters, received the experimental diets for 14 weeks after weaning before being paired on one occasion to give the $F_2$ litters. Treatment continued until termination of males after successful littering and of females after weaning of their litters. | |
| | | **Oral** | |
| 3.3.4 | Type | In Food *ad libitum* | |
| 3.3.5 | Concentration | 0, 100, 300 or 1000 ppm | **X** |
| 3.3.6 | Vehicle | None | |
| 3.3.7 | Concentration in vehicle | Not applicable | |
| 3.3.8 | Total volume applied | Not applicable | |
| 3.3.9 | Controls | Plain diet | |
| **3.4** | **Examinations** | | |
| 3.4.1 | Clinical signs | All animals were examined daily throughout the study and any visible signs of reaction to treatment were recorded, with details of type, severity, time of onset and duration.<br>Any animals found dead were subjected to a thorough macroscopic examination and specimens of tissues considered abnormal were retained. | |
| 3.4.2 | Body weight | Males were weighed at commencement and weekly until termination. Females were weighed at commencement and weekly until mating was detected, on Days 0, 6, 13 and 20 post coitum and on Days 1, 4, 7, 14 and 21 post partum | |
| 3.4.3 | Food/water consumption | Food and water consumption was recorded weekly until the animals were paired for mating. | |
| 3.4.4 | Oestrus cycle | Vaginal smears were taken for ten days before pairing to produce the $F_1$a and $F_2$ matings in order to assess the regularity and duration of the oestrous cycle. This was continued after pairing with the male until evidence of mating was observed. | |
| 3.4.5 | Sperm parameters | No | |
| 3.4.6 | Offspring | Number and sex of pups, still births, live births, presence of gross anomalies, weight gain, physical or behavioural abnormalities | |
| 3.4.7 | Organ weights P and F1 | Uterus, ovaries, mammary glands, vagina, testes, epididymus, prostate, seminal vesicles, pituitary | |
| 3.4.8 | Histopathology P and F1 | Uterus, ovaries, mammary glands, vagina, testes, epididymus, prostate, seminal vesicles, pituitary | |

| | | | |
|---|---|---|---|
| 3.4.9 | Histopathology F1 not selected for mating, F2 | Uterus, ovaries, mammary glands, vagina, testes, epididymus, prostate, seminal vesicles, pituitary | |
| **3.5** | **Further remarks** | | |
| | | **4. RESULTS AND DISCUSSION** | |
| **4.1** | **Effects** | | |
| 4.1.1 | Parent males | Bodyweight gain of $F_0$ males in all treated groups was marginally superior of that of the control group throughout.<br>Food and water intake during maturation showed no effects attributable to treatment.<br>Mating performance was similar in all groups.<br>Absolute and relative weights, and histopathological examination of the reproductive organs showed no evidence of response to cyphenothrin<br>No macroscopic findings were recorded | |
| 4.1.2 | Parent females | Bodyweight gain of females receiving 1000 ppm was slightly but significantly reduced during maturation, whereas female bodyweight performance during $F_1$a and $F_1$b gestation and lactation phases was unaffected.<br>Food and water intake during maturation showed no effects attributable to treatment.<br>Regularity of oestrus, conception rate, fertility index, gestation length and gestation index were similar in all groups<br>Litter size and the survival, growth, and development of offspring were unaffected by cyphenothrin<br>Regularity of oestrus, mating performance, conception rate, fertility index, gestation length and gestation index were similar in all groups<br>No macroscopic findings were recorded<br>. | **X** |
| 4.1.3 | F1 males and females | Necropsy of $F_1$ offspring culled on Day 4 post partum, the few offspring that died, and of weanlings showed no treatment-related macroscopic changes<br>The general condition of F2 males and females was unaffected by treatment. One male and one female receiving 1000 ppm died during Week 5 of age but no unequivocal relationship with cyphenothrin could be established<br>Bodyweight gain of females receiving the highest level (1000 ppm) was slightly but significantly reduced during maturation. Bodyweight gain of males throughout and weight change of females during gestation and lactation, were unaffected by treatment.<br>Food and water intakes and food conversion efficiency of males and females were comparable in all groups.<br>Oestrous cycles, mating performance, conception rate, fertility index, gestation length and gestation index were similar in all groups.<br>Litter sizes and the survival, growth and development of F offspring to weaning were unaffected by treatment | |

| | | | |
|---|---|---|---|
| 4.1.4 | F2 males and females | A Necropsy of $F_2$ offspring culled on Day 4 post partum, the few offspring that died, and of weanlings showed no treatment-related macroscopic changes | |
| **4.2** | **Other** | | |
| | | **5. APPLICANT'S SUMMARY AND CONCLUSION** | |
| **5.1** | **Materials and methods** | Sprague Dawley rats (24/sex/dose) were fed diets containing 0, 100, 300 or 1000 ppm cyphenothrin [redacted] throughout 2 successive generations. $F_0$ animals were treated for 71 days prior to mating. $F_0$ were mated twice (with 10 d recovery post-weaning allowed between matings) to produce 2 litters - $F_1$a and $F_1$b. $F_1$a animals were treated for a minimum of 14 weeks from the time of weaning, and then mated together to produce the $F_2$ litter. | |
| **5.2** | **Results and discussion** | There were no treatment-related deaths or overt signs of toxicity in either $F_0$ or $F_1$a parents. Body weight gain was slightly reduced (during maturation only) in $F_0$ and $F_1$a females treated at 1000 ppm. There were no other treatment-related effects on body weights, or any effects on food and water consumption in $F_0$ and $F_1$a parents. There were no treatment-related effects on oestrous cycles, mating performance, gestation of $F_0$ parents or on $F_1$a and $F_1$b litters. Physical development, auditory and visual functions and sex ratios of $F_1$a and $F_1$b offspring were not affected by treatment. Macroscopic and microscopic evaluation of $F_1$a and $F_1$b offspring and of $F_0$ adults revealed no treatment-related findings. $F_1$a parents and $F_2$ offspring were evaluated in the same way and there were no treatment-related findings.<br><br>The no effect level was 300 ppm equivalent to approximately 25 mg mg/kg/day, based on slightly reduced body weight gains of $F_0$ and $F_1$a females | |
| **5.3** | **Conclusion** | | |
| 5.3.1 | LO(A)EL maternal toxic effects | | **X** |
| 5.3.1.1 | Parent males | None established | |
| 5.3.1.2 | Parent females | 1000 ppm | |
| 5.3.1.3 | F1 males | None established | |
| 5.3.1.4 | F1 females | 1000 ppm | |
| 5.3.1.5 | F2 males | None established | |
| 5.3.1.6 | F2 females | None established | |
| 5.3.2 | NO(A)EL | | **X** |
| 5.3.2.1 | Parent males | ≥ 1000 ppm | **X** |
| 5.3.2.2 | Parent females | 300 ppm (equivalent to 23.7 mg mg/kg/day) | |
| 5.3.2.3 | F1 males | ≥ 1000 ppm | **X** |
| 5.3.2.4 | F1 females | 300 ppm(equivalent to 23.7 mg mg/kg/day) | |
| 5.3.2.5 | F2 males | ≥ 1000 ppm | **X** |
| 5.3.2.6 | F2 females | ≥ 1000 ppm | **X** |
| 5.3.3 | Reliability | 1 | |
| 5.3.4 | Deficiencies | No | |

**EVALUATION BY COMPETENT AUTHORITIES**

**EVALUATION BY RAPPORTEUR MEMBER STATE**

**Date**

November, 2017

**Materials and methods**

Point 3.1.2.2: The purity of the test substance is [redacted] as stated in the study report (cyphenothrin purity is reported again under point 5.1).
Points 3.2.6 & 3.2.10: There is a typographical error. The number of animals used in the study was 24/sex/group. All animals were treated on a daily basis.
Point 3.3.5: The RMS considers that the chemical intake (mg/Kg bw/day) for each group should be presented, as follows:

Table 6.8.2-1: Cyphenothrin intake for $F_0$ males and females *

| **Dose (ppm)** | 0 | 100 | 300 | 1000 |
|---|---|---|---|---|
| **Compound intake – males** | 0 | 6.4 | 19.2 | 63.9 |
| **Compound intake – females** | 0 | 7.6 | 23.7 | 76.8 |

* compound intake was calculated as mean values for the 10-week growth/premating period

**Results and Discussion**

The applicant's opinion is adopted.
Clarification:
Point 4.1.2: It should be noted that, as stated in the study report, with regard to $F_0$ generation paired to produce $F_{1A}$ litters *"due to a technical error, parturition observations were not correctly performed on one day durng the littering phase, affecting the calculation of gestation length and offspring development for a number of litters in each group"*. Thus, the observations related to these parameters refer mostly to $F_0$ and $F_1$ females paired to produce $F_{1B}$ and $F_2$ litters, respectively.

**Conclusion**

Point 5.3.1: The RMS considers that LOAEL values should be set for systemic parental, reproductive and offsring toxicity as follows:
$LOAEL_{parental\ systemic}$ = 1000 ppm (equivalent to76.8 mg/Kg bw/day) based on decreased body weight gains of $F_0$ and $F_{1A}$ females.
$LOAEL_{reproductive}$ > 1000 ppm, since no adverse effects were noted on reproductive parameters.
$LOAEL_{offspring}$ > 1000 ppm, since no adverse effects were noted on offspring development.

Point 5.3.2: The NOAEL values proposed by the RMS are as follows:
$NOAEL_{parental\ systemic}$ = 300 ppm (equivalent to 23.7 mg/Kg bw/day).
$NOAEL_{reproductive}$ = $NOAEL_{offspring}$ = 1000 ppm.

**Reliability**

2 (certain reproductive parameters were not assessed in $F_0$ generation paired to produce $F_{1A}$ litters, due to a technical error)

**Acceptability**

Acceptable.

**Remarks**

Points 2.1: The RMS considers that the OECD Guideline 416 (equivalent to the EU testing method B.35) is the suitable experimental protocol for a multigeneration reproduction toxicity study.