Section A6.9 (02) Delayed Neurotoxicity

Annex Point IIA6.9 **Neurobehavioral screening – Functional Observation Battery testing in the rat**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]; Utility of a neurobehavioral screening battery for differentiating the effect of two pyrethroids, Permethrin and cypermethrin. [redacted].	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	Not applicable	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No current guidelines available for this type of study. Details of the protocol are included in the report.	
2.2	**GLP**	Not mentioned	
2.3	**Deviations**	Not applicable. Protocol was based on previous work published by the authors.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cypermethrin	
3.1.1	Lot/Batch number	Not specified (supplied by FMC Corp., Princeton, NJ)	
3.1.2	Specification	97% purity, approx. equal quantities of cis and trans isomers	
3.2	**Reference Substance (positive control)**	No positive control used in this study. However the synthetic pyrethroid Permethrin was also investigated in this study.	
3.3	**Test Animals**		
3.3.1	Species	Rat	
3.3.2	Strain	Long Evans	
3.3.3	Source	[redacted]	
3.3.4	Sex	Males and Females	
3.3.5	Rearing conditions	Prior to study initiation, rats used in the FOB study were singly housed in polycarbonate cages. Rats used in the motor activity study were housed in wire mesh cages. All rats were allowed access to feed and deionised water *ad libitum*. Temperature was maintained at 22 ± 1 ºC, 55 ± 5% humidity with a 12 hour light / 12 hour dark photoperiod.	
3.3.6	Age/weight at study initiation	70-90 days old	
3.3.7	Number of animals per group	8 rats of each sex	
3.3.8	Control animals	Yes (vehicle only)	
3.4	**Administration**		
3.4.1	Exposure	Oral (gavage)	

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3.4.2	Dose Levels	0 (vehicle only), 20, 60 or 120 mg/kg bw (FOB study) 0 (vehicle only), 20, 60 or 100 mg/kg bw (motor activity study)
3.4.3	Vehicle	Corn oil
3.4.4	Concentration in vehicle	Not specified
3.4.5	Total volume applied	1 ml/kg
3.4.6	Post exposure period	48 hours
3.4.7	Anticholinergic substances used	Not applicable
3.4.8	Controls	Vehicle only
3.5	**Examinations**	
3.5.1	Body Weight	Yes, measured during study
3.5.2	Signs of Toxicity	Effects on specific domains of neurological function: autonomic (salivation, increased urination), excitability (removal resistance, decreased arousal, choreoathetosis), neuromuscular (splayed limbs, flattened posture, decreased grip strength, altered righting, increased landing foot splay) and sensorimotor (increased click response, decreased touch response, decreased tail pinch response) based on a 1-4 severity scoring system.
3.5.3	Observation schedule	In the FOB study, animals were observed 1.5 and 3 hours after dosing (times of peak effect determined during preliminary arousal and gait score assessments) and after 24 and 48 hours.
3.5.4	Motor activity study	Motor activity experiments were performed separately. Eight rats of each sex were dosed with either 0 (vehicle only), 20, 60 or 100 mg/kg bw (the top dose was reduced due to a lethality noted in the FOB study). Motor activity testing took place after 3, 24 and 48 hours after dosing using a maze composed of interconnecting alleys in a figure of eight design with two blind alleys projecting from the centre. Six phototransmitter/diode pairs were equally spaced around the maze, including each of the blind alleys. Motor activity was recorded by a microprocessor as the number of photocell interruptions over a 1 hour session.
3.5.5	Statistical analysis	Two-way ANOVA followed by Dunnett's t-test
3.6	**Further remarks**	-

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4 RESULTS AND DISCUSSION

4.1 Body Weight

Body weight loss was seen in rats of both sexes and was still evident 48 hours after dosing. Rats in the high dose group losing a maximum of 7% and 9% of the pre-dosing weight in male and female rats respectively at 24h.

4.2 Signs of toxicity

In the FOB study, 1 male and 6 females died in the top dose group (120 mg/kg). These rats were subsequently replaced in order to allow the study to continue. The top dose was reduced to 100 mg/kg for the motor activity test, where 2 males died at this dose.

4.3 Functional Changes

Two phases of toxicity were evident. Salivation and increased removal activity (males) were more evident at 1.5 hours. At 3 hours these signs were subsiding and pronounced motor and sensory effects were apparent.

See Table A6_9_02-1

4.4 Motor Activity

Motor activity was markedly depressed on the day of dosing and the effects on total counts showed that 3 h after dosing, all doses significantly decreased activity in both sexes, and the high dose was still effective at 24 h. Furthermore, the low dose (20 mg/kg) was closed to the ED50 value for this measure, in that it decreased activity by 46% and 43% in males and females, respectively. No effects were obtained at 48 h.

4.5 Behavioural effects

After cypermethrin administration, rats displayed pawing and burrowing behavior even while on the open field. The swollen muzzles may have been a direct effect of cypermethrin or due to irritation produced by the excessive burrowing actions. Spontaneous vocalization was noted.

The behavior changes included: increased sensitivity to external stimuli, splayed hindlimbs and abnormal locomotion, decrease of sensorimotor reactivity, with exeption of increased click response. Neuromuscular changes were decreased grip strengths, gait changes and altered righting ability. Muscle tone was decreased as evidenced by splayed leggs, increased landing foot spread, and flattened posture.

The behavioral effects were significant at both the middle and the high dose.

4.6 Other

Hypothermia was observed in rats of both sexes.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A functional observation battery (FOB) study was performed to assess the effects of cypermethrin on neurological function and behaviour in the rat. 8 Adult Long-Evans rats/sex/dose received by gavage, cypermethrin (97%) in corn oil at 20, 60 or 120 mg/kg bw and were observed 1.5 and 3 h , 24 and 48 h after dosing. Motor activity experiments were preformed at 20, 60 and 100 mg/kg bw after 3, 24 and 48 h after dosing.

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5.2	**Results and discussion**	After cypermethrin administration, rats displayed pawing and burrowing behavior excessive burrowing actions, spontaneous vocalization, increased sensitivity to external stimuli, splayed hindlimbs and abnormal locomotion, decrease of sensorimotor reactivity, with exeption of increased click response. Neuromuscular changes were decreased: grip strengths, gait changes and altered righting ability. Muscle tone was decreased as evidenced by splayed legs, increased landing foot spread, and flattened posture. The behavioral effects were significant at both the middle and the high dose.
5.3	**Conclusion**	Behavior representing all of the functional domains assessed were affected, indicating the broad neurological activity of cypermethrin.
5.3.1	LOAEL	Not determined
5.3.2	NOAEL	20 mg/kg bw
5.3.3	Reliability	2
5.3.4	Deficiencies	Although this is a non-guideline study, the published report contains a high level of detail including the protocol used, purity and source of the test substance and full details of the experimental conditions. This report was identified in the monograph for cypermethrin under Directive 91/414/EC and an NOAEL of 20 mg/kg assigned by the RMS.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May, 2007.
Materials and Methods	The applicant's version is acceptable.
Results and discussion	The applicant's version is adopted with the following amendments: Neuromuscular dysfunction produced by cypermethrin was evident at even the low dose. Two phases of toxicity were evident. Salivation and increased removal reactivity (males) were most evident at 1.5h. By 3h these signs were subsiding, and the pronounced motor and sensory effects were apparent. Several motor effects (gait changes, lowered grip strenghts, decreased motor activity) were still evident 1-2 days later (especially females).
Conclusion	The applicant's version is adopted. NOAEL = 20 mg/kg bw
Reliability	2
Acceptability	Acceptable.
Remarks	

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	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_9_02-1: Effects of Cypermethrin on measures of the FOB

Data for only the time at wich the peak effect occurred								
FOB tests /dose	**0 mg/kg bw**		**20 mg/kg bw**		**60 mg/kg bw**		**120 mg/kg bw**	
	♂	♀	♂	♀	♂	♀	♂	♀
Salivation 1.5 h	1	1	1	1	1.5*	2*	2.5*	3*
Urination 1.5 h	0.13	0.13	0.13	0	0.63	0.38	0.86*	0.38
Arousal 3 h	3	4	3	4	2.5*	2.5*	2*	2*
Abnormal motor movements: 1.5 h	0/8	0/8	1/8	0/8	6/8*	4/8*	7/8*	7/8*
3 h	0/8	0/8	1/8	0/8	7/8*	4/8*	7/8*	8/8*
24 h	0/8	0/8	0/8	0/8	2/8*	0/8	2/8*	1/6
Forelimb grip strength 3h	1.012± 0.69	0.993± 0.044	0.973± 0.074	1.022± 0.053	0.654± 0.155*	0.835± 0.114	0.533± 0.152*	0.275± 0.051*
Hindlimb grip strength 3 h	0.966± 0.054	0.866± 0.033	0.851± 0.034	0.825± 0.054	0.521± 0.066*	0.582± 0.063*	0.511± 0.107*	0.379± 0.05*
Landing foot splay 24 h	66.1±4.1	59.3±4.4	65.2±4	58.5±3.9	81.6±8.4*	62.4±4	80.8±6.1*	78.5±4.5*
Righting reflex 3 h	1	1	1	1	3.5*	3*	4*	4*
Touch response 3 h	3	3	3	2*	1.5*	2.5	1.5*	1*
Tail pinch response 3 h	4	3	4	2	1.5*	4	2*	1*

* Statistically different from vehicle control group