Section A6.9 (01) Delayed Neurotoxicity

Annex Point IIA6.9 **Neurotoxic potential in the hen**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]; Toxicity of Pyrethroid Insecticides: Investigation of the Nerotoxic Potential of WL 43467 (cypermethrin) to Adult Domestic Hens; [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac-Agriphar s.a.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guidelines available for this type of study at the time it was conducted. However the protocol appears to be partially in compliance with OECD guideline 418 (1984).	
2.2	**GLP**	No. GLP was not compulsory at the time this study was conducted.	
2.3	**Deviations**	Yes Duration of treatment is not in accordance with OECD 418, birds were treated for 5 days and after 3 weeks the dosing regime was repeated and the birds killed after a further 3 weeks.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	WL 43467 (cypermethrin)	
3.1.1	Lot/Batch number	Batch no. 30 from Shell Biosciences Laboratory	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.2.1	Description	Not specified in report	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Not mentioned in report	
3.2	**Reference Substance (positive control)**	TOTP (Tri-o-tolyl phosphate)	

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3.3	**Test Animals**	
3.3.1	Species	Domestic Laying Hen (Gallus gallus domesticus)
3.3.2	Strain	Arbor Acre laying hens
3.3.3	Source	[redacted]
3.3.4	Sex	Not specified in report
3.3.5	Rearing conditions	Animals were healthy free-range laying hens and were maintained for one week prior to dosing in floor pens with sawdust and woodchip litter.
3.3.6	Age/weight at study initiation	Approximately 1 year old, weight not specified in report
3.3.7	Number of animals per group	3 experimental groups of six birds: Group I – dosed with test substance Group II – Positive control group Group III – Negative control group
3.3.8	Control animals	Yes
3.4	**Administration**	Oral by gavage
3.4.1	Exposure	Repeated oral dosing by gavage, administration once daily for 5 days. Dosing regime was repeated after 3 weeks.
3.4.2	Dose Levels	One positive control (TOTP), one negative control (no treatment), one treatment group
3.4.3	Vehicle	Dimethyl sulphoxide (DMSO) (due to low water solubility of cypermethrin)
3.4.4	Concentration in vehicle	1 g/kg bw
3.4.5	Total volume applied	Not specified in report, dose was prepared as a 50% w/v solution
3.4.6	Postexposure period	3 weeks
3.4.7	Anticholinergic substances used	Not specified in report
3.4.8	Controls	Negative control group received no treatment.
3.5	**Examinations**	
3.5.1	Body Weight	Not determined
3.5.2	Signs of Toxicity	Birds were observed daily for signs of ataxia, and at intervals tested for their ability to land without staggering when forced to fly.
3.5.3	Observation schedule	All birds observed daily

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3.5.4	Clinical Chemistry	No	
3.5.5	Pathology	Yes	
		Organs:	brain and sciatic nerve
3.5.6	Histopathology	Yes	
		Organs:	Cervical, thoracic and lumber cords, sciatic nerve, cerebellum and medulla oblongata.
3.6	**Further remarks**	-	
		4 RESULTS AND DISCUSSION	
4.1	**Body Weight**	Not specified in report	
4.2	**Clinical signs of toxicity**	In the group dosed with cypermethrin there were no signs of intoxication at any time during the study. See Table A6.9_01_1.	
4.3	**Clinical Chemistry**	Not included in study	
4.4	**Pathology**	No pathological lesions found in any of the birds dosed with cypermethrin	
4.5	**Histopathology**	No histological lesions were found in the peripheral or central nervous system tissues in any of the birds treated with cypermethrin.	
4.6	**Other**	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	6 Adult domestic hens were given 5 daily oral doses of 1 g/kg bw of cypermethrin, Wl43467 (b.n°.30; 98%) dissolved in DMSO. After 3 weeks, the dosing regime was repeated and a further 3 weeks later the birds were killed. A positive control received pralidoxine chloride and atropine sulphate and dosed once with 0.5 ml/kg of tri-ortho-tolyl phosphate.	
5.2	**Results and discussion**	In the group dosed with cypermethrin there were no deaths and no signs of intoxication at any time. No histological lesions were found in the peripheral or central nervous system	
5.3	**Conclusion**		
5.3.1	LOAEL		
5.3.2	NOAEL	1000 mg/kg bw	
5.3.3	Reliability	2	
5.3.4	Deficiencies	Yes. Duration of treatment was not strictly in accordance with the published guideline, however the study was conducted at an established facility and using cypermethrin of known purity. Study evaluated and accepted under Directive 91/414/EC.	

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Annex Point IIA6.9 **Neurotoxic potential in the hen**

	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.
Conclusion	The applicant's version is adopted. NOAEL = 1000 mg/kg bw/d
Reliability	Based on the assessment of materials and methods include appropriate reliability indicator
Acceptability	Acceptable.
Remarks	Repeated dosing regime; no body weight data; no biochemistry measurements, no vehicle control group included.
	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	

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Table A6.9_01_1. Table for delayed neurotoxicity in the hen

	untreated control	1 g/kg cypermethrin dose group	positive control
Number of animals at the start	6	6	6
Deaths	0	0	Hens sent for pathological examination after 18, 23, 29 and 57 days after dosing
Showing lesions	0	0	6 (typical sciatic lesions)
Showing effects in behaviour	0	0	6 (ataxia and/or paresis)
Showing other effect, state other effect	0	0	Typical signs of neurological disturbance