Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

Spirotetramat (ISO)

EC number: not allocated
CAS number: 203313-25-1

CLH-O-0000001688-63-02/A1

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

10 September 2013

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Spirotetramat (ISO)

EC Number: **not allocated**

CAS Number: **203313-25-1**

Index Number:

Contact details for dossier submitter:

Version number: **Date:** **09 December 2010**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**Spirotetramat**
EC number:	**Not allocated**
CAS number:	**203313-25-1**
Annex VI Index number:	**-**
Degree of purity:	**96%**
Impurities:	**No relevant impurities.**

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	No current entry	No current entry
Current proposal for consideration by RAC	Cat. 2, H319 Cat. 1, H317 Repr. Cat. 2, H361fd H400 Very toxic to aquatic life	Xn, R36 Xn, R43 Xn, R62 Xn, R63 N Dangerous for the environment R51/53 Toxic to aquatic organisms, may cause long-term adverse effect in the aquatic environment.
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)		

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Directive 67/548/EEC:

Symbols: Xn, Xi, N
Risk phrases: R 36 , R 43; R51/53
Repr. Cat.3, R 62, R 63

Safety phrases: S2, 13, 20/21, 24/25, 27/28, 36/37/39, S56, S66, S60, S61

Regulation EC 1272/2008:

Signal words: Warning

Symbols: 

Hazard statements: H317, H319, H361fd, H400, EUH401

Precautionary statements: P201, P202, P260, P263, P264, P270, P272, P273, P280, P391, P501

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Not explosive.
2.2.	Flammable gases				No gas.
2.3.	Flammable aerosols				No aerosol.
2.4.	Oxidising gases				No gas.
2.5.	Gases under pressure				No gas.
2.6.	Flammable liquids				No liquid.
2.7.	Flammable solids				Not a highly flammable solid.
2.8.	Self-reactive substances and mixtures				Not applicable.
2.9.	Pyrophoric liquids				No liquid.
2.10.	Pyrophoric solids				Not applicable. Stable.
2.11.	Self-heating substances and mixtures				Not applicable. Stable.
2.12.	Substances and mixtures which in contact with water emit flammable gases				Not applicable. Stable.
2.13.	Oxidising liquids				No liquid.
2.14.	Oxidising solids				No oxidizing properties.
2.15.	Organic peroxides				
2.16.	Substance and mixtures corrosive to metals				
3.1.	Acute toxicity - oral	No classification	-	-	Conclusive, but not sufficient for classification
	Acute toxicity - dermal	No classification	-	-	Conclusive, but not sufficient for classification
	Acute toxicity - inhalation	No classification	-	-	Conclusive, but not sufficient for classification
3.2.	Skin corrosion / irritation	No classification	-	-	Conclusive, but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Cat. 2, H319			
3.4.	Respiratory sensitisation	No classification	-	-	Conclusive, but not sufficient for classification
3.4.	Skin sensitisation	Cat. 1, H317			
3.5.	Germ cell mutagenicity	No	-	-	Conclusive, but not sufficient for classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
		classification			
3.6.	Carcinogenicity	No classification	-	-	Conclusive, but not sufficient for classification
3.7.	Reproductive toxicity	Cat. 2, H361fd			
3.8.	Specific target organ toxicity – single exposure	No classification	-	-	Conclusive, but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	No classification	-	-	Conclusive, but not sufficient for classification
3.10.	Aspiration hazard	No classification	-	-	Conclusive, but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Acute categorie 1			
5.1.	Hazardous to the ozone layer				Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors

[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word:	Warning
Hazard statements:	H317, H319, H361fd, H400
Precautionary statements:	P201, 202, 260, 263, 264, 270, 272, 280, P373, P391, P501
Suppl. Hazard:	EUH401

Proposed notes assigned to an entry:

Table 4: Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness				Not explosive.
Oxidising properties				No oxidizing properties.
Flammability				No self-ignition temperature was observed up to the melting point (142°C) or up to the maximum test temperature of 401°C.
Other physico-chemical properties *[Add rows when relevant]*				
Thermal stability				Endothermic effect in temp. range of 135 – 165°C (meling) and two exothermal effects in the range of 200 – 300°C and 380 – 450 °C with a maximum overall energy of 246 J/g.
Acute toxicity	No classification	-	-	Conclusive, but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	No classification	-	-	Conclusive, but not sufficient for classification
Repeated dose toxicity	No classification	-	-	Conclusive, but not sufficient for classification
Irritation / Corrosion	Xi, R36			
Sensitisation	Xi, R43			
Carcinogenicity	No classification	-	-	Conclusive, but not sufficient for classification
Mutagenicity – Genetic toxicity	No classification	-	-	Conclusive, but not sufficient for classification
Toxicity to reproduction – fertility	Xn, R62			
Toxicity to reproduction – development	Xn, R63			
Toxicity to reproduction – breastfed babies. Effects on or via lactation	No classification	-	-	Conclusive, but not sufficient for classification
Environment	R51/53			

[1)] Including SCLs

[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:	Indication of danger:	Xn, Xi, N
	R-phrases:	R36, R43, R62, R63, R51/53
	S-phrases:	S2, 13, 20/21, 24/25, 27/28, 36/37/39, S56, S57, S60, S61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Spirotetramat was applied as new active substance (Insecticide ; Chemical group: Tetramic acid) under Council Directive 91/414/EEC, and as a technical grade active ingredient under the Pest Control Products Act by the Pest Management Regulatory Authority (PMRA) of Canada and under the Federal Insecticide Fungicide and Rodenticide Act by the Environmental Protection Agency of the United States of America.

Spirotetramat has been evaluated and assessed in an OECD Joint Review Project, in co-operation with the regulatory Authorties of Canada (PMRA), United States of America (US EPA) and Austria (AGES - Austrian Agency of Health and Foodsafety). The evaluation of the Spirotetramat dossier is shared between these three regulatory authorities. The participating countries peer reviewed the evaluation conducted by each of the other regulatory authorities. The end product, this joint monograph, is used as the basis for regulatory decisions.

In accordance with Article 36(2) of the CLP Regulation, Spirotetramat should now be considered for harmonised classification and labelling. Therefore, this proposal considers all physical and chemical properties, human health and environmental endpoints. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of Spirotetramat under Directive 91/414/EEC. This assessment (DAR) was based on one full data package submitted by one company.

Spirotetramat is not currently listed in Annex VI of Regulation EC 1272/2008 (CLP Regulation). Following evaluation of the data this proposal seeks to propose classification for human health and the environment. No classification for physical and chemical properties is proposed.

2.2 Short summary of the scientific justification for the CLH proposal

Spirotetramat technical demonstrated moderate to low acute toxicity via the oral (LD_{50}>2000 mg/kg bw), dermal (LD_{50}>2000 mg/kg bw), and inhalation (LC_{50} >4.183 mg/L) routes. Spirotetramat is non-irritating to the skin, although it is an irritant to the eyes (acute hazard category 2, H319) and exhibits a skin-sensitization potential under the conditions of the guinea pig maximization test and the local lymph node assay, but not the Buehler patch test (acute hazard category 1, H317). The sensitization potential of spirotetramat was supported by two cases of Type IV hypersensitivity that were reported in spirotetramat manufacturing plant personnel .

In rats, the testes were the target organ following subchronic oral treatment at a high dose. Abnormal spermatozoa and hypospermia in the epididymis, decreased testicular weight, and testicular degeneration and vacuolation in males were observed in males after 90 days of exposure at 10000 ppm (616 mg/kg bw/day). These effects proved to be reversible in most animals after cessation of treatment. In rats, abnormal sperm or spermatid degeneration in the testes and germ cell exfoliated debris in the epididymis were observed in male rats at high doses (7500 ppm; 414 or 373 mg/kg bw/day) following 12 (not statistically significant) or 24 months of oral exposure.

Unlike the rat, no adverse effects of any kind were observed in mice tested orally up to the limit dose. In vitro results from a comparative metabolism study using hepatocytes from male rats, mice, and humans revealed species differences in the metabolism of spirotetramat. Specifically, mouse hepatocytes were better able than rat or human liver cells to metabolize BYI 08330-enol via glucuronidation. Potentially lower levels of the enol metabolite in mice in vivo may account for the lack of testicular toxicity observed in this

species.
The reproductive and developmental toxicity potentials of spirotetramat were tested in a multigeneration study in rats and in developmental toxicity studies in rats and rabbits. The observed effects on male reproductive function and performance triggered the conduct of several mechanistic studies and pharmacokinetic investigations. The developmental toxicity study in rats was supplemented by a second study to further elucidate the findings of the first (main) study.
In addition to testicular histopathology observed following subchronic and chronic exposure of male rats to spirotetramat, evidence of male reproductive toxicity was provided in the 2-generation reproductive toxicity study. Abnormal sperm cells were reported in F1-generation male rats treated with 419 mg/kg bw/day spirotetramat in the diet, and decreased reproductive performance was also observed in one of these males. Similar results were obtained in the 1-generation reproductive toxicity range-finding study, in which decreased sperm motility and progression and increased abnormal sperm cells in the epididymides were observed in F1 males at 320 mg/kg bw/day (hazard category 2 for reproductive toxicity, hazard statement H361, Suspected of damaging fertility).
In the developmental toxicity study in rats, reduced fetal weight and increased incidences of malformations and skeletal deviations were observed at 1000 mg/kg bw/day. Malformations at the high dose included single cases of cleft palate, co-arctation of aortic arch, atrial septal defect of the heart, microphthalmia, and supernumerary lumbar vertebra, four cases of dysplastic forelimb bones, and three cases of malformed sacral vertebral arches with pelvic shift. Skeletal findings at this dose were primarily incomplete or delayed ossification and skeletal variations (hazard category 2 for reproductive toxicity, hazard statement H361, Suspected of damaging the unborn child).

Regarding classification criteria for spirotetramat for **aquatic environment hazards acute category 1** (very toxic to aquatic organisms) is proposed.

2.3 Current harmonised classification and labelling

Spirotetramat has not been previously discussed or agreed at TC C&L (Dir. 67/548/EEC); no harmonised classification and labelling exist.

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

2.4 Current self-classification and labelling

No current self-classification and labelling based on CLP Regulation criteria.

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

2.4.2 Current self-classification and labelling based on DSD criteria

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

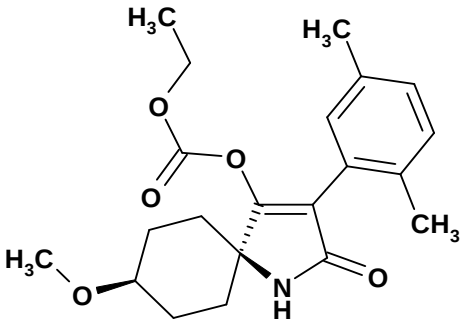
Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	**Not allocated.**
EC name:	**Not allocated.**
CAS number (EC inventory):	
CAS number:	**203313-25-1**
CAS name:	**cis-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl carbonate**
IUPAC name:	**cis-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro[4.5]dec-3-en-2-one**
CLP Annex VI Index number:	
Molecular formula:	**$C_{21}H_{27}NO_5$** 
Molecular weight range:	**373.45 g/mol**

Structural formula:

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
$C_{21}H_{27}NO_5$	96% (w/w) min. purity	No range since minimal purity.	

Current Annex VI entry:

No entry available.

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
None			

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

Physico-chemical properties: purity of tested technical material in the range from 99.2 % to 97.5%)

Human health hazard assessment: purity of tested technical material in the range from 93.1 to 99.0 %

Environmental hazard assessment: purity of tested technical material in the range from 97.1% to 98%

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

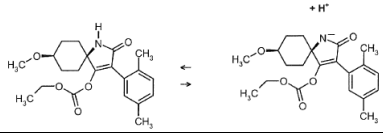
Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
B.2.1.1 Melting point, freezing point or solidification point, purified a.s. IIA 2.1.1	EC A.1, OECD 102	BYI 08330, mix-batch 08045/0003, purity 99.2 %	The melting point of Spirotetramat at atmospheric pressure is 142 °C.	The result is acceptable	Franke, J.; 2004 M-063268-01-1
B.2.1.2 Boiling point of purified active substance IIA 2.1.2	EC A.2, OECD 103	BYI 08330, mix-batch 08045/0003, purity 99.2 %	Spirotetramat has no boiling point at atmospheric pressure. Spirotetramat decomposed at a temperature of 235 °C.	The test material is a solid.	Franke, J.; 2004 M-063268-01-1
B.2.1.3 Temperature at which decomposition or sublimation occurs IIA 2.1.3	OECD 113	BYI 08330, mix-batch 08045/0003, purity 99.2 %	Spirotetramat showed an endo-thermic effect in the temperature range 135 – 165 °C (melting) and a two exothermal effects in the temperature range 200 – 300 °C and 380 – 450 °C with a maximum overall energy of 246 J/g.	The result is acceptable	Franke, J.; 2004 M-063268-01-1
B.2.1.4 Relative density of purified active substance IIA 2.2	EC A.3, OECD 109 (pycnometer method) EC A.3, OECD 109, OPPTS 830.7300 (pycnometer method)	BYI 08330, mix-batch 08045/0003, purity 99.1 % BYI 08330, PFV0586001, purity 97.5 %	Active substance, pure: D_4^{20} = 1.23 Active substance as manufactured: D_4^{20} = 1.22	The results are acceptable	Muehlberger, B., Lemke, G.; 2004 M-063293-01-1 Bogdoll, B., Lemke, G.; 2006 M-270041-01-1

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
B.2.1.5 **Vapour pressure of purified active substance** **IIA 2.3.1**	EC A.4, OECD 104 effusion method (vapour pressure balance)	BYI 08330, mix-batch 08045/0003, purity 99.2 %	Extrapolated: 5.6 x 10^{-9} Pa for 20 °C 1.5 x 10^{-8} Pa for 25 °C 1.5 x 10^{-6} Pa for 50 °C	The gas saturation method should be used instead of vapour pressure balance method.	Franke, J.; 2004 M-066171-01-1
B.2.1.6 **Henry's law constant** **IIA 2.3.2**	Calculation		Henry's law constants at 20 °C at different pH values: at pH 4: 6.24 x 10^{-8} Pa x m^3 x mol^{-1} at pH 7: 6.99 x 10^{-8} Pa x m^3 x mol^{-1} at pH 9: 1.09 x 10^{-7} Pa x m^3 x mol^{-1}	The results are acceptable	Bogdoll, B.; 2005 M-262215-01-1
B.2.1.7 **B.2.1.8** **Description of the physical state and colour, pur. and techn. a.s.** **IIA 2.4.1**	OPPTS 830.6302, OPPTS 830.6303 OPPTS 830.6302, OPPTS 830.6303	BYI 08330, mix-batch 08045/0003, purity 99.1 % BYI 08330, PFV0586001, purity 97.5 %	Active substance, pure: light beige powder Active substance as manufactured: white powder	The results are acceptable	Muehlberger, B.; 2003 M-103239-01-1 Bogdoll, B., Lemke, G.; 2006 M-270051-01-1
B.2.1.9 **Description of the odour - purified and technical active substance** **IIA 2.4.2**	OPPTS 830.6304 OPPTS 830.6304	BYI 08330, mix-batch 08045/0003, purity 99.1 % BYI 08330, PFV0586001, purity 97.5 %	Active substance, pure: no characteristic odor Active substance as manufactured: no characteristic odor	Acceptable	Muehlberger, B.; 2003 M-103239-01-1 Bogdoll, B., Lemke, G.; 2006 M-270051-01-1
B.2.1.10.1 **UV/VIS** **IIA 2.5.1.1**		BYI 08330, M26802, purity 99.2 %	UV (acetonitrile) The UV spectrum of BYI 08330 is shown in Figure 1.	Acceptable spectra were provided Spectra in alkaline and acid media are required	Kaussmann, M.; 2004 M-182543-02-1
B.2.1.10.2 **IIA 2.5.1.2** **IR**		BYI 08330, M26802, purity 99.2 %	The IR spectrum for BYI 08330 (Figure 2) was obtained using Bruker FTIR-Spectrometer TENSOR 37. Absorption bands and assignments are given in Table 1.	Key bands are consistent with the structure. The results are acceptable	Kaussmann, M.; 2004 M-182543-02-1
B.2.1.10.3 **IIA 2.5.1.3** **NMR**		BYI 08330, M26802, purity 99.2 %	^{1}H and ^{13}C spectra were obtained using a Bruker Avance 400 spectrometer. ^{1}H-NMR (400.13 MHz, $CDCl_3$) ^{13}C-NMR (100.61 MHz, $CDCl_3$) The structure and the assignement of chemical shifts of carbons and protons in ppm are given in Figure 3 and tables 2 and 3	The spectra are consistent with the structure of BYI 08330.	Kaussmann, M.; 2004 M-182543-02-1

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
B.2.1.10.4 **IIA 2.5.1.4** **MS**		BYI 08330, M26802, purity 99.2 %	LC-MS/ESI$^+$ was performed using HPLC Agilent 1100 with DAD and autosampler coupled to Waters ZMD MS with ESI Z-Spray source. The results showed the $[M+H]^+$ ion m/z 374.3 and $[2M+H]^+$ ion m/z 747.4. These are consistent with a molecular weight of 373.48 (Figure 4).	MS confirmed the structure of BYI 08330.	Kaussmann, M.; 2004 M-182543-02-1
IIA 2.5.1.5 **Wavelengths at which UV/VIS molar extinction occurs, max >290 nm**		BYI 08330, M26802, purity 99.2 %	Peak maxima [nm]: 211; 276 molar absorptivity [1000 cm²/mol]: 22.0×10^3; 0.8×10^3	The results are acceptable	Kaussmann, M.; 2004 M-182543-02-1
B.2.1.10.5 **Optical purity** **IIA 2.5.1.6**				Not relevant as the active substance is not a resolved optical isomer	
IIA 2.5.2.1 **UV/VIS**			No data presented	None of the impurities present in the active substance as manufactured is of toxicological or environmental significance	
IIA 2.5.2.2 **IR**			No data presented	None of the impurities present in the active substance as manufactured is of toxicological or environmental significance	
IIA 2.5.2.3 **NMR**			No data presented	None of the impurities present in the active substance as manufactured is of toxicological or environmental significance	

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
IIA 2.5.2.4 **MS**			No data presented	None of the impurities present in the active substance as manufactured is of toxicological or environmental significance	
B.2.1.12 **Solubility of purified active substance in wate r (pH 4-10)** **IIA 2.6**	EC A.6, OECD 105 (Flask method) EC A.6, OECD 105, OPPTS 830.7840	BYI 08330, mix-batch 08045/0003, purity 99.1 % BYI 08330, SPT0378-1, purity 99.4 %	pH 4 33.5 mg/L at 20°C pH 7 29.9 mg/L at 20°C pH 9 19.1 mg/L at 20°C The HPLC chromatograms shows that the test item is unstable in aqueous solution at pH 9 (formation of a degradation product). The degradation does increase with longer stirring time. In distilled water: pH 6.0 - 6.3 33.4 mg/L at 20°C	The results are acceptable	Muehlberger, B., Strunk, B.; 2003 M-103256-01-1 Bogdoll, B., Lemke, G.; 2006 M-270060-01-1
B.2.1.13 **Solubility in organic solvents at 15 to 25°C** **IIA 2.7**	EC A.6, OECD 105 (Flask method)	BYI 08330, mix-batch 08045/0003, purity 99.1 %	[g/L at 20 °C] ethanol 44 n-hexane 0.055 toluene 60 dichloromethane > 600 acetone 100 - 120 ethyl acetate 67 dimethyl sulfoxide 200 - 300	The results are acceptable	Muehlberger, B., Eyrich, U.; 2004 M-122802-01-1
B.2.1.14 **n-Octanol/water partition coefficient** **IIA 2.8.1**	EC A.8, OECD 117 (HPLC-method)	BYI 08330, mix-batch 08045/0003, purity 99.1 %	Determination of the partition coefficient of Spirotetramat in 1-octanol / water at 20 °C Pow log Pow pH 7 324 2.51	The results are acceptable	Lemke, G., Muehlberger, B.; 2003 M-103244-01-1
Effect of pH (4 to 10) on the n-octanol/ water partition coefficient **IIA 2.8.2**	EC A.8, OECD 117 (HPLC-method)	BYI 08330, mix-batch 08045/0003, purity 99.1 %	The partition coefficient (1-octanol / water) of Spirotetramat different pH-values was: Pow log Pow pH 4 324 2.51 pH 7 324 2.51 pH 9 316 2.50	The results are acceptable	Lemke, G., Muehlberger, B.; 2003 M-103244-01-1

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
B.2.1.15 **Hydrolysis rate at pH 4, 7 and 9 under sterile and dark conditions** **IIA 2.9.1**	OECD 111, EPA 161-1, DACO 8.2.3.2, MAFF 8147	[Azaspirodecenyl-3-^{14}C]-cis-BYI 08330, radiochemical purity ≥ 99 %, specific radioactivity 3.71 MBq/mg [Azaspirodecenyl-5-^{14}C]-cis-BYI 08330, radiochemical purity 98 %, specific radioactivity 4.03 MBq/mg	BYI 08330 is hydrolytically labile under acidic, neutral and alkaline conditions at ambient temperature. The fastest degradation was observed at pH 9. The experimental half-lives of the test substance at pH 7 were 8.6 days (25 °C) and 13 days (20 °C). The test substance also was unstable under acidic conditions (half-lives of 32.5 days (25 °C) and 48 days (20 °C) at pH 4) and degraded with a half-life of 7.6 hours at pH 9 (25 °C). The hydrolytic degradation was strongly temperature dependent. One major degradation product occurred in the total pH range tested the formation of BYI 08330-enol as a common hydrolysis product was observed (for structure see appendix 1).	The results are acceptable.	Heinemann, O.; 2004 M-093124-01-2
B.2.1.16 **Direct phototransformation in sterile water using artificial light** **IIA 2.9.2**	EPA 162-1, DACO 8.2.3.3.2	[Azaspirodecenyl-3-^{14}C]-BYI 08330, radiochemical purity > 98 %, specific radioactivity 3.67 MBq/mg [Azaspirodecenyl-5-^{14}C]-BYI 08330, radiochemical purity > 98 %, specific radioactivity 4.03 MBq/mg	Spirotetramat was degraded in sterile aqueous 0.01 M acetate buffer pH 5 under conditions of direct phototransformation. Four major phototransformation products were found and identified as products of re-arrangement reactions (for structures see appendix 2). P6 was the main metabolite and increased to max. 39.2 % of the AR at DAT-7; P7 was max. 22.1 % at DAT-4; P8 increased to 11.1 % after 3 days exposure to light and decreased then to 5.3 % at the end; P9 was max. 18.2 % of the AR at DAT-6. Based on the experimental DT50 of 2.7 days for BYI 08330 the predicted environmental DT50 is calculated to be e.g. 12.9 solar summer days at Phoenix, AZ, USA or 19.9 summer days at Athens, Greece. Under dark conditions the half life under experimental conditions is 26 days, which is regarded as hydrolysis half-life, and BYI 08330-enol is formed, only.	The results are acceptable.	Stupp, H.-P.; 2005 M-266695-01-1

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
B.2.1.17 **Quantum yield of direct phototransformation** **IIA 2.9.3**	ECETOC, EPA 161-1	BYI 08330, batch M26802, purity 99.2 %	A degradation of BYI 08330 of approx. 56 % was measured by HPLC-UV during the maximum irradiation period of 240 minutes in water. This indicates that BYI 08330 is not stable against direct photo-transformation in aqueous solution relative to other compounds irrated under the same study conditions. Using the UV absorption data and the degration kinetics of both the experiments a mean quantum yield of Φ = 0.00571 was calculated. Estimates based on two different arithmetic models (GC-Solar and Frank & Kloepffer) by means of the resulting quantum yield and the light absorption in a range of wavelengths relevant for the environment are given. They are well comparable when considering identical marginal conditions. "Environmental direct phototransformation half-lives" of BYI 08330 of 0.5 days to about one week during the period of main use can be assessed. However, this assessment does not consider any indirect mechanisms, which may enhance the photodegradation in natural water and which compete with the direct phototransformation to a great extent. This is addressed in more detail in chapter IIA 7.6.	The results are acceptable.	Heinemann, O.; 2004 M-092941-01-2
B.2.1.18 **Lifetime in the top layer of aqueous systems (calculated and real)** **IIA 2.9.4**	ECETOC, EPA 161-2 EPA 162-1, DACO 8.2.3.3.2	BYI 08330, batch M26802, purity 99.2 % [Azaspirodecenyl-3-^{14}C]-BYI 08330, radiochemical purity > 98 %, specific radioactivity 3.67 MBq/mg [Azaspirodecenyl-5-^{14}C]-BYI 08330, radiochemical purity > 98 %, specific radioactivity 4.03 MBq/mg	This point is covered by point IIA 2.9.3 and in more detail also considering natural water by point IIA 7.6.	The result is acceptable.	Heinemann, O.; 2004 M-092941-01-2 Stupp, H-.P.; 2005 M-266753-01-1
B.2.1.19 **Dissociation in water of purified active substance** **IIA 2.9.5**	OECD 112 (spectrophotometric method)	BYI 08330, mix-batch 08045/0003, purity 99.1 %	The dissociation constant of Spirotetramat was: pKa = 10.7 	The result is acceptable.	Muehlberger, B., Eyrich, U.; 2005 M-261598-01-1

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
B.2.1.20 Estimated photochemical oxidative degradation IIA 2.10	Calculation according to Atkinson		The chemical lifetime of spirotetramat in the air is assessed based on the calculation according to Atkinson by AOPWIN (version 1.91). A value of at the most 3 hours corresponding to a half-life of approx. 2 hours results, with respect to the OH radical and ozone reaction, only. Based on these values no long-range transport and no accumulation in air are expected for spirotetramat. Reaction with OH radicals and ozone contribute to the degradation of spirotetramat in the air to a high extent. The chemical stability of spirotetramat in air is not determined by an attack at one single site, but at different parts of the molecule. This should result in the formation of various primary radicals leading to secondary oxidation products, which can be eliminated from the air by wet and/or dry deposition.	The results are acceptable.	Hellpointner, E.; 2004 M-092840-01-1
B.2.1.21 Flammability of the active substance as manufactured IIA 2.11.1	EC A.10	BYI 08330, PFV0586001, purity 97.5 %	Spirotetramat is not a highly flammable solid in the sense of EC guideline A.10.	The results are acceptable.	Smeykal, H.; 2006 M-269989-01-1
B.2.1.22 Auto-flammability of the active substance as manufactured IIA 2.11.2	EC A.16 BCC-Test	BYI 08330, PFV0586001, purity 97.5 % BYI 08330, PFV0586001, purity 97.5 %	No self-ignition temperature was observed up to the melting point or up to the maximum test temperature of 401 °C. Spirotetramat is not classified in Division 4.2 (a negative result was obtained in the test using a 10 cm cube sample at 140 °C).	The results are acceptable. The study is considered as additional information by EU	Smeykal, H.; 2006 M-270010-01-1 Smeykal, H.; 2006 M-270036-01-1
B.2.1.23 Flash point of the active substance as manufactured IIA 2.12			Not applicable. The active substance is a solid; its melting point is > 40 °C.	The results are acceptable.	
B.2.1.24 Explosive properties of the active substance as manufactured IIA 2.13	EC A.14	BYI 08330, PFV0586001, purity 97.5 %	Not explosive in the sense of EC guideline A.14.	The results are acceptable.	Smeykal, H.; 2006 M-269992-01-1

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
B.2.1.25 **Surface tension of the active substance as manufactured** **IIA 2.14**	EC A.5, OECD 115 EC A.5, OECD 115	BYI 08330, mix-batch 08045/0003, purity 99.1 % BYI 08330, PFV0586001, purity 97.5 %	Pure substance: 61.65 mN/m at 20°C Technical substance: 60.5 mN/m at 20°C the concentration used: 90% of the saturation concentration	Spirotetramat is classified to be non-surface active according to EC Guideline A.5. This study is considered as additional information by EU This study is not required by Canada (PMRA) The study is accepted by EU.	Muehlberger, B., Lemke, G.; 2004 M-063303-01-1 Muehleberger, B., Lemke, G.; 2004 M-063303-01-1 Bogdoll, B., Lemke, G.; 2006 M-270037-01-1
B.2.1.26 **Oxidizing properties of the active substance as manufactured** **IIA 2.15**	EC A.17	BYI 08330, PFV0586001, purity 97.5 %	Spirotetramat has no oxidizing properties in the sense of EC guideline A.17.	The result is acceptable.	Smeykal, H.; 2006 M-270029-01-1
B.2.1.2.27 **pH** **IIA 2.16**	OPPTS 830.7000	BYI 08330, batch SPT0378-1, purity 99.4 % BYI 08330, batch PFV0586001, purity 97.5 %	Pure substance: 6.3 (1 % suspension in distilled water) Technical substance: 6.3 (1 % suspension in distilled water)	The result is acceptable This study is not required according to directive 91/414.	Bogdoll, B., Lemke, G.; 2006 M-269907-01-1
B.2.1.2.28 **Storage stability** **IIA 2.17.1**	OPPTS 830.6317	BYI 08330, mix-batch 08045/0014, purity 97.75 %	The results from a twelve months 30 °C stability study showed Spirotetramat to be stable in Polypropylene and Polyethylene containers. After completion of the accelerated storage test no decomposition of Spirotetramat could be established. There are no visible corrosive effects on the containers themselves after twelve months of storage.	This study is not required by Canada (PMRA) This study is not required according to directive 91/414.	Olenik, B.; 2006 M-272433-03-1
B.2.1.2.29 **Stability (temperature, metals)** **IIA 2.17.2**	OPPTS 830.6313	BYI 08330, mix-batch 08045/0014, purity 97.7 %	The results from a two weeks 54 °C stability study showed Spirotetramat to be stable in presence of the metals and metal ions. After completion of the accelerated storage test no decomposition of Spirotetramat could be established. In the presence of metals (iron and aluminum) changes in color during the test period could not be observed. The deviation of the analytical measurement of the samples with aluminum flakes are based on the inhomogeneity of these samples.	The result is acceptable This study is not required according to directive 91/414.	Olenik, B.; 2006 M-271221-02-1

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
B.2.1.2.30 Other/special studies IIA 2.18 **Oxidation or reduction properties**	OPPTS 830.6314	BYI 08330, batch PFV0586001, purity 97.5 %	Technical BYI 08330 was found to be non-reactive with ammonium dihydrogen phosphate, metallic iron or aqueous solution of 0.1N potassium permanganate in terms of significant temperature increase or evolution of gas. No significant temperature increase was observed for all applied test mixtures, i.e. the temperature increase was clearly below 5 °C in the course of the experiments and never exceeded ambient conditions.	This study is not required by Canada (PMRA) This study is not required according to directive 91/414.	Bogdoll, B., Lemke, G.; 2006 M-270048-01-1

Table 10: Addendum: Physchem properties, Volume 3, Annex B, December 2008

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	GLP Y/N	Reference
B.2.1.10.6 UV/VIS at pH 2 and pH 10 2.5.1.6		BYI 08330, purity 99.2 %	at pH 2: λ max [nm] — molar absorptivity [1000 cm²/mol] 201 — 26823.64 213 — 20704.35 at pH 10: λ max [nm] — molar absorptivity [1000 cm²/mol] 212 — 22417.81	Acceptable	Y	Olenik, B. (2008) M-306462-01-1

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for C & L.

2.2 Identified uses

Spirotetramat (BYI 08330) is intended to be used as an insecticide in agriculture on a range of crops such as citrus, pome and stone fruits; grape; strawberries, fruiting and leafy vegetables (indoor and outdoor); brassica and bulb vegetables, hops but also on tubers and tropical fruits such as pineapple, mangoes, bananas, tree nuts and cotton. In the EU the representative uses for Annex-1-inclusion consist of lettuce as a low crop and citrus as a high crop.

Spirotetramat is very effective against aphids, scales, mealy bugs, psyllids, white flies and selected thrips. It has a slow initial but an excellent residual activity and acts mainly against immature stages and requires oral ingestion. In many cases it outperforms most of the currently used products, as for example control of the Californian red scale (Aonidiella aurantii) on citrus, or control of the sweet potato whitefly (Bemisia tabaci) on vegetables. It is an important tool in resistance management, as it has a new mode of action.

Spirotetramat is highly systemic and possesses not only xylem-, but also phloem-mobility. Once taken up by the plant, the active substance moves acropetal in the xylem and acropetal and basipetal in the phloem. After treatment, even the new shoots or the roots are protected from sap-sucking pests due to this specific biological property.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 11: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference

3.1 *[Insert hazard class when relevant and repeat section if needed]*

3.1.1 Summary and discussion of

Spirotetramat is a white powder. The purified active substance has a melting point of 142°C and a vapour pressure of 5.6 x 10-9 Pa for 20°C. The active substance is quite soluble in polar organic solvents such as dichloromethane, dimethyl sulfoxide or acetone and slightly soluble in non polar solvents like n-hexane. Its water solubility depends on the pH of water and ranges from 19.1 mg/L at pH 9 to 33.5 mg/L at pH 4. Spirotetramat has a Log Kow value of 2.51.

It is not explosive and has no oxidizing properties. Its pH is within the range that naturally occurs e.g. in soil. Its stability allows storage under practical and commercial conditions. Its technical properties indicate that no particular problems have to be expected, when it is used as recommended.
A shelf-life (2 year) storage stability study is currently in progress. The end of storage is scheduled for 2007-12-03.
No physical/chemical classification is proposed.

3.1.2 Comparison with criteria

Criteria for classification and labelling according to DSD or CLP are not met.

3.1.3 Conclusions on classification and labelling

No classification and labelling for physico chemical properties is proposed.

RAC evaluation of physical hazards
Summary of the Dossier submitter's proposal No physical/chemical classification is proposed by the Dossier Submitter (DS) based on

the following observations:

- Flammability: Spirotetramat is not a highly flammable solid in the sense of EC guideline A.10.
  Auto-flammability: No self-ignition temperature was observed up to the melting point or up to the maximum test temperature of 401 °C. Spirotetramat is not classified in Division 4.2 (a negative result was obtained in the test using a 10 cm cube sample at 140 °C).
- Flash point: The active substance is a solid; its melting point is > 40 °C; therefore, criteria regarding the flash point are not applicable.
- Explosive: Not explosive in the sense of EC guideline A.14.
- Oxidising: Spirotetramat has no oxidizing properties in the sense of EC guideline A.17.
  Oxidation or reduction properties: Technical Spirotetramat was found to be non-reactive with ammonium dihydrogen phosphate, metallic iron or an aqueous solution of 0.1N potassium permanganate in terms of significant temperature increase or evolution of gas. No significant temperature increase was observed for all applied test mixtures, i.e. the temperature increase was clearly below 5 °C in the course of the experiments and never exceeded ambient conditions.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

RAC supports the proposal of DS not to classify Spirotetramat for physical hazards.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The disposition of spirotetramat (BYI 08330) was investigated in one in vitro and three in vivo studies. Gastrointestinal absorption of spirotetramat was rapid following oral administration to male and female rats at a single dose of 2 or 100 mg/kg bw or repeated doses of 2 mg/kg bw for 14 days. The absorption rate in all tests was between 89 and 98% of the total recovered radioactivity, based upon recovery from the urine and carcass (without the gastrointestinal tract). No significant differences in the absorption rate were observed among any of the dose groups. The absorption rate in the single low dose test was 95% for male rats and 96% for female rats. The maximum of plasma concentration was reached for all dose groups within 0.09 to 2.03 hours after administration (values calculated by pharmacokinetic modelling). The radioactivity concentrations in plasma declined steadily by several orders of magnitude within 48 hours for all dose groups. Radioactivity in tissues and organs at the 48-hour termination were very low (<0.2% and below the limit of detection for some organs/tissues). Urinary excretion was very rapid (essentially complete in 24 hrs) and was the major excretion route at these doses. Faecal excretion accounted for 2-11% of the dose in male and female rats. The excretion behavior was similar for all dose groups. Spirotetramat was completely metabolized by the rat in this study; no parent compound was detected in the excreta. Identified metabolites accounted for 87-95% of the administered dose. Only very minor metabolites (<0.7%

of the dose) were not identified. The main metabolic reaction was cleavage of the ester group which resulted in the formation of the primary and most predominant metabolite BYI 08330-enol (53-87% of the administered dose). All other identified metabolites could be derived from the enol intermediate. The second prominent metabolic transformation was oxidative demethylation of the 8-methoxy group to BYI 08330-desmethyl-enol (5-37% of the administered dose). Oxidation of the azaspiro moiety to BYI 08330-ketohydroxy and BYI 08330-desmethyl-ketohydroxy were detected as minor pathways. Other minor metabolic transformations were conjugation of the enol with glucuronic acid to BYI 08330-enol-GA and oxidation of the aromatic methyl group of the enol metabolite to BYI 08330-enol-alcohol. A sex-related difference was observed in metabolism in this study with male rats showing much higher rates of demethylation to BYI 08330-desmethyl-enol compared to female rats. Similar results were obtained when male and female rats were administered a single dose of 3 mg/kg bw in another disposition study using quantitative whole body autoradiography. The highest equivalent concentrations were observed in the liver, kidney, and blood in that study.

Absorption in male rats administered a single dose of 1000 mg/kg bw spirotetramat was much lower with only 27% of the dose excreted in the urine after 24 hours. Excretion was also distinctively less than for lower-dose animals, and radioactivity in plasma was slightly higher than in liver and kidney. These findings were consistent with saturation of cellular transport mechanisms. In addition, decline of tissue radioactivity was minimal from 1 h to 8 h post dose and increased only slightly at 24 hours. The metabolism profile was qualitatively similar to that of the lower doses; however, BYI 08330-desmethyl-enol occurred at lower levels at 1000 mg/kg bw, and this may be a reflection of the decreased absorption at this dose. Similar to the low dose groups, BYI 08330-desmethyl-enol levels were greater in urine than in plasma and organs. The highest percentage of BYI 08330-desmethyl-enol was detected in liver and kidney. Results of this study indicate that saturation of active transport mechanisms occurs at a dose of 1000 mg/kg but not 2 mg/kg. This results in decreased excretion via urine and faeces and a potential for accumulation of BYI 08330 related residues in the body following repeated high doses. The results of pharmacologically based pharmacokinetic (PBPK) simulations supported this conclusion and suggested that repeated daily doses of ≥300 mg spirotetramat/kg bw lead to non-linear elimination kinetics, resulting in a high body burden in multiple-dose toxicological studies. This assumes, however, that spirotetramat enters the systemic circulation as the metabolite BYI 08330-enol.

In a comparative in vitro metabolism study using hepatocytes from male rats, mice, and humans, differences in the proportions of several down-stream metabolites were observed; however, BYI 08330-enol was the first and most prominent metabolite and accounted for 66-100% of all metabolites across species.

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

Absorption, distribution, excretion and metabolism (toxicokinetics)

Rate and extent of oral absorption ‡	95 % absorbed (rat study, 2 mg/kg bw)
Distribution ‡	highest levels found in plasma, liver and kidney

Potential for accumulation ‡	no evidence for bioaccumulation
Rate and extent of excretion ‡	rapid, mainly via urine (90% within 24 hours)
Metabolism in animals ‡	Cleavage of esther group and O-demethylation of 8-methoxy group
Toxicologically relevant compounds ‡ (animals and plants)	Spirotetramat
Toxicologically relevant compounds ‡ (environment)	Spirotetramat

4.2 Acute toxicity

Table 12: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute oral toxicity	Wistar Hanover Rat: f > 2000 mg/kg bw	No treatment-related findings.	*Eigenberg, 2004*
Acute dermal toxicity	Wistar Hanover Rat: m/f > 2000 mg/kg bw	Day 1 – 3: red stain – nose, wetness – urogenital area, yellow stain – urogenital area and red zone – back. No clinical signs were observed after Day 3.	*Eigenberg, 2004*
Acute inhalation toxicity	Wistar Rat: LC50 > 4183 mg/m³. (4h, nose only)	ungroomed hair-coat, piloerection, bradypnea, labored breathing pattern, breathing sounds, nostrils reddened, nasal discharge, nostrils: red encrustations, nose/snout region: red encrustations, stridor, motility reduced, limp and high-legged gain	*Pauluhn, 2002*

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In an **acute oral toxicity study**, five young adult female Wistar Hanover rats (age: 10 weeks; weight: 155-159 g; source: Charles River Laboratories, Inc., Raleigh, NC) were given a single oral dose of spirotetramat (Purity: 93.1%; Batch No. NLL6425-9; beige powder) at 2000 mg/kg body weight. The test material was formulated as a suspension in 0.5% aqueous carboxymethylcellulose. The test material was given as a dose volume of 10 ml/kg to fasted rats.

Body weights were recorded on the Day(s) 1, 7 and 14. On Day 1, the dosed animal was observed for clinical observations: 1) within 30 minutes of dosing, 2) two observations were made between 30 minutes and four hours after dosing, and 3) in the afternoon on the Day 1. Following Day 1, clinical observations were made once each morning and a mortality check was performed each afternoon until the end of the study. All animals were necropsied and examined for any gross lesions.
All animals survived the test and gained body weight during the study. No clinical signs or gross

pathological findings were observed.
Oral LD_{50} Females > 2000 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

In an **acute inhalation toxicity study**, two groups of young adult Wistar (Hsd Cpb:WU) rats (5/sex) (age: 2 months; weight: 183-203 g males; 158-181 g females; source: Harlan-Winkelmann GmbH, Borchen, Germany) were exposed via the inhalation (nose-only exposure route) to spirotetramat (Purity: 96.5%; Batch No. NLL 6425-9; beige powder) for 4 hours at a concentrations of 1.1 mg/L (MMAD = 3.68 µm; GSD = 2.29) and 4.183 mg/L (MMAD= 5.13 µm; GSD = 2.37).
Body weights were taken before exposure, on Days 3 and 7 and weekly thereafter. On the day of exposure, the test animals were observed several times for clinical signs of toxicity and at the least once daily thereafter. All animals were necropsied and examined for any gross lesions.

1.1 mg/L dose group:
There was no mortality noted. in this dose group. By Day 3, 4/5 males and 5/5 females had either lost body weight or not gained any weight. By Day 7 all of the males and females had surpassed their initial body weights and continued to gain weight till the end of the study. Clinical signs observed in this group included the following: ungroomed hair-coat, piloerection, bradypnea, labored breathing pattern and nasal discharge (serous). No clinical signs were observed after Day 5. No gross pathological findings were observed.

4.183 mg/L dose group:
There was no mortality noted in this dose group. By Day 3 all the males and females had lost body weight. By Day 7, 4/5 males and 4/5 females had surpassed their initial body weight and by day 14 all animals exceeded their initial body weights. Clinical signs observed in this group included the following: ungroomed hair-coat, piloerection, bradypnea, labored breathing pattern, breathing sounds, nostrils reddened, nasal discharge , nostrils: red encrustations, nose/snout region: red encrustations, stridor, motility reduced and limp high-legged gain. No clinical signs were noted after Day 8. A battery of reflex measurements was made on the first post exposure day. Rats from this group experienced abnormal reflexes (reduced grip strength, reduced tonus and impaired righting response). No gross pathological findings were observed.
LC_{50} Combined Males/Females > 4.183 mg/L.

4.2.1.3 Acute toxicity: dermal

In an **acute dermal toxicity study**, 5 young Wistar Hanover rats of each sex (Crl:WI[Glx/BRL/Han]IGS BR) and (age: 10 weeks; weight: 271-319 g males; 182-195 g females; source: Charles River Laboratories, Inc., Raleigh, NC) were exposed to a single dermal application of spirotetramat (Purity: 93.1%; Batch No. NLL6425-9; beige powder) at 2000 mg/kg bw for 24 hours.
The test material was mixed with aqueous 0.5% carboxymethylcellulose in approximately a 1:1 ratio to form a dry paste. The paste was placed on a 2 inch by 2 inch piece of gauze and applied to the shaved area on the back of the animals. The gauze was held in place with medical tape and the torso of the animal was wrapped with porous medical tape. After the 24 hour exposure period, the wrappings were removed and the dose site was gently wiped with water-dampened and dry paper towels to remove as much of the test material as possible without damaging the skin.

Individual body weights were recorded on the Day(s) 1, 7 and 14. Clinical observations were made once each morning, except on the first two days of study, when clinical observations were performed in the afternoon. All animals were necropsied and examined for any gross lesions.
All animals survived the test and gained body weight during the study. Clinical signs observed on days 1-3 included the following: red stain – nose, wetness – urogenital area, yellow stain – urogenital area and red zone – back. No clinical signs were observed after Day 3. No gross pathological findings were observed.
Dermal LD_{50} Combined Males/Females > 2000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

No information on other routes.

4.2.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.2.3 Summary and discussion of acute toxicity

Spirotetramat was found to be of low acute toxicity to the rat by the oral route. No treatment-related findings were observed at the limit dose level of 2000 mg/kg bw. Treatment-related findings in the acute dermal toxicity were limited to minor clinical signs (resolved by day 3) at the dose level of 2000 mg/kg bw. Treatment-related findings in the acute inhalation toxicity study were limited to minor bodyweights and clinical signs (resolved by day 8) at the dose levels of 1.1 and 4.183 mg/L.

4.2.4 Comparison with criteria

All estimated LD_{50} values are above the criteria for classification and labelling (both DSD and CLP).

4.2.5 Conclusions on classification and labelling

No classification and labelling is proposed regarding acute toxicity.

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal <u>Acute toxicity: oral</u> No classification is proposed based on the absence of mortality or any treatment-related findings at the limit dose of 2000 mg/kg in an OECD TG 425 study in rats. In this study, the LD_{50} of Spirotetramat in rat by the oral route exceeded 2000 mg/kg. <u>Acute toxicity: inhalation</u> Rats were exposed (nose-only) to 1.1 or 4.183 mg/L of Spirotetramat (as an aerosol) for four hours in an OECD TG 402 study. No mortality was observed in any dose group. In both groups, some animals experienced a transient effect on body weight gain. Clinical signs consisting of ungroomed hair-coat, piloerection, bradypnea, laboured breathing and serous nasal discharge were observed up to day 4 at 1.1 mg/L. These clinical signs were also observed at 4.183 mg/L up to day 7, together with breathing sounds, reddened nostrils, nasal discharge, red encrustations in the nostrils and nose/snout region, stridor, reduced motility and limp high-legged gait. Abnormal reflexes were also reported. From

this study, the LC_{50} of Spirotetramat in rat by inhalation exceeds 4.183 mg/L. No classification was proposed by the DS.

Acute toxicity: dermal
Rats were exposed to a limit dose of 2000 mg/kg in an OECD TG 403 study and no mortality, effect on body weight gain or gross pathological findings were observed. Clinical signs consisting of red stain (nose), wetness (urogenital area), yellow stain (urogenital area) and red zone (back) were reported from day 1 to 3. From this study, the LD_{50} of Spirotetramat in the rat by the dermal route exceeds 2000 mg/kg. No classification was proposed by the DS.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

RAC supports the proposal of DS not to classify Spirotetramat for physical hazards.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Acute toxicity: oral
The LD_{50} of Spirotetramat in the rat is greater than 2000 mg/kg, below which classification for acute toxicity by the oral route applies according to the criteria of both the CLP Regulation and DSD.

Acute toxicity: inhalation
The available study provides no evidence that the LC_{50} of spiroteramat in rats is below the 5 mg/L trigger for classification for acute toxicity by inhalation for aerosols under the criteria of both the CLP Regulation and DSD.

Acute toxicity: dermal
The LD_{50} of Spirotetramat in rat is greater than 2000 mg/kg, below which classification for acute toxicity by the dermal route applies according to the criteria of both the CLP Regulation and DSD.

RAC supports the proposal of the DS not to classify Spirotetramat for acute toxicity.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

No specific, non lethal, target organ toxicity after single exposure was observed in acute toxicity studies. In addition, no human data are available that would support classification for this endpoint. No classification as STOT-SE under the CLP Regulation is proposed.

4.3.2 Comparison with criteria

No effects observed in acute toxicity studies would trigger criteria for classification and labelling STOT SE.

4.3.3 Conclusions on classification and labelling

No classification and labelling is proposed regarding specific target organ toxicity after single exposure.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

Spirotetramat was found to be of low acute toxicity to the rat by the oral route. No treatment-related findings were observed at the limit dose of 2000 mg/kg bw. Treatment-related acute dermal toxicity findings were limited to minor clinical signs (resolved by day 3) at the dose of 2000 mg/kg bw. Treatment-related findings in the acute inhalation toxicity study were limited to minor effects on bodyweight gain and clinical signs (resolved by day 8) at the doses of 1.1 and 4.183 mg/L.

The DS concluded that no specific, non-lethal, target organ toxicity after single exposure was observed in the acute toxicity studies. In addition, no human data are available that would support classification for this endpoint. No classification as STOT SE under the CLP Regulation was proposed by the DS.

Comments received during public consultation

No specific comments were received.

Additional key elements

In the acute inhalation study, clinical signs indicating respiratory irritation are reported. At 1.1 mg/L bradypnea, laboured breathing pattern and nasal discharge (serous) were reported. It is not known when each symptom started and ended, but all clinical signs were resolved after day 5. At 4.183 mg/L bradypnea, laboured breathing pattern, breathing sounds, reddened nostrils, nasal discharge, red incrustations in the nostrils and in the nose/snout region and stridor were reported. The same uncertainty related to the time of onset and duration of symptoms applies, but all clinical signs were resolved after day 8.
No gross pathological findings were observed.

It is noted that except for the acute inhalation study, no other inhalation data is available for Spirotetramat. No human data are available on inhalation.

Assessment and comparison with the classification criteria

The criteria for STOT SE 3 for respiratory irritation states that "this evaluation is primarily based on human data" but that "useful information may be obtained from the single and repeated inhalation toxicity tests" in animals. Effects that are relevant to consider for respiratory irritation according to the CLP criteria are clinical signs such as dyspnea and rhinitis, and histopathology findings such as hyperaemia, oedema, minimal inflammation, thickened mucous layer, which are reversible effects.

In the absence of relevant human data for Spirotetramat, the acute inhalation study provides some relevant evidence of respiratory irritation. No histopathological findings were observed but it is noted that gross necropsy was performed 14 days after exposure and hence effects, if present, may have reversed. The only indication of respiratory irritation therefore comes from the clinical signs observed. Reversible breathing

difficulties, nasal discharge and red incrustation in the nostrils and nose/snout region in the acute inhalation are interpreted as signs of acute respiratory tract irritation.
As the test substance is a white solid, it cannot be excluded that the mechanical effect of solid particles contributed to the irritation observed at high concentrations.
However, clinical signs indicating respiratory irritation are not only observed at the high dose of 4.183 mg/L, but also at 1.1 mg/L, and therefore mechanical irritation may not fully explain the observed clinical signs.

On this basis, RAC proposes to classify Spirotetramat with STOT SE 3 – H335 according to the CLP Regulation and Xi; R37 according to DSD.

4.4 Irritation

4.4.1 Skin irritation

Table 13: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation study	Himalayan Rabbit, males; not irritating	No dermal or systemic intolerance reactions.	Leuschner, 2002

4.4.1.1 Non-human information

In a **primary dermal irritation study**, 500 mg of spirotetramat (Purity: 96.5%; Batch No. NLL 6425-9; beige powder) was applied to the skin of three young healthy male Himalayan rabbits.
A dose of 500 mg was applied to the test site (approximately 6 cm^2). Pulverised solids were moistened sufficiently with water to ensure good contact with skin. The test material was applied to the test site and covered with a gauze patch. The patch was held in place with semi-occlusive dressing for the exposure period. After the 4-hour exposure period the patch was removed and the skin sites were evaluated. Scores were taken at 1, 24, 48 and 72 hours after patch removal.

No dermal irritation was noted at any of the examination time points.

4.4.1.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.4.1.3 Summary and discussion of skin irritation

Spirotetramat was not irritating to rabbit skin.

4.4.1.4 Comparison with criteria

Estimated skin irritation scores are below the criteria for classification and labelling (according to both DSD and CLP).

4.4.1.5 Conclusions on classification and labelling

No classification and labelling is proposed for Spirotetramat regarding skin irritation.

RAC evaluation of skin corrosion/irritation
Summary of the Dossier submitter's proposal In an OECD TG 404 study, Spirotetramat (as a powder, moistened with water) was applied to the skin of three rabbits for 4 hours under semi-occlusive conditions. No irritation was observed at any time point in any animal (scores of 0). The DS concluded that Spirotetramat is not irritating to the rabbit skin. **Comments received during public consultation** No specific comments were received. **Additional key elements** It is also noted that no local effect were observed in a 28-day study in rat after cutaneous exposure to Spirotetramat up to 1000 mg/kg. A red zone on the back of animals that persisted for three days (day 1 to 3) was observed in the acute dermal study and could indicate erythema. Incidence and severity are however not known and it is noted that this reaction was observed after an exposure duration of 24 hours, which exceeds the reference duration of four hours in guideline studies to determine skin irritation potential. **Assessment and comparison with the classification criteria** In the absence of any sign of irritation in a guideline-compliant study, Spirotetramat does not fulfil the criteria for skin irritation under the CLP Regulation or DSD, either in terms of severity of scores or in terms of irreversibility. RAC supports the proposal of the DS not to classify Spirotetramat for skin irritation/corrosion.

4.4.2 Eye irritation

Table 14: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye Irritation Study	Himalayan Rabbit, males; irritating	corneal opacity, Iritis, Conjunctival redness and chemosis	Leuschner, 2002

4.4.2.1 Non-human information

In a **primary eye irritation study**, 100 mg of spirotetramat (Purity: 96.5%; Batch No. NLL 6425-9; beige powder) was instilled into the conjunctival sac of the right eye in each of the three young healthy male Himalayan rabbits (age: 6.5 months; weight: 2.3-2.5 kg; source: LPT Laboratory of Pharmacology and Toxicology KG, D-24601 Lohndorf/Post Wankendorf). The left eye was untreated and served as control. The eyes were examined ophthalmoscopically with a slit lamp prior to instillation and also at 1, 24, 48 and 72 hours and on days 4 to 8. The eye reactions were observed and recorded. Twenty-four hours and 7 days after instillation, the eyes were treated additionally with fluorescein and examined.
Corneal opacity (grade 1) was observed in all eyes from 24 hours to 6 days after instillation and in two eyes until 7 days after instillation. No corneal opacity was observed on day 8. Iritis (grade 1) was noted in two eyes at 24 and 48 hours, in three eyes at 72 hours and days 4 and 5, and in one eye on day 6 after instillation. No iritis was observed on day 7. Conjunctival redness (grade 1) was noted in all eyes 1 hour to 72 hours after instillation and in two eyes until 4 days after instillation. Conjunctival chemosis (grade 1) was noted in one eye at 24 and 48 hours after instillation. All conjunctival irritation was resolved by day 5.

Based on the corneal opacity observed up to day 7 after instillation, spirotetramat is classified as an eye irritant.

4.4.2.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.4.2.3 Summary and discussion of eye irritation

In the primary eye irritation study, corneal opacity was observed in all eyes from 24 hours to 6 days after instillation and in two eyes until 7 days after instillation. No corneal opacity was observed on day 8. Iritis was noted in two eyes at 24 and 48 hours, in three eyes at 72 hours and days 4 and 5, and in one eye on day 6 after instillation. No iritis was observed on day 7. Conjunctival redness was noted in all eyes 1 hour to 72 hours after instillation and in two eyes until 4 days after instillation. Conjunctival chemosis was noted in one eye at 24 and 48 hours after instillation. All conjunctival irritation was resolved by day 5.

4.4.2.4 Conclusions on classification and labelling

According to Annex VI of the EC Council Directive 67/548/EEC, Spirotetramat has to be classified as irritating to the eyes (Hazard symbol Xi, risk phrase R 36).

According to Regulation EC 1272/2008, Spirotetramat should be classified in acute hazard category 2 for eye irritation and labeled with signal word “Warning” and hazard statement H319 (Causes serious eye irritation).

RAC evaluation of eye corrosion/irritation
Summary of the Dossier submitter’s proposal

In an OECD TG 405 study, Spirotetramat was instilled into the conjunctival sac of three rabbits. Corneal opacity (grade 1) was observed in all eyes from 24 hours to 6 days after instillation and in two eyes until 7 days after instillation. No corneal opacity was observed on day 8. Iritis (grade 1) was noted in two eyes at 24 and 48 hours, in three eyes at 72 hours and days 4 and 5, and in one eye on day 6 after instillation. No iritis was observed on day 7.
Conjunctival redness (grade 1) was noted in all eyes from 1 to 72 hours after instillation and in two eyes until 4 days after instillation. Conjunctival chemosis (grade 1) was noted in one eye at 24 and 48 hours after instillation. All conjunctival irritation was resolved by day 5.
The DS concluded that the effects observed on corneal opacity, iritis, conjunctival redness and conjunctival chemosis warrants classification of Spirotetramat as Eye Irrit. 2 – H319 according to the CLP Regulation and Xi; R36 according to DSD.

Comments received during public consultation

Three MSCA agreed with the proposed classification as Eye Irrit. 2 – H319 without further comments.

Additional key elements

The individual scores observed in the study and their comparison with the CLP and DSD criteria are summarised in the table below. Under both legislations, the criteria are met when at least two out of three animals exhibit mean scores equal to or above the mean scores stated in the criteria.

	Observation times									N° of animals fulfilling criteria	Result
	1h	24h	48h	72h	D4	D5	D6	D7	D8		
Corneal opacity											
Animal 1	0	**1**	**1**	**1**	1	1	1	1	0	CLP: mean 24/48/72h score ≥ 1 in 3/3 animals DSD: mean 24/48/72h score ≥ 2 in 0/3 animals	CLP + DSD -
Animal 2	0	**1**	**1**	**1**	1	1	1	1	0		
Animal 3	0	**1**	**1**	**1**	1	1	1	0	0		
Iritis											
Animal 1	0	1	1	**1**	**1**	**1**	1	0	0	CLP: mean score over 3 consecutive days ≥ 1 in 3/3 animals DSD: mean score over 3 consecutive days ≥ 1 in 3/3 animals	CLP + DSD +
Animal 2	0	1	1	**1**	**1**	**1**	0	0	0		
Animal 3	0	0	0	**1**	**1**	**1**	0	0	0		
Conjunctival redness											
Animal 1	1	1	1	1	1	0	0	0	0	CLP: mean	CLP –

Animal 2	1	1	1	1	1	0	0	0	0	24/48/72h score ≥ 2 in 0/3 animals DSD: mean 24/48/72h score ≥ 2.5 in 0/3 animals	DSD -
Animal 3	1	1	1	1	0	0	0	0	0		
Conjunctival oedema (chemosis)											
Animal 1	0	1	1	0	0	0	0	0	0	CLP: mean 24/48/72h score ≥ 2 in 0/3 animals DSD: mean 24/48/72h score ≥ 2 in 0/3 animals	CLP – DSD -
Animal 2	0	0	0	0	0	0	0	0	0		
Animal 3	0	0	0	0	0	0	0	0	0		

CLP+=fulfils the criteria for classification according to CLP
CLP-=does not fulfil the criteria according to CLP
DSD+=fulfils the criteria for classification according to DSD
DSD-=does not fulfil the criteria according to DSD

It is noted that the criteria point towards consideration of mean scores over 24, 48 and 72 hours. However, the CLP guidance (section 3.3.2.3.2.2) also refers to "average scores over three consecutive days (usually 24, 48 and 72 hours)" and the DSD criteria to "significant ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours". The mean score of 1 for iritis that is observed in the three animals from 72 hours after exposure and persists over two days is therefore considered to meet the criteria under both CLP and DSD.

Assessment and comparison with the classification criteria

RAC supports classification as Eye Irrit. 2 – H319 according to the CLP Regulation on the basis of three animals with corneal opacity scores of 1, and three animals with iritis scores of 1 over three consecutive days, and as Xi; R36 according to the DSD on the basis of three animals with iritis scores of 1 over three consecutive days.

As all effects were reversible before the end of the observation period, a more stringent classification is not justified.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

There is no specific information regarding the ability of Spirotetramat to cause irritation to the respiratory tract during the acute inhalation toxicity study.

4.4.3.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.4.3.3 Summary and discussion of respiratory tract irritation

No classification is proposed for respiratory tract irritation.

RAC evaluation of respiratory sensitisation
Summary of the Dossier submitter's proposal Based on the data from the acute inhalation studies, the DS concluded that Spirotetramat is not a respiratory sensitiser. **Comments received during public consultation** One MSCA agreed with the proposal not to classify for respiratory irritation without including any further comments. **Assessment and comparison with the classification criteria** In the absence of any relevant data to compare with existing criteria, RAC considers that a conclusion on classification for respiratory sensitisation is not possible.

4.5 Corrosivity

Based on the data from the skin and eye irritation studies it can be concluded that Spirotetramat is not corrosive.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 15: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Skin Sensitization Effect of BYI 08330 in Guinea Pigs (Guinea Pig Maximization Test According to Magnusson and Kligman)	Guinea pigs, female, sensitizing	Challenge with 25% test material formulation led to dermal effects (grade 1-3) in 95% in the test material group.	Vohr, 2002
Study for Skin Sensitization Effect of BYI 08330 in Guinea Pigs (Buehler Patch Test).	Guinea pigs, female, not sensitizing	No dermal effects during induction treatments in control and test groups. Challenge with 71% test material paste produced no dermal effects in test group and in control group.	Vohr, 2004
Evaluation of Potential Dermal Sensitization in the Local Lymph Node Assay.	Mice, female, sensitizing		Esdaile, 2004

4.6.1.1 Non-human information

Skin Sensitization Effect of Spirotetramat (BYI 08330) in Guinea Pigs (Guinea Pig Maximization Test According to Magnusson and Kligman)

The Guinea Pig Maximization Test was performed on 30 female Hsd Poc:DH guinea pigs (20 animals for the test group and 10 animals for the control group) (age: 5-6 weeks; weight: 292-366 g; source: Harlan Winkelmann GmbH Laboratory Animal Breeders, Borchen) to determine whether spirotetramat (Purity 96.5%; Batch No. NLL6425-9; beige powder) exhibits skin-sensitizing properties. The test material was formulated in polyethylene glycol 400 to yield a suspension or a paste. An additional two animals were used for dose-finding for the challenge concentration.

The study was conducted according to OECD Guideline No. 406/ EC Guideline 96/54/EC (22nd Adaptation of Guideline 67/548/EEC)/Health Effects Test Guideline, OPPTS 870.2600 with the following test material concentrations:

Intradermal induction: 5% (= 20 mg test material/animal)

Topical induction: 50% (= 250 mg test material/animal)

Challenge: 25% (= 125 mg test material/animal)

One animal from the test group died on day 15 of the study. After the intradermal induction the animal in the control group and in the test group showed strong effect up to encrustation at the injection sites of the first induction. The challenge with the 25% test material formulation led to dermal effects (grade 1-3) in 18 of 19 animals (95%) in the test material group. No dermal effects were observed in the control group animals.

In this study, **s**pirotetramat exhibits a dermal sensitization potential.

Study for Skin Sensitization Effect of Spirotetramat (BYI 08330) in Guinea Pigs (Buehler Patch Test)

The Buehler epicutaneous patch test was performed on 30 female Crl:HA guinea pigs (20 animals for the test group and 10 animals for the control group) (age: 4-5 weeks; weight: 309-375 g; source: Charles River Laboratory Animal Breeders. KiBlegg, Germany) to determine whether **s**pirotetramat (Purity: 97.1%; Batch No. Mix-Batch 08045/0014; white powder) exhibits skin-sensitizing properties. The test material was formulated in polyethylene glycol 400 to yield a suspension or a paste. An additional two animals were used for dose-finding for the challenge concentration.

The study was conducted according to OECD Guideline No. 406/ EC Guideline 96/54/EC (22nd Adaptation of Guideline 67/548/EEC); Health Effects Test Guideline, OPPTS 870.2600 with the following test material concentrations:

1^{st} to 3^{rd} induction: 71%

Challenge: 71%

In the control and test groups there were no dermal effects during the induction treatments. The challenge with the 71% test material paste produced no dermal effects in the test group and there were no dermal effects in the control group.

In this study, **s**pirotetramat exhibits no dermal sensitization potential.

Evaluation of Potential Dermal Sensitization in the Local Lymph Node Assay

In a dermal sensitization study with **s**pirotetramat (Purity: 97.2%; Batch No. 08045/0014), forty five female CBA/J mice (age: 8 weeks; weight: 21.1-24.0 g; source: R. Janvier, Le Genest St Isle, France) were tested using the Local Lymph Node Assay. The mice were allocated to nine groups of five animals each: four groups receiving the test material each at a concentration of 10, 5, 2.5 or 1%; four positive control groups receiving the reference substance (Isoeugenol) at a concentration of 5, 2.5, 1 or 0.5% and one control group receiving the vehicle, Dimethylformamide (DMF).

The test material, positive control and the vehicle were applied on external surfaces of each ear (i.e. 50 µl/animal) for three consecutive days (Days 0, 1 and 2) at the appropriate concentration. On Day 5, animals were intravenously injected via the tail vein with 250 µl of 0.9% sodium chloride containing 20µCi if ^{3}H methyl thymidine. Five hours after intravenous injection the mice were sacrificed and the two auricular lymph nodes were removed from each mouse. The lymph nodes from each mouse were placed in an individual tube containing physiological saline and were disaggregated by crushing with a plastic piston. A single cell suspension was obtained, free of connective tissue. Cell suspensions were washed with 6 ml of 0.9% physiological saline, centrifuged for 20 minutes at 1800 rpm and pellets obtained were resuspended in 4 ml of 5% trichloroacetic acid (TCA) and stored overnight at +4°C. After a final centrifugation, the pellets were resuspended in 1 ml of saline, mixed and then placed for 25 minutes in an Ultrasonic Bath to ensure a thoroughly dispersed suspension. Once prepared cell suspensions were added to numbered scintillation pots containing 10 ml of scintillation fluid and counts were taken in a beta-counter. The results were expressed as disintegrations per minute (DPM) per animal.

Animals were weighed before treatment and at study termination. The site of application was examined for signs of irritation. The animals were checked for clinical signs and mortality at least once a day during the study.

The criterion for a positive response was a statistically significant increase in cell proliferation in the test concentration groups compared to the vehicle control group and/or SIs greater than or equal to 3.0.

No mortality and no clinical signs were observed during the study. Animals in 10, 2.5 and 1% test groups gained body weight while animals in the 5% group lost body weight. No dermal reactions were observed in the vehicle, reference control or treated groups.

The stimulation index values of the test material were 5.9, 5.4, 4.3 and 3.4 at treatment concentrations of 10, 5, 2.5 and 1%, respectively. The stimulation indices of the positive control were 3.4, 1.8, 1.3 and 0.8 at treatment concentrations of 5, 2.5, 1 and 0.5%, respectively.
In this study, **s**pirotetramat exhibited dermal sensitization potential.
In comparison with the positive control substance, **s**pirotetramat had a skin sensitization potential of approximately five times that of Isoeugenol.

4.6.1.2 Human information

The sensitization potential of Spirotetramat was supported by two cases of Type IV hypersensitivity that were reported in spirotetramat manufacturing plant personnel.

4.6.1.3 Summary and discussion of skin sensitisation

Three dermal sensitization studies were conducted with spirotetramat. In two of the studies, Maximization Test according to Magnusson & Kligman and Local Lymph Node Assay (LLNA), spirotetramat exhibited a dermal sensitization potential. In the third study, Buehler Patch Test, spirotetramat exhibited no dermal sensitization potential.

4.6.1.4 Conclusions on classification and labelling

According to Annex VI of the EC Council Directive 67/548/EEC, spirotetramat has to be classified as a sensitizer (hazard symbol Xi, risk phrase R 43).

According to Regulation EC 1272/2008, spirotetramat should be classified in acute hazard category 1 for dermal sensitization and labeled with signal word "Warning" and hazard statement H317 (May cause an allergic skin reaction).

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal The skin sensitising potential of Spirotetramat was tested in three different studies conducted according to OECD test guidelines. A Guinea pig maximisation test (GPMT) was conducted with Spirotetramat in polyethylene glycol 400, using an intradermal induction concentration of 5%, a topical induction concentration of 50% and a challenge topical concentration of 25%. 95% of animals (18/19) exhibited dermal reactions (grade 1-3) while no reactions were observed in the control group. A Buehler Guinea pig test was conducted with Spirotetramat in polyethylene glycol 400, using a topical induction concentration of 71% and a challenge topical concentration of 71%. No reactions were observed either in the test group or in the control group. A Local Lymph Node Assay (LLNA) was conducted with Spirotetramat in dimethylformamide, using a topical concentration of 10, 5, 2.5 or 1%. The stimulation index (SI) values of Spirotetramat were 5.9, 5.4, 4.3 and 3.4 while SI values for the positive control (isoeugenol) were 3.4, 1.8, 1.3 and 0.8% at concentrations of 5, 2.5, 1 and 0.5%, respectively.

The sensitisation potential of Spirotetramat was supported by two cases of Type IV hypersensitivity that were reported in Spirotetramat manufacturing plant personnel. No further details are given in the Report on medical surveillance of manufacturing plant personnel.

Based on the positive GPMT and LLNA, the DS concluded that Spirotetramat should be classified as Skin Sens. 1A – H317 according to the CLP Regulation and Xi; R43 according to DSD.

Comments received during public consultation

Three MSCAs agreed with the proposed classification as Skin Sens. 1A – H 317 without further comments. Two additional MSCAs also agreed with classification in category 1 but one commented that available data do not enable sub-categorisation as 1A and the other that the rationale supporting sub-categorisation in 1A should be clarified.

Additional key elements

The database for skin sensitisation consists of several tests with diverging results: a clear positive response is identified in a GPMT and in an LLNA test, but no sensitisation is observed in a Buehler test. Experimental conditions alone cannot explain the negative result in the Buehler test, since higher concentrations than in the GPMT and the LLNA were applied topically and the vehicle was similar to that in the GPMT. However, it is noted that the GPMT is designed to optimise detection of sensitisation reactions using both intradermal and topical exposure during induction, and using Freund's complete adjuvant to potentiate sensitisation. Therefore, GPMT may be more sensitive than a Buehler test and this may explain the difference in results between the two assays. It is also noted that some false positives have been reported with both the GPMT and the LLNA with skin irritant substances; however, given the absence of skin irritation potential of Spirotetramat and the clear response observed in the GPMT and LLNA, a false positive response is not likely. The positive results in these two assays are therefore considered as reliable.
It is noted that two human cases of type IV hypersensitivity have been reported in Spirotetramat manufacturing plant personnel. However, in the absence of details in the reporting of these cases, and in the absence of specific studies investigating the sensitisation potential of Spirotetramat in humans, no conclusion on classification can be drawn on the basis of human data.

Skin sensitisation potency is assessed in the LLNA using the concentration that elicits a stimulation index of 3 (EC3). This value was not derived in the available test but an SI above 3 was reported at all concentrations tested, including an SI of 3.4 at the lowest tested concentration of 1%. It can be concluded that the EC3 in this test is below 1%, which fulfils the criteria for subcategory 1A.

In the GPMT, a single intradermal concentration of 5% was tested for induction, and an incidence of 95% of animal sensitised was observed. This fulfils the criteria for subcategory 1B (more than 30% of animals sensitised at an intradermal induction concentration above 1%). Given the high incidence of sensitisation observed, it can however not be excluded that Spirotetramat may induce a significant level of sensitisation at concentrations below 1% in a GPMT, which may indicate a potency in line with subcategory 1A.

Assessment and comparison with the classification criteria

On the basis of the positive results observed in the GPMT and in the LLNA, RAC considers that Spirotetramat should be classified as Skin Sens. 1 – H317 according to the CLP Regulation and Xi; R43 according to DSD.

The skin sensitisation potency of Spirotetramat, as evaluated in the LLNA, indicates that a sub-categorisation in 1A is justified. The GPMT results indicate that Spirotetramat has at least a moderate potency but the study did not use experimental conditions that could identify a high potency. The GPMT results are therefore not inconsistent with the high potency identified in the LLNA.

RAC therefore agrees with the DS that Spirotetramat should be classified as Skin Sens. 1A – H317 according to the CLP Regulation.

RAC also concludes that the results justify a specific concentration limit of 0.1% for Xi; R43 according to the DSD.

4.6.2 Respiratory sensitisation

Based on the data from the acute inhalative studies, it can be concluded that Spirotetramat is not a respiratory sensitizer.

4.7 Repeated dose toxicity

Table 16: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
BYI 08330: Subacute study with male mice (four-week dietary treatment, 500 and 5000 ppm)	500 and 5000 ppm: no treatment-related effects.	Study did not follow GLP Standards	Schladt, 2001
BYI 08330: Subacute study with female rats (four-week dietary treatment, 500 and 5000 ppm)	500 and 5000 ppm: no treatment-related effects.	Study did not follow GLP Standards	Krotlinger and Mihail, 1998
BYI 08330: A Subacute Toxicity Feeding Study in the Beagle Dog (four-week dietary treatment, 0, 100, 400, 1600, 6400 ppm)	NOAEL: 100 ppm (3 mg/kg/day [♂/♀])	LOAEL: 400 ppm (13/12 mg/kg/day [♂/♀]):thyroid hormone levels ↓.	Eigenberg, 2004
BYI 08330: Subchronic Toxicity Testing Study in the Mouse (90 days, 0, 70, 350, 1700, 7000 ppm)	NOAEL: 7000 ppm (1305/1515 mg/kg/day [♂/♀]).	LOAEL not established	Wahle, 2005
BYI 08330: Subchronic Toxicity Testing Study in the Rat (90 days, 0, 150, 600, 2500, 10000 ppm)	NOAEL: 2500 ppm (148/188 mg/kg bw/day [♂/♀])	LOAEL: 10000 ppm (616/752 mg/kg bw/day [♂/♀]):body weight ↓ absolute testicular weight ↓, testicular tubular degeneration, abnormal epididymal spermatozoa and hypospermia, alveolar macrophages in lungs [♂/♀].	Wahle, 2005
BYI 08330: 1 Year Toxicity Feeding Study in the Rat (0, 250, 3500, 7500/12000 ppm [♂/♀])	NOAEL 250 ppm (13.2 mg/kg bw/day [♂]) 3500 ppm (255 mg/kg bw/day [♀])	LOAEL: 3500/12000 ppm (189/890 mg/kg bw/day [♂/♀]) ♂: -alveolar macrophages in lungs ♀:-bodyweight ↓ -bodyweight gain ↓ -relative liver weight ↑ -relative kidney weight ↑ -discoloration of lungs -alveolar macrophages in lungs -yellow/brown staining of perigenital area and tail	Wahle, 2005

BYI 08330: 90-Day Subchronic Toxicity Feeding Study in the Beagle Dog (0, 150, 300, 1200, 2500 ppm)	NOAEL: 300 ppm (9/10 mg/kg bw/day [♂/♀])	LOAEL: 1200 ppm (33/32 mg/kg/day [♂/♀]): thyroid hormone T4 in both sexes ↓.	Eigenberg, 2005
BYI 08330: 1 Year Toxicity Feeding Study in the Beagle Dog (0, 200, 600, 1800 ppm)	NOAEL: 200 ppm (6/5 mg/kg bw/day [♂/♀])	LOAEL: 600 ppm (20 mg/kg/day, [♂]): thymus involution, brain dilation. 600 ppm (19 mg/kg/day, [♀]):thyroid hormone levels ↓.	Eigenberg, 2006
BYI 08330: Subacute (28 days) dermal toxicity study in rats (0, 100, 300, 1000 mg/kg bw/day)	NOAEL dermal and systemic: 1000 mg/kg bw/day	LOAEL: Not established, > 1000 mg/kg bw/day	Eigenberg, 2006

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

28 day treatment of male mice (strain: Crl:CD-1(ICR)BR) with spirotetramat (BYI 08330) at dietary concentrations of 500 and 5000 ppm, 136.5 and 1415 mg/kg bw/d respectively *(Schladt, 2001)* resulted in no treatment-related effects. The study did not follow the GLP Standards and many details regarding the methods, material, and parameters measured were not provided. No rationale was provided for using only the male mouse. These deficiencies could affect the interpretation of study results.

28 day dietary treatment of female rats (strain: Hsd/Win:WU) with spirotetramat at dosages 500 and 5000 ppm, 47.3 and 502 mg/kg bw/d respectively *(Krotlinger and Mihail, 1998)* resulted in no treatment-related effects. The slight decrease in mean triglyceride level at 500 and 5000 ppm was not dose-dependent or substantiated by any microscopic findings. Therefore, this finding was not considered toxicologically relevant or indicative of an influence on lipid metabolism.

In a **28 day Subacute Toxicity Feeding Study in Beagle Dogs** *(Eigenberg, 2004)* spirotetramat was administered in the diet at doses of 0, 100, 400, 1600 and 6400 ppm (0, 3, 13, 42, 104 mg/kg bw/d males, 0, 3, 12, 70, 127 mg/kg bw/d females, respectively). Neither males nor females tolerated the 6400 ppm dietary level. The dogs lost body weight, food consumption was reduced and appearance emaciated. Apparent secondary effects in some of these animals included decreased albumin and calcium in both sexes, and atrophy of the parotid salivary gland and sexual immaturity in one male.
Reduced thymus size and weight (described microscopically as involution) were seen in both sexes at 6400 ppm. The effect on the thymus might have been stress-related since there was no evidence of lymphocytic changes (degeneration, necrosis, depletion) in any of the other lymphocytic organs.
Partially statistically significant ($p<0.05$) decreases in circulating levels of T4 and T3 were observed at 400, 1600 and 6400 ppm in males and at 6400 ppm in females and were considered to be adverse. Non-statistical decreased TSH levels were also seen at 6400 ppm in both sexes. There was no evidence of correlative effects on the pituitary-thyroid endocrine axis at gross necropsy or histopathology.
The LOAEL is 400 ppm (13 and 12 mg/kg/day in males and females, respectively) based on decreased thyroid hormone levels. The NOAEL is 100 ppm (3 mg/kg/day in males and females).

In a **Subchronic Toxicity Study (90 days) in the Mouse** (strain: CD-1 [ICR]/BR), doses of 0, 70, 350, 1,700, and 7,000 ppm were chosen *(Wahle, 2005)*.
No treatment-related clinical signs or mortalities were observed. Body weight was not affected at any dose level in either sex. No relevant effect on feed intake was determined. Hematologic and clinical chemistry parameters were not affected. No treatment-related macroscopic and microscopic changes were noted. Absolute and relative organ weights showed no treatment-related changes.
A LOAEL was not established. The NOAEL was 7000 ppm (1305/1515 mg/kg/day [M/F]).

In a **Subchronic Toxicity Testing Study (90 days) in the Rat** (strain: Wistar Hanover, (Crl:WI [Glx/BRL/Han] IGS BR) doses of 0, 150, 600, 2500, and 10000 ppm were chosen *(Wahle, 2005)*.

Study design:

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg; x±Std)	Male	Female
Control (A)	0	0	20	20
Low (B)	150	males 8.9 ± 1.4 females 11.4 ± 1.2	10	10
Mid 1 (C)	600	males 35.9 + 5.7 females 46.1 + 4.5	10	10
Mid 2 (D)	2,500	males 147.9 + 23.2 females 188.3 ± 24.2	10	10
High (E)	10,000	males 615.9 ± 91.6 females 752.0 ± 84.6	20	20

No treatment-related macroscopic changes were seen. Treatment-related effects in the high dose (10000 ppm, 616 mg/kg bw/day in males, 752 mg/kg bw/day in females, respectively) were reduced body weight, decreased absolute testes (6.5%) weights and kidney weights (11%) in males. Kidney weight for high dose males was similar to controls after recovery. Kidney weight in females was decreased by 6% at termination and equivalent to the control after recovery.
Histopathology:
At the end of the treatment period, an increased incidence of minimal to severe abnormal spermatozoa and hypospermia were noted in the epididymides of 10000-ppm males. Multi-nucleated giant cells, spherocytes (immature sloughed germ cells), and tissue debris (recorded as abnormal spermatozoa) were present within the duct of the epididymis at a level well above that found in control males. Only one recovery male each showed abnormal spermatozoa or hypospermia at the end of the 4-week recovery period suggesting reversibility of lesions.
A minimal to moderate tubular degeneration was noted in the testes of 10000-ppm males. Tubular degeneration and vacuolization in the 10000 ppm dose group was generally multifocal in distribution, and occurred in the germinal epithelial layer of seminiferous tubules with degeneration and loss of epithelial cells. This finding correlated with the slight decrease in absolute testicular weight. Only one recovery male showed tubular degeneration in the testes at the end of the 4-week recovery period suggesting reversibility of lesions.
An increased incidence of minimal to slight accumulation of alveolar macrophages of the lungs was noted in both sexes at 10000 ppm and were considered to be affected by compound administration. At the end of the recovery period, reversibility was suggested for the affected parameter.

2500 ppm (equal to 148 or 188 mg/kg bw/day) in males or females, respectively can be considered as NOAEL under the conditions of this study.

In a **1 year Toxicity Study in the Rat** (strain: Wistar Hanover Crl:WI[Glx/BRL/Han]IGS BR), doses of 0, 250, 3500 and 7500 (males)/12000 (females) ppm were chosen *(Wahle, 2005)*.

Study design:

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg; x±Std)	Main Study 12 months	
			Male	Female
Control	0	0	25	25
Low (LDT)	250	males 13.2 ± 3.4 females 18.0 ± 3.4	25	25
Mid (MDT)	3,500	males 189 + 49 females 255 + 39	25	25
High (HDT)	males 7,500 females 12,000	males 414 ± 106 females 890 ± 156	25	25

Treatment-related effects in the high dose were slightly reduced body weights in 12000 ppm females at end of treatment. Overall mean body weight gain in this group was 86% of controls. Male body weights were not affected. Feed intake was not toxicologically relevantly affected.
Neurobehavioral parameters (e.g. weekly open field assessments; FOB conducted during Month 12) provided no indication of a neurological effect attributable to exposure to the test substance.
Gross necropsy showed an increased incidence of discoloration of the lung in 12000-ppm females. An increase in relative liver weights was noted in 7500-ppm males and in 12000-ppm females which was considered treatment-related. Relative kidney weights were also slightly increased in females at 12000 ppm.
Histopathology:
Testis/epididymis: An increased incidence of exfoliated germ cells/debris was observed in the epididymis of males at 7500 ppm (3/25 vs. 0/24 in controls). Abnormal spermatozoa were also observed in high dose males (2/25 vs. 0/25 in controls). Although the increases were not statitistically significant, they were considered toxicologically relevant, since testicular/epididymal histopathology was observed at higher incidences following treatment with spirotetramat for 24 months.
Lung: An increased incidence of minimal to slight accumulation of alveolar macrophages was noted in the lungs of 3500 and 7500 ppm males and 12000 ppm females. This finding correlated with the discoloration of the lung in the 12000 ppm females (see above). The increased incidence noted in this study was attributed to exposure to the test substance. No other indication of microscopic changes attributable to exposure to the test substance was observed.
The chronic NOAEL was established at 250 ppm (equal to 13.2 mg/kg bw/day) in male rats, based on an increased incidence of accumulation of alveolar macrophages at 3500 ppm (equal to 189 mg/kg bw/day). Some evidence of testicular toxicity was observed in a total number of 25 male rats at 7500 ppm (equal to 414 mg/kg bw/day) examined histopathologically after one year of treatment.
The chronic NOAEL in females was established at 3500 ppm (equal to 255 mg/kg bw/day), based on decreased body weight and body weight gain, yellow and brown staining in the perigenital area and tail,

discoloration of the lung and increased incidence of accumulation of alveolar macrophages at 12000 ppm (equal to 890 mg/kg bw/day).

In a **Subchronic Toxicity Study (90 days) in beagle dogs** *(Eigenberg, 2005)*, dose levels of 0, 150, 300, 1200 and 2500 ppm were chosen.

Study design:

Dose Group (ppm)	No. Animals/ Dose/Sex	Males (mg/kg/day)	Females (mg/kg/day)
0 (control)[a]	4	0	0
150	4	5	6
300	4	9	10
1,200	4	33	32
2,500*	4	81**	72**

At the dose level of 2500 ppm, body weight gain and food consumption were reduced and can be considered as compound-related effects.
Slight declines in mean values for RBC count, hemoglobin concentration and hematocrit were seen at the 2500 ppm dietary level in females at 58 days. The mean values for these parameters were further decreased at test day 86 (16-17%), and the difference from control was statistically significant for each parameter. The noted compound-related effect on red blood cell parameters at 2500 ppm in females is considered toxicologically significant.
There was a treatment-related decrease in T_4 in the 1200 and 2500 ppm male and female dose groups. There was a slight, but statistically significant decrease in T_3 in the 2500 ppm male and female dose groups on study days 30 and 58 which is possibly considered to be treatment-related. However, on study day 86, T_3 levels were comparable to the control mean. Despite percentage declines in T_4 at some time points of greater than 50%, no changes in thyroid weight, thyroid pathology, and no compensating increases in thyrotropin (TSH) were seen.
Gross necropsy, organ weights:
There were no treatment-related effects on organ weights. The statistically significant increase in relative liver weights for males in the 150, 300, and 2500 ppm groups is considered to be incidental as there is no dose response, the magnitude of differences were slight, there were no correlative clinical chemistry or histopathological changes and there was no statistical difference in the absolute liver weights. Likewise, statistically significantly ($p<0.05$) lower absolute pituitary weights in 1200 and 2500 ppm females are not considered compound-related; corresponding weights relative to body weights were not significantly different from the control.
The only notable compound-related histopathologic change was thymus atrophy in one female at 2500 ppm.
Based on statistically significant decrease in thyroid hormone T_4 observed at 1200 ppm in both sexes, this dose level is considered to be the LOAEL (33/32 mg/kg/day in males/females).
The NOAEL is 300 ppm (9/10 mg/kg/day in males/females).

In a **1 year Toxicity Study in beagle dogs** *(Eigenberg, 2006)* dose levels of 0, 200, 600 and 1800 ppm were chosen.

Study design:

Dose Group (ppm)	No. Animals/ Dose/Sex	Males (mg/kg/day)	Females (mg/kg/day)
0 (control)[a]	4	0	0
200	4	6	5
600	4	20	19

1,800	4	55	48

There were no compound-related effects on food consumption and body weights. There were no toxicologically significant findings in mean values for RBC count, hemoglobin concentration and hematocrit. Thyroxine (T_4) was statistically decreased in males at 1800 ppm for all post-treatment time points and at 600 ppm at termination of study. In females, T_4 was statistically decreased for most time points at 600 and 1800 ppm. Triiodthyronine (T_3) decreases were less pronounced and occurred in males at 1800 ppm for all time points. There was a tendency for T_4 values to fall below the historical control range at 1800 ppm in males and females; the same tendency was seen in T_3 values at 1800 ppm in males. Despite declines in thyroid hormones at some time points, no changes in thyroid weight or compensating increases in thyrotropin (TSH) were seen at ≤ 600 ppm.
Reduced thymus size and dilated brain were observed in males at 600 and 1800 ppm; brain dilation was also noted in females, but only at 600 ppm.
Gross necropsy, organ weights:
Organ weights were not affected by treatment. Mean thyroid absolute and relative organ weights were not statistically different from controls. Mean absolute heart weight was statistically low ($p<0.05$) at 1800 ppm in males, however, there was no statistical difference in mean relative weight and correlative histopathologic changes were not found. Absolute and relative thymus weights were low (not statistically) in males at 600 ppm only.
Histopathology:
Two 1800 ppm males had a slight reduction in the size of the peripheral thyroid follicles. Two additional step-sections from the blocks were also evaluated for all control and 1800 ppm males and females. None of these additional evaluations changed the follicular morphology observed in the original slides.
Brain dilation was seen at 600 ppm in 1 male (mild) and 1 female (moderate) as well as at 1800 ppm in 1 male (moderate); mild axonal degeneration was also detected in 1 female at 1800 ppm. Thymus involution was graded mild in 1 male at 600 ppm and moderate in 1 male at 1800 ppm.
In this study, the LOAEL for male dogs is considered to be 600 ppm (20 mg/kg/day) based on thymus involution and brain dilation, and 600 ppm (19 mg/kg/day) in females based on decreased thyroid hormone levels. The NOAEL for males and females is 200 ppm (6/5 mg/kg/day in males/females, respectively).

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

In a **Subacute dermal toxicity study in rats** (Wistar Hanover CRL:WI [GLX/BRL/HAN]IGS BR) for a period of 28 days, animals received dose levels of 0, 100, 300, 1000 mg/kg bw/day by dermal application. The test substance was applied undiluted and only moistened with water immediately before application.
No effects on body weight development, food consumption, clinical signs, or local skin reactions were observed. Statistically significant differences observed in treated groups in hematology (increased white blood cell count, activated partial prothromboblastin time, prothrombin time, differential cell counts) and clinical chemistry (decreased creatine kinase, lactate dehydrogenase, aspartate aminotransferase) measurements were not considered toxicologically or biologically significant since they did not have a clear dose-response pattern, were within historical control ranges, and/or no histological correlations were found. Only tissues from the control and high dose groups were examined microscopically, and no

treatment-related findings were reported.
The systemic and dermal NOAEL for male and female rats is 1000 mg/kg/day.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

No data available.

4.7.1.7 Summary and discussion of repeated dose toxicity

The insecticidal mode of action (lipid biosynthesis inhibition) was not reflected in the results of the short-term toxicological studies in rodents and dogs. Rats, mice, and dogs did not exhibit changes in plasma lipid parameters such as plasma triglycerides and plasma cholesterol. The thyroid and thymus glands were target organs in oral subchronic toxicity studies in the dog. Subchronic (90-day and 1 year) exposure of dogs to spirotetramat was characterized by statistically significant declines in circulating thyroid hormones (T_4) at ≥1200 ppm (32 mg/kg bw/day) and 600 ppm (20 mg/kg/day) respectively; decreased T_3 was observed at the highest dose tested (2500 ppm, 72 mg/kg bw/day, 90-day and 1800 ppm, 60 mg/kg bw/d respectively). Despite declines in thyroid hormones at some time points, no changes in thyroid weight or compensating increases in thyrotropin (TSH) were seen at ≤ 600 ppm. After 1 year exposure of dogs to spirotetramat, thymus involution and dilated brain were observed in males at 600 and 1800 ppm.
In rats, the testes were the target organ following subchronic oral treatment at a high dose. Abnormal spermatozoa and hypospermia in the epididymis, decreased testicular weight, and testicular degeneration and vacuolation in males were observed in males after 90 days of exposure at 10000 ppm (616 mg/kg bw/day). These effects proved to be reversible in most animals after cessation of treatment. Other effects in subchronically treated rats were limited to declines in terminal body weight in 10000 ppm male rats and an increased incidence of accumulation of alveolar macrophages in both sexes at 10000 ppm. It is noteworthy that the thyroid and thymus were unaffected in rats at any dose, while testicular histopathology was unobserved in dogs.
Chronic toxicity was tested in rats following application of spirotetramat for one year, respectively. Target organs in rats were the kidney (both sexes) at mid and high doses and the liver (females) at the highest dose only. These results are consistent with the excretory and/or detoxification roles of the kidney and liver. Consistent with the subchronic study the lung demonstrated treatment-related presence of alveolar macrophages in mid and high dose males and high dose females.
Exfoliated germ cells/debris in the epididymis and abnormal spermatozoa were observed in high dose males which were considered as toxicologically relevant.
In the last month of the one year chronic toxicity study, 10 rats/sex/dose were evaluated in an FOB assessment that included evaluation of motor activity and responses to sensory stimuli. Treatment-related effects in the FOB assessments were not observed.
Unlike the rat, no adverse effects of any kind were observed in mice tested orally up to the limit dose. In vitro results from a comparative metabolism study using hepatocytes from male rats, mice, and humans

revealed species differences in the metabolism of spirotetramat. Specifically, mouse hepatocytes were better able than rat or human liver cells to metabolize BYI 08330-enol via glucuronidation. Potentially lower levels of the enol metabolite in mice in vivo may account for the lack of testicular toxicity observed in this species.
Subchronic exposure of rats by the dermal route yielded no evidence of systemic toxicity when spirotetramat was tested up to 1000 mg/kg bw/day. This result may in part be a reflection of the low dermal absorption (approximately 10%) in rats.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

Effects observed in the subchronic studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

Effects observed in the subchronic studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Effects observed in the subchronic/chronic studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Effects observed in the subchronic studies in rat, mouse and dog do not trigger the criteria for classification and labelling for Specific target organ toxicity after repeated exposure.

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)
Summary of the Dossier submitter's proposal The insecticidal mode of action (lipid biosynthesis inhibition) was not reflected in the results of the short-term toxicological studies in rodents and dogs. Rats, mice and dogs did not exhibit changes in plasma lipid parameters such as plasma triglycerides and plasma cholesterol. The thyroid and thymus glands were the target organs in oral (sub)chronic toxicity studies in the dog. (Sub)chronic (90-day and 1 year) exposure of dogs to Spirotetramat was characterised by statistically significant reductions in circulating thyroid hormones (T4) at ≥1200 ppm (32 mg/kg bw/day) and 600 ppm (20 mg/kg bw/day) respectively; decreased T3 was observed at the highest dose tested (2500 ppm in the 90-day study, and 1800 ppm in the 1 year study, respectively). Despite reductions in thyroid hormone levels at some time points, no changes in thyroid weight or compensating increases in thyrotropin (TSH) were seen at ≤ 600 ppm. After exposure of dogs to Spirotetramat for 1 year, thymus involution and dilated brain were observed in males at 600 and 1800 ppm.

In rats, the testis was the target organ following subchronic oral treatment at a high dose. Abnormal spermatozoa and hypospermia in the epididymis, decreased testicular weight, and testicular degeneration and vacuolation in males were observed after 90 days of exposure at 10000 ppm (616 mg/kg bw/day). These effects proved to be reversible in most animals after cessation of treatment. Other effects in subchronically treated rats were limited to reductions in terminal body weight in 10000 ppm male rats and an increased incidence of accumulation of alveolar macrophages in both sexes at 10000 ppm. It is noteworthy that the thyroid and thymus were unaffected in rats at all doses, while testicular histopathology was not observed in dogs.

Chronic toxicity was tested in rats following oral exposure of Spirotetramat for one year. Target organs in rats were the kidney (both sexes) at mid and high doses and the liver (females) at the highest dose only. These results are consistent with the excretory and/or detoxification roles of the kidney and liver. Consistent with the subchronic study, the lung demonstrated treatment-related presence of alveolar macrophages in mid and high dose males and high dose females. Exfoliated germ cells/debris in the epididymis and abnormal spermatozoa were observed in high dose males and these findings were considered toxicologically relevant. In the last month of the one year chronic toxicity study, 10 rats/sex/dose were evaluated in weekly, open field, Functional Observational Battery (FOB) assessments that included evaluation of motor activity and responses to sensory stimuli. Treatment-related effects in the FOB assessments were not observed.

Unlike the rat, no adverse effects of any kind were observed in mice tested orally up to the limit dose. Results from a comparative *in vitro* metabolism study using hepatocytes from male rats, mice, and humans revealed species differences in the metabolism of Spirotetramat. Specifically, mouse hepatocytes were better able to metabolise BYI 08330-enol via glucuronidation compared to rat and human liver cells. Based on these findings, it was hypothesised that potentially lower levels of the enol metabolite in mice *in vivo* might account for the lack of testicular toxicity observed in this species.
Subchronic exposure of rats by the dermal route yielded no evidence of systemic toxicity when Spirotetramat was tested up to 1000 mg/kg bw/day. This result may in part be a reflection of the low dermal absorption (approximately 10%) in rats.

The DS concluded that Spirotetramat did not induce significant toxic effects at doses relevant for classification.

Comments received during public consultation

No specific comments were received.

Additional key elements

Repeated dose toxicity - oral route:

28-day studies (to be compared with the classification criteria range of 30-300 mg/kg bw/day for STOT RE 2 under the CLP Regulation, and 15-150 mg/kg bw/day for Xn; R48 under DSD)

No treatment-related toxicity was reported in male mice up to 1415 mg/kg bw/day and in female rats up to 502 mg/kg bw/day in 28-day studies. It is however noted that deficiencies in the 28-day study in mice could affect the interpretation of the study

results.

In a 28-day study, Beagle dogs (2 animals/sex/dose) were exposed through diet to 100, 400, 1600 or 6400 ppm Spirotetramat daily (corresponding to 3/3, 13/12, 42/70 or 104/127 mg/kg bw/day in males/females, respectively). A decrease in body weight was observed at the two highest doses of 1600 and 6400 ppm and reached -12% and –33% in males and -8% and -29% in females, respectively. It was accompanied by a decrease in food consumption, which was marked at the highest dose. Decreases of calcium and albumin levels were reported at the highest dose but these were considered secondary to emaciation.

Dose-related significant decreases in T3 and T4 were observed from 400 ppm in males and at 6400 ppm in females. A decrease in TSH was seen in both sexes at 6400 ppm but this was not statistically significant (see Table 1 below). No effect was observed on thyroid weight or thyroid tissue. No histological effect was observed on the pituitary-thyroid-endocrine axis.

Table 1 - T3, T4 and TSH levels at day 23 in the 28-day dog study.

	0 ppm	100 ppm	400 ppm	1600 ppm	6400 ppm
Males					
T4	1.5	1.5	0.9*	0.6*	0.3*
T3	0.9	0.9	0.7	0.6	0.4
TSH	0.24	0.10	0.13	0.12	0.06
Females					
T4	1.9	1.7	1.0	1.4	0.3*
T3	1.0	0.8	0.9	1.0	0.4*
TSH	0.24	0.13	0.22	0.29	0.02

*Statistically significant (p-level not known)

Thymus weights were markedly reduced at 6400 ppm in males (-53% and -32% of absolute and relative weight, respectively) and in females (-83% and -76% of absolute and relative weight, respectively). No histological lesions were observed and there was no evidence of lymphocytic changes in any of the other lymphocytic organs. It could potentially be attributed to a non-specific stress response stemming from the dogs' general state.
One male at the highest dose showed atrophy of the parotid salivary gland and sexual immaturity. Based on the very small number of animals investigated in this study, the significance of this finding is unknown.

<u>90-day studies (to be compared with the classification criteria range of 10-100 mg/kg bw/day for STOT RE 2 under the CLP Regulation, and 5-50 mg/kg bw/day for Xn; R48 under DSD)</u>

In a 90-day study, Wistar rats were exposed through diet to 150, 600, 2500 or 10000 ppm Spirotetramat daily (corresponding to 9/11, 36/46, 148/188 or 616/752 mg/kg bw/day in males/females, respectively). In order to determine the reversibility of possible effects, additional recovery groups (0 and 10000 ppm) with an equal number of rats were observed for additional 4 weeks without treatment. Treatment-related effects were only reported at the highest dose of 10000 ppm. A reduction of 8.4% was noted in the body weight of males at the end of treatment.

A decrease in kidney weight was noted in males (-11%) and females (-6%) and was comparable to controls after 4 weeks of recovery. No histological findings were reported in the kidney. A decrease in testis weight was noted in males (-6.5%). Histopathological findings in the testis (tubular degeneration and vacuolisation) and in the epididymides (abnormal spermatozoa, hypospermia), were reported at this high dose in several animals. Only one recovery-group male showed tubular degeneration in the testes at the end of the 4-week recovery period, suggesting that the lesions were reversible. An increased incidence of minimal to slight accumulation of alveolar macrophages was noted in the lung of both males and females.

It is also noted that information on subchronic toxicity in rats by the oral route is provided in a 2-generation study (Young, 2006). In this study decreases in absolute and/or relative kidney weights and minimal to moderate tubular dilatation in kidneys were reported in males and females at the dose of 6000 ppm (corresponding to 487 mg/kg bw/day in premating males, and 539/435/931 mg/kg bw/day in premating/pregnant/lactating females). Effects on reproductive organs and function are detailed in the fertility part of the opinion.

In a 90-day study, CD-1 mice were exposed through diet to 70, 350, 1700 or 7000 ppm Spirotetramat daily (corresponding to 13/16, 60/72, 300/389 or 1305/1515 mg/kg bw/day in males/females, respectively). No effect was observed.

In a 90-day study, Beagle dogs were exposed through diet to 150, 300, 1200 or 4000 ppm Spirotetramat daily. This corresponds to 5/6, 9/10, 33/32 or 81/71 mg/kg bw/day in males/females, respectively. Due to a reduction of body weight in males and females during the two first weeks at 4000 ppm, the dose was decreased to 2500 ppm. While males recovered, females still had 17% lower body weights compared to controls at termination of the study. This was accompanied by a decrease in food consumption in females throughout the study (magnitude not known). At the high dose in females, reductions in mean values for red blood cell (RBC) count, haemoglobin concentration and haematocrit were seen from day 58 and gained statistical significance at day 86 (respectively -17, 17 and 16% of control values).

There was a treatment-related decrease in T4 in the 1200 and 2500 ppm males and females (see table 2). A slight decrease in T3 was observed at days 30 and 58 in males and females of the 2500 ppm group but not on day 86. No change in thyroid weight, thyroid pathology or TSH levels was observed.

Table 2 - T3, T4 and TSH levels at day 86 in the 90-day dog study.

	0 ppm	150 ppm	300 ppm	1200 ppm	2500 ppm
Males					
T4	1.85±0.51	1.70±0.68	1.42±0.30	0.75±0.25*	0.45±0.38*
T3	0.97±0.19	0.98±0.21	0.97±0.11	0.90±0.12	0.79±0.15
TSH	0.07±0.03	0.06±0.02	0.07±0.05	0.13±0.10	0.06±0.04
Females					
T4	2.75±1.08	1.29±0.38*	1.50±0.60	1.34±0.71*	1.00±0.16*
T3	1.03±0.18	0.75±0.13	0.91±0.14	0.86±0.19*	0.72±0.04
TSH	0.08±0.06	0.08±0.09	0.11±0.09	0.05±0.01	0.24±0.37

*Statistically significant (p-level not known)

The only compound-related histological change was thymus atrophy in one female at

2500 ppm.

1-year studies (to be compared with the classification criteria range of 2.5-25 mg/kg bw/day for STOT RE 2 under the CLP Regulation, and 1.25-12.5 mg/kg bw/day for Xn; R48 under DSD)

In a 1-year study, Wistar rats were exposed through diet to 250, 3500 or 7500 ppm Spirotetramat daily in males and 12000 ppm in females (corresponding to 13/18, 189/255 or 414/890 mg/kg bw/day in males/females, respectively). Treatment-related effects were reported only at the mid and top doses. A reduction of 6.6% was noted in the body weights of females at the end of treatment at the highest dose. An increase in relative kidney weight was noted in high dose females (+8%). No histological findings were reported in the kidneys. An increase in relative liver weight was noted in high dose males (+9%) and females (+15%). No histological findings were reported in the liver.
No effect was observed on testis or epididymides weight but histopathological findings were reported in some high dose males in the testis (abnormal spermatozoa) and in the epididymides (exfoliated germ cells/debris). An increased incidence of minimal to slight accumulation of alveolar macrophages was noted in the lung of high dose females and males at 3500 and 7500 ppm.

In a 1-year study, Beagle dogs were exposed through diet to 200, 600 or 1800 ppm Spirotetramat daily, corresponding to 6/5, 20/19 or 55/48 mg/kg bw/day in males/females, respectively.
No effect was observed on body weight or haematology parameters.
There was a treatment-related decrease in T4 in the 600 and 1800 ppm males and females (see table 3). No change in TSH levels or in thyroid weight was observed. At histology, slight reduction in size of peripheral thyroid follicles was reported in 2 of the 4 high dose males.

Table 3 - T3, T4 and TSH levels at day 357 in the 1-year dog study.

	0 ppm	200 ppm	600 ppm	1800 ppm
Males				
T4	1.85±0.65	1.16±0.25	1.02±0.12*	0.58±0.14*
T3	0.65±0.09	0.57±0.05	0.67±0.09	0.46±0.02*
TSH	0.16±0.13	0.18±0.17	0.11±0.09	0.06±0.05
Females				
T4	2.88±0.53	1.79±0.52*[a]	1.11±0.27*	0.85±0.23*
T3	0.73±0.09	0.62±0.06	0.63±0.07	0.61±0.11
TSH	0.14±0.08	0.10±0.08	0.08±0.05	0.06±0.03

*statistically significant ($p<0.05$)
[a]not considered to be treatment-related as control value was unexpectedly high

Reduced thymus size was observed in males at 600 ppm and 1800 ppm, but absolute and relative thymus weights were low (not statistically) in males only at 600 ppm. Thymus involution graded as mild were reported in 1 male at 600 ppm and involution graded as moderate was reported in 1 male at 1800 ppm.
Some effects were reported in the brain. At gross necropsy, dilated brain was noted in males and females at 600 ppm and in males only at 1800 ppm. At histopathology, brain dilation was seen at 600 ppm in 1 male (mild) and 1 female (moderate) as well as at 1800 ppm in 1 male (moderate). Mild axonal degeneration was also detected in 1 female at 1800 ppm.

2-year studies (to be compared with the classification criteria range of 1.25-12.5 mg/kg bw/day for STOT RE 2 under the CLP Regulation, and 0.65-6.50 mg/kg bw/day for Xn; R48 under DSD)

In a 2-year study, Wistar rats (n=55/sex/group) were exposed through diet to 250, 3500 or 7500 ppm Spirotetramat daily in males and 12000 ppm in females (corresponding to 12/17, 169/229 or 373/823 mg/kg bw/day in males/females, respectively). A reduction in the body weight of 14% in females and 10% in males was noted at the end of treatment at the respective highest dose.

Absolute kidney weights were decreased in both sexes at 3500 and 7500/12000 ppm but relative kidney weights were decreased only in females at 3500 ppm. A dose-related increase in incidence of animals with kidney tubular dilatation was noted from 3500 ppm in males and females (see table 4). An increase in relative liver weight was noted in high dose females (+12%). No histological findings were reported in the liver. In the bile duct, a non- statistical increased incidence of hyperplasia/fibrosis with associated minimal periportal mononuclear cell infiltrate was noted in 12000-ppm females. No effect was observed on testis weight. There was a high background incidence of typical aging-related changes including testicular degeneration of variable severity but in the 7500-ppm males, there were a few animals with more subtle changes characterised by depletion, asynchrony and degeneration of the latter stage spermatids. An increased incidence of immature/exfoliated germ cells/debris was observed in the lumen contents of the head, body and tail of the epididymides at 7500 ppm. An increase in relative lung weight was noted in high dose males (+19%) and females (+14%). An increased incidence of accumulation of alveolar macrophages was noted in the lung of males at 250 and 3500 ppm, but not at the highest dose (see table 4). Interstitial pneumonia was also reported in high dose males as well as from 250 ppm in females, with a dose-related increase in incidence and/or severity. It is noted that the alveolar macrophages and interstitial pneumonia did not occur in the same animals in female control, low and mid dose groups.

Table 4 –Incidence and severity (in parenthesis) of histopathology findings in the lung in the 2-year rat study.

	0 ppm	250 ppm	3500 ppm	7500/12000 ppm
Males				
Lung, alveolar macrophages	7 (1.3)	17 (1.2)*	17 (1.4)*	3 (1.0)
Lung, interstitial pneumonia	22 (1.6)	15 (1.7)	19 (1.7)	44 (2.3)*
Combined	29	32	36	47*
Females				
Lung, alveolar macrophages	20 (1.3)	14 (1.1)	18 (1.1)	3 (1.7)
Lung, interstitial pneumonia	4 (1.5)	13 (1.4)*	14 (2.1)*	52 (3.4)*
Combined	24	27	32	55*

*Statistically significant ($p<0.05$)

In an 18-month study, CD-1 mice were exposed through diet up to 7000 ppm

(corresponding to 1022/1319 mg/kg bw/day in males/females, respectively). No effects were reported except for two lipomatous neoplasms in high dose males (see the Carcinogenicity section).

Repeated dose toxicity - dermal route:

28-day studies (to be compared with the classification criteria range of 60-600 mg/kg bw/day for category 2 under CLP and 30-300 mg/kg bw/day for Xn; R48 under DSD)

No treatment-related findings were reported in a rat 28-day study up to 1000 mg/kg bw/day.

Assessment and comparison with the classification criteria

By the oral route, no toxicity was observed in mice after exposure to Spirotetramat for 28 days up to 1415 mg/kg bw/day, for 90 days up to 1305/1515 mg/kg bw/day or for 18 months up to 1022/1319 mg/kg bw/day.

In rats, the main target organs were the testis and epididymides, lung, kidney and liver. All effects were, however, observed at doses exceeding the criteria for classification, except for effects reported in the lung in the two-year study. In this latter study, an increased incidence in accumulation of alveolar macrophages was observed from 250 ppm in males. It is noted that this effect was not observed in males at the highest dose and no increase was noted in females at any dose. However, an increased incidence in interstitial pneumonia was reported in the high dose males and in the females from 250 ppm with a dose-related increase in incidence and severity; the findings reflected an inflammatory response in the lung, occurring with a dose-related increase in incidence in both males and females. Besides, an increased incidence of accumulation of macrophages was also reported in the 90-day and the 1-year study, therefore its relationship to treatment is not in doubt. It is however noted that:

- 250 ppm (corresponding to 12 mg/kg bw/day in males and 17 mg/kg bw/day in females) is at the upper limit dose of 12.5 mg/kg bw/day relevant for classification under CLP and exceeds the limit dose of 6.5 mg/kg bw/day relevant for classification under DSD;
- the severity of both findings at 250 ppm was not marked (severity of 1.2 and 1.1 for accumulation of macrophages and 1.7 and 1.4 for interstitial pneumonia, in males and females, respectively) and was similar to that seen in the controls. It is therefore considered that it does not provide clear evidence of marked organ dysfunction at this dose.

On this basis, RAC considers that the effect of Spirotetramat on the lung in the rat does not justify classification for repeated dose toxicity.

The dog is the most sensitive species tested, with the main effects observed on thyroid hormone T4 (and in some studies on T3) and on the thymus, these being consistently reported in a 28-day, a 90-day and a 1-year study. The following effects were observed at doses relevant for classification according to CLP:

- A marked effect on body weight in males (-33%) and females (-29%) was observed at the high dose (104/127 mg/kg bw/day, respectively) in the 28-day study. A 17% decrease in body weight was also observed in females at 2500 ppm

(72 mg/kg bw/day) but not in males in the 90-day study. Such effects were not found at doses relevant for classification in the 1-year study. In all cases, decreases in body weights were accompanied by decreases in food consumption. No clinical signs indicating a poor general health status were observed (only some animals were reported as thin). Body weight reduction may therefore be secondary to palatability problems due to the presence of high levels of the substance in the diet and may well not be an indication of morbidity associated with toxicity of the substance. It is not therefore considered that classification is justified.

- A decrease of 17% in the RBC count, 17% in the haemoglobin concentration and 16% in haematocrit in high dose females (2500 ppm or 71 mg/kg bw/day) in the 90-day study was observed. No effect on haematology parameters were however reported in the two other dog studies making the interpretation of this finding uncertain and it was not considered sufficient to justify classification.
- In the 1-year dog study, significant decreases in T4 levels were reported in males and females from 600 ppm (corresponding to 20 and 19 mg/kg bw/day, respectively). A reduction in T4 and as well as sometimes in T3 was identified in studies of shorter durations at high doses. It was not associated with effect on thyroid weight in any study. Histological findings were restricted to two out of four males with reductions in the size of the peripheral thyroid follicles at the high dose of 1800 ppm (48 mg/kg bw/day), which were above classification cut-offs, in the 1-year study. No functional, morphological or histological effect related to the perturbation in T4 levels was identified at a dose relevant for classification.
- In the 1-year study, males of the 600 ppm group (20 mg/kg bw/day) had reduced thymus size, non-significantly lowered thymus weight and 1 animal (out of 4) with thymus involution graded as mild. Considering the low incidence and severity of this lesion it is not considered sufficient to indicate a significant toxic effect triggering classification.
- In the 1-year study, gross necropsy revealed dilated brain at 600 ppm in males and females, which was confirmed following histopathological examination of brain dilatation in one male (mild) and one female (moderate). It was however not accompanied by any clear histopathological alterations. Such an effect was not reported in females at the high dose or in studies of shorter duration. In addition, brain ventricular dilatation is occasionally reported to occur spontaneously in the strain of dogs used in the test and this finding is considered to be of unclear toxicological significance.

RAC therefore agreed with the DS that classification is not justified for repeated toxicity under neither CLP nor DSD.

4.9 Germ cell mutagenicity (Mutagenicity)

The mutagenicity of spirotetramat has been adequately investigated *in vitro* and *in vivo*.

Table 17: Summary table of relevant in vitro and in vivo mutagenicity studies

Type of Study	Test system	Dose levels	Results	Reference
In vitro studies				
Salmonella/microsome test	*Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537)	0 - 5000 µg/plate	Negative	Herbold, 2006

Salmonella/microsome test	*Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537)	0 - 5000 µg/plate	Negative	Herbold, 2002
In vitro Chromosome aberration test	Chinese hamster V79 cells	0 - 750 µg/ml	Weakly positive at cytotoxic concentrations only.	Herbold, 2002
Mammalian cell gene mutation	Chinese hamster V79 cells	0 - 140 µg/ml	Negative	Herbold, 2002
In vivo studies				
Mouse Micronucleus Test	bone marrow of male mice	500mg/kg	Negative	Herbold, 2002
Chromosomal aberration assay in mice	bone marrow of male mice	500mg/kg	Negative	Herbold, 2003
Unscheduled DNA synthesis – rat liver	liver of male rats	2000 mg/kg	Negative	Brendler-Schwaab, 2003

4.9.1 Non-human information

4.9.1.1 In vitro data

Assays for point mutations and chromosomal aberrations in vitro were negative for spirotetramat. A weak positive finding was noted in a single in vitro chromosomal aberration test, but at cytotoxic concentrations only. Negative results were observed in one in vitro unscheduled DNA synthesis assay using rat hepatocytes also.

4.9.1.2 In vivo data

Assays for point mutations and chromosomal aberrations in vivo were negative for spirotetramat. Negative findings in two in vivo chromosomal aberration studies and one in vivo unscheduled DNA synthesis assay using rat hepatocytes do not suggest a genotoxic concern for spirotetramat.

4.9.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

Effects observed in the *in vitro* and *in vivo* mutagenicity studies do not trigger the criteria for classification and labelling for mutagenicity.

4.9.5 Comparison with criteria

Effects observed in the *in vitro* and *in vivo* mutagenicity studies do not trigger the criteria for classification and labelling for mutagenicity.

4.9.6 Conclusions on classification and labelling

There is no evidence of a genotoxic potential of spirotetramat, therefore, no classification is proposed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

In vitro assays for point mutations and chromosomal aberrations were negative in the presence of Spirotetramat. A weak positive finding was noted in a single *in vitro* chromosomal aberration test, but at cytotoxic concentrations only. Negative results were observed in one *in vitro* unscheduled DNA synthesis assay also using rat hepatocytes.

In vivo assays for point mutations and chromosomal aberrations were negative in the presence of Spirotetramat. Negative findings in two *in vivo* chromosomal aberration studies and one *in vivo* unscheduled DNA synthesis assay using rat hepatocytes do not suggest a genotoxic concern for Spirotetramat and no classification is proposed by DS.

Table 5 – Summary of mutagenicity tests

Type of Study	Test system	Dose	Results	Reference
In vitro				
Salmonella/microsome test	*Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537)	0 - 5000 µg/plate	Negative	Herbold, 2006
Salmonella/microsome test	*Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537)	0 - 5000 µg/plate	Negative	Herbold, 2002
In vitro Chromosome aberration test	Chinese hamster V79 cells	0 - 750 µg/ml	Weakly positive at cytotoxic concentrations only.	Herbold, 2002
Mammalian cell gene mutation	Chinese hamster V79 cells	0 - 140 µg/ml	Negative	Herbold, 2002
In vivo				

Mouse Micronucleus Test	bone marrow of male mice	500mg/kg	Negative	Herbold, 2002
Chromosomal aberration assay in mice	bone marrow of male mice	500mg/kg	Negative	Herbold, 2003
Unscheduled DNA synthesis – rat liver	liver of male rats	2000 mg/kg	Negative	Brendler-Schwaab, 2003

Comments received during public consultation

One MSCA agreed with the no classification being proposed for mutagenicity without further comments.

Assessment and comparison with the classification criteria

Based on the three negative *in vivo* studies (micronucleus test, chromosomal aberration test and UDS test) Spirotetramat is considered to be non-mutagenic *in vivo*.

RAC therefore agreed that a classification for mutagenicity was not warranted.

4.10 Carcinogenicity

Table 18: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
BYI 08330: 2 Year Oral Cancerogenicity Study in the Rat (0, 250, 3500, 7500/12000 ppm [♂/♀])	NOAEL 250 ppm (12.5/16.8 mg/kg bw/day [♂/♀])	♂/♀: absolute kidney weight ↓, tubular dilation ♂/♀: interstitial pneumonia ♂: alveolar macrophages in lungs ♀: discoloration of lungs ♀:-yellow/brown staining of perigenital area and tail	Wahle, 2006
BYI 08330: 18 Months Oral Cancerogenicity Study in the Mouse (0, 70, 1700, 7000/6000 ppm [♂/♀])	NOAEL 7000/6000 ppm (1022/1319 mg/kg bw/day [♂/♀])	No toxicologically significant changes at the highest dose tested	Wahle, 2006

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

In an **Oncogenicity Testing Study in the Rat** (Wistar Hanover rats (Crl:WI[Glx/BRL/Han]IGS BR), dose levels of 0, 250, 3500, 7500/12000 ppm [♂/♀] were chosen *(Wahle, 2006)*.

Study design:

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg; x±Std)	Main Study 24 months	
			Male	Female
Control	0	0	55	55
Low (LDT)	250	males 12.5 ± 4.4 females 16.8 ± 3.9	55	55
Mid (MDT)	3,500	males 169 + 60 females 229 + 53	55	55
High (HDT)	males 7,500 females 12,000	males 373 ± 134 females 823 ± 157	55	55

Gross necropsy showed an increased incidence of focal to multifocal (1-4 mm) discoloured zones (sometimes raised) of the lung in 12000 ppm females which were attributed to compound administration.

Relative lung weight was slightly increased in the 7500 ppm male and the 12000 ppm female group. The lung weight changes in 12000 ppm females correlated with increased focal to multifocal discolorations observed in the lungs at necropsy. Absolute kidney weights were decreased in both sexes at 3500 and 7500/12000 ppm and were associated with microscopic findings noted in the same groups.

Histopathology:
Non-neoplastic changes: Statistically significant changes in the incidence of non-neoplastic lesions were detected in the lung, kidneys, testis/epididymis and liver.
Lung: An increased incidence of alveolar macrophage accumulation, a common background lesion in the rat, and a complex of changes described as interstitial pneumonia was noted in the lungs of 7500-ppm males and 12000 ppm females. Both findings were attributable to exposure to the test substance and were interpreted as a continuum of morphologic changes (correlating with the gross lung observations noted in 12000 ppm females) and were evaluated together. Macrophage accumulation without additional change was coded as such. Interstitial pneumonia was diagnosed with the presence of one or more of the following changes: presence of lymphocytes, cholesterol clefts, interstitial thickening of the alveolar septa by connective tissue, or increased alveolar pneumocytes and presence of occasional extravasation of erythrocytes (micro hemorrhage). The lung effects described above were focal to multifocal in distribution and involved a small overall portion of the lung tissue triggering no respiratory symptoms.
Kidney: An increased incidence of tubular dilatation was observed in the outer medulla (presumed to be in the loop of Henley) in both sexes of the 3500 ppm groups and in the 7500/12,000 ppm groups. The affected renal tubules were dilated and were either empty or contained pale eosinophilic proteinacious material, usually granular. Negligible cellular death, regeneration, or inflammatory responses to the tubular dilatation was present. In addition, there were no associated tubular casts in more distal tubular locations such as the collecting ducts. This lesion in the 3500 and 7500/12000 ppm animals was considered treatment-related and was presumed to be reflected in the decreased kidney weights although there was no associated loss of tissue. It was also stated that the high background incidence of chronic nephropathy greatly influenced the variability of the kidney weights measured in this study which is commonly noted in 2-year rat studies.
Testis: In males, there was a background incidence of typical aging changes including variably severe testicular degeneration, oftentimes noted grossly as soft, brown, or reduced in size. In addition to this change in the 7500-ppm males, there were animals with generally slight morphologic testicular change, more subtle than distinct tubular degeneration, characterized by a depletion, asynchrony, and degeneration of the latter stage spermatids (0,0,0, and 9 for the control, low, mid and high dose groups, respectively).
Epididymis: An increased incidence of immature/exfoliated germ cells/debris was observed in the lumen contents of the head, body, and tail of the epididymides at 7500 ppm (6,10,6, and 31 for the control, low, mid and high dose groups, respectively), which correlates with the testicular change. There was no morphologic change noted in the anatomical structure of the epididymis tissue in conjunction with the luminal content of exfoliated immature/abnormal testicular stages.
Liver: In the bile duct, a nonstatistical increased incidence of hyperplasia/fibrosis with associated minimal periportal mononuclear cell infiltrate was noted in 12000 ppm females.

Neoplastic changes:
Type, incidence and organ distribution of all neoplastic lesions was not significantly different between treated and control rats.

In an **18 Months Oncogenicity Testing Study in the Mouse** (strain: CD-1; (CD-1 [ICR]/BR), concentrations of spirotetramat chosen were 0, 70, 1700 and 7000/6000 ppm [♂/♀]*(Wahle, 2006)*.

Study design:

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg; ±SD)	Main Study 18 months	
			Male	Female
Control	0	0	55	55
Low (LDT)	70	m: 10.9±1.4 f: 13.7±2.2	55	55
Mid (MDT)	1,700	m: 263±35 f: 331±49	55	55
High (HDT)	7,000 (1)	m: 1022±196 f: 1319±290	55	55

Clinical observations attributable to exposure to the test substance were not observed in either sex at any dose tested. The general observation profile was consistent with that of the aging mouse.
There were no treatment-related effects on body weight and food consumption in either sex at any dose tested. Evaluation of the general profile of absolute and relative organ weights as well as of gross lesions observed in this study did not indicate a BYI 08330-induced change in either sex at any dose tested.
Histopathology:
Non-neoplastic and Neoplastic changes:
Microscopic findings attributable to exposure to the test substance were not observed in this study.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.10.3 Other relevant information

No data available.

4.10.4 Summary and discussion of carcinogenicity

Carcinogenicity was tested in rats and mice following application of spirotetramat for two years or 18 months, respectively.
The chronic (24 months) NOAEL was established at 250 ppm (equal to 12.5 mg/kg bw/day) in male rats, based on decreased absolute kidney weights and an increased incidence of renal tubular dilatation at 3500 ppm (equal to 169 mg/kg bw/day). There was a slightly increased incidence of testicular tubular degeneration and immature/exfoliated germ cells in the epididymal lumen in 7500 ppm male rats after two years of treatment (equal to 373 mg/kg bw/day).
The chronic (24 months) NOAEL in females was established at 250 ppm (equal to 16.8 mg/kg bw/day), based on decreased absolute kidney weights and an increased incidence of renal tubular dilatation at 3500 ppm (equal to 229 mg/kg bw/day).
Based on type, incidence and organ distribution of neoplastic lesions in treated and control rats, there was no indication for an oncogenic effect of spirotetramat.
The NOAEL of the chronic feeding study in mice was established at 7000/6000 ppm (equal to 1022 or 1319 mg/kg bw/day in males or females, respectively), based on the absence of compound-induced toxicological responses.
Based on type, incidence and organ distribution of neoplastic lesions in treated and control mice, there was no indication for an oncogenic effect of spirotetramat.

4.10.5 Comparison with criteria

No oncogenic effects were observed in studies conducted with spirotetramat, neither in rat nor in mouse carcinogenicity studies (according to both DSD and CLP).

4.10.6 Conclusions on classification and labelling

Spirotetramat can be regarded to have no oncogenic potential.

RAC evaluation of carcinogenicity
Summary of the Dossier submitter's proposal Carcinogenicity was tested in rats and mice following application of Spirotetramat for two years or 18 months, respectively. Based on type, incidence and organ distribution of neoplastic lesions in treated and control rats and mice, there was no indication for an oncogenic effect of Spirotetramat in either of the species tested. The DS concluded that no oncogenic effect was observed in studies conducted with Spirotetramat, neither in rat nor in mouse carcinogenicity studies and proposed no classification for carcinogenicity (according to both CLP and DSD). **Comments received during public consultation** One MSCA agreed with the no classification being proposed for carcinogenicity without further comments. **Additional key elements**

No carcinogenic effect was observed in the rat 2-year study.

In the mouse 18-month study, two cases of tumours arising from brown fat were noted in high-dose males (DAR, 2008). Microspically the tumours were determined to be benign and were described with the term "lipomatous neoplasm". In both cases, the tumours were noted in gross macroscopic observations (1.5 to 2.0 cm in size) and were located in the interscapular region. Both animals lived until study termination. Similar tumours were not observed in females. The report of this tumour is rare.

It was suggested in the DAR that these tumours may be linked to the use of implantable identification transponders (ID chips), which were placed on the animals of all groups (scapula, subcutaneously) prior to study. Whether there is a direct role of the chips in the formation of the tumours was not documented in the study according to the DAR. In the literature, as pointed out by the DS, some studies report that implantable responders (microchips) have been associated with the formation of various lesions and tumours in rodents (Rao Ghanta & Edmondson, 1990; Elcock *et al*., 2001). All were of mesenchymal origin (malignant schwannoma, fibrosarcoma, anaplastic sarcoma and histiocytic sarcoma) and contained embedded microchip devices.

It is noted that lipomatous neoplasms were not reported in these studies but given the profile of induction of rare and isolated tumours of mesenchymal origin observed in rat, it is not excluded that lipomatous neoplasm may be linked to a foreign-body induced reaction. In the absence of any information on a potential direct involvement of ID chips in the Spirotetramat study, such a link is however not demonstrated.

It is noted that the insecticidal mode of action of Spirotetramat involves inhibition of lipid biosynthesis. However, in rats, mice and dogs, no impact on plasma lipid parameters was observed and hence a link between the insecticidal mode of action and the induction of lipomatous neoplasms in mice has not been shown. It is also noted that toxicokinetic data provides no indication of any accumulation of Spirotetramat in fat.

Overall, considering that they consist only of benign tumours, that they are found only in males, only at the high dose and with a low incidence (4.5%), RAC considers that the link between these tumours and treatment is uncertain and does not provide clear evidence of a carcinogenic effect.

Assessment and comparison with the classification criteria

RAC agreed that:

- no carcinogenic effect was observed in a rat 2-year study;
- the unclear evidence of carcinogenicity provided in the mouse 18-month study based on the observation of two lipomatous tumours is not sufficient to justify a classification for carcinogenicity.
-

RAC agreed with the DS that Spirotetramat should not be classified for carcinogenicity.

4.11 Toxicity for reproduction

Table 19: Summary table of relevant reproductive toxicity studies

Study; Reference	Dose levels	NOAEL	Relevant effects
Dose range-finding Multigeneration, rats Young A. D.; 2006	F_0: 0, 200, 500, 6000 and 10000 ppm /diet (equivalent to 0, 10, 28, 320 and 538 mg/kg bw (♂) and 0, 13, 31, 384 and 645 mg/kg bw (♀) F_1: 0, 13, 33, 400 and 667 mg/kg bw using a conversion factor of 15.	Parental: 500 ppm (28 mg/kg bw ♂, 31 mg/kg bw ♀) Reproductive/ Developmental: 500 ppm (33 mg/kg bw)	parental: -body weight ↓ -body weight gain ↓ reproductive: -abnormal sperm cells -spermmotility ↓ -spermprogression ↓ developmental: -body weight ↓ -body weight gain ↓
Multigeneration, rats Young A. D.; 2006	0, 250, 1000, 6000 ppm/diet (equivalent to 0, 17, 70 and 419 mg/kg bw (♂) and 0, 20, 82, 485 mg/kg bw (♀))	Parental/reproductive/ developmental: 1000 ppm (70 mg/kg bw ♂, 82 mg/kg bw ♀)	parental: -body weight ↓ -body weight gain ↓ -food consumption ↓ -kidney weight↓ -tubular dilation reproductive: -abnormal sperm cells -reproductive performance ↓ developmental: -body weight ↓ -body weight gain ↓
Developmental toxicity, rats Klaus A. M.; 2004	0, 20, 140, 1000 mg/kg bw	maternal/ developmental: 140 mg/kg bw	maternal: -food consumption ↓ -body weight loss developmental: -fetal weight ↓ -skeletal variations ↑ -retarded ossification ↑ -skeletal malformations ↑
Supplementary developmental toxicity, rats Klaus A. M.; 2004	0, 10, 35, 140 mg/kg bw	maternal/ developmental: 140 mg/kg bw	No toxicologically significant changes at the highest dose tested
Developmental toxicity, rabbits Klaus A. M.; 2004	0, 10, 40, 160 mg/kg bw	maternal: 10 mg/kg bw developmental: 160 mg/kg bw	maternal: -food consumption ↓ -body weight loss -abortion ↑ -clinical signs developmental: no effects

4.11.1 Effects on fertility

4.11.1.1 Non-human information

In a ***Dose range-finding reproductive toxicity study in the Wistar rat*** (strain: Wistar Hanover rats Crl:WI(Glx/BRL/Han)IGS BR), dose levels of 0, 200, 500, 6000 and 10000 ppm *(Young, 2006)* were chosen, equivalent to 0, 10, 28, 320 and 538 mg/kg bw (♂) and 0, 13, 31, 384 and 645 mg/kg bw (♀) in parental animals (P = F_0) . As this test was a preliminary dose finding study the actual feed consumption of the F_1 generation animals had not been measured. According to the Guideline for the preparation of pesticides residues (Geneva December 2002) a conversion factor of 15 should be used in multigeneration studies for calculating the substance intake when food consumption data are not available. Extrapolating to the intake of spirotetramat for the F_1 generation when using a conversion factor of 15, dose levels of 0, 200, 500, 6000 and 10000 ppm would be equivalent to 0, 13, 33, 400 and 667 mg/kg bw/d.

Study design:

Test Group	Dose in Diet (ppm)	Animals/group			
		P Males	P Females	F_1 Males Day 21/Days 59-64	F_1 Females Day 21/Days 37-42
Control	0 ppm	10	10	**14/17**	**11/15**
Low (LDT)	200 ppm	10	10	**12/13**	**13/13**
Mid 1 (MDT 1)	1,000 ppm	10	10	**16/16**	**15/16**
Mid 2 (MDT 2)	6,000 ppm	10	10	**12/15**	**16/18**
High (HDT)	10,000 ppm	10	10	**0/0**	**0/0**

Findings:

Parental toxicity:

General observations:

There were no treatment-related mortalities or clinical signs observed during the course of the study. Hair thinning was noted on three males at 10000 ppm. During premating, no effects were observed on body weight, body weight gain, or food consumption in parental animals up to 500 ppm. At 10000 ppm, decreased mean premating body weight gain was observed in male (10%) and female (12%) parental animals. Females showed nonsignificant body weight gain declines at 6000 ppm during premating (11%) and lactation (12%). Females of the 10000 ppm group were sacrificed after the gestation phase due to non-pregnancy.

Reproductive function:

In P generation males a significant decline in both motility and progression of sperm was noted in the 10000 ppm dose group as well as a slight decline in epididymal counts and a significant increase in abnormal sperm. The abnormal sperm observed in the morphological exam presented as amorphous sperm heads. These findings resulted in no pregnancies at this dietary level. Sperm analysis revealed no effects in P generation males at 6000 ppm and below.

In F_1 eight-to-nine week old interim males, slight declines in motility and progression of sperm was observed in the 6000 ppm dose group. Morphology showed an increase in abnormal sperm presenting as amorphous sperm heads. There were no sperm effects observed in F1 eight-to-nine week old interim animals at 500 ppm and below.

Reproductive performance:

The 10000 ppm dietary level was associated with no fertility in the P generation animals. There were no implantation sites noted in the females at this same level. At the 200, 500 and 6000-ppm dietary levels, there were no compound-related effects on any parameter (e.g., mating, fertility, or gestation indices, days to insemination, gestation length, or the median number of implants). The litter parameters determined at birth (litter size, percentage of males born, birth index, live birth index, viability index, lactation index), were also not affected.

Gross pathology, organ weights:

No treatment-related macroscopic changes were observed at necropsy in P generation rats. Absolute and relative weights of the cauda epididymis were decreased by 29% and 27%, respectively, in the 10000 ppm males. In the females of the 10000 ppm dose group, terminal body weight declines as well as declines in absolute and relative kidney and liver weights were observed.

Histopathology:

P generation males in the 10000 ppm group showed abnormal sperm in the epididymis (10/10) and cauda epididymis (9/9). The abnormal sperm consisted of what appears to be the retention of the residual body to the tail of the spermatozoa. Severity grade consisted of minimal to moderate change. There was no evidence of change in the epithelium lining the seminiferous tubules of the testes in 10000 ppm generation males. There were no abnormal sperm cells observed in P generation males at 6000 ppm and below.

Neonatal toxicity:

General observations:

No treatment-related clinical signs and no malformations were observed in F_1 pups. Pup weights at birth were not affected. At 6000 ppm, significant declines in offspring body weight (14%) were noted at lactation day 21 with a total decline in body weight gain of 22% and 15% for lactation days 14-21 and 4-21, respectively.

Gross pathology, organ weights:

In F_1 pups of 21 days of age, no treatment-related macroscopic alterations were observed and no significant differences in terminal body weights or absolute and relative organ weights were noted at any dietary level tested. In F_1 eight-to-nine week old males, a significant decline (8%) in terminal body weight was observed at 6000 ppm. There were no statistically significant effects observed on the organs weighed (testis, epididymis, cauda epididymis) at any dietary level tested.

Histopathology:

In F_1 weanlings the testis and epididymis of the control and 6000 ppm animals did not contain sperm due to the immaturity of the animals. The epithelium appeared normal.

F_1 eight-to-nine week old interim animals of the 6000 ppm dose group showed abnormal sperm in the epididymis (4/15) and cauda epididymis (4/14). Severity grade consisted of minimal or moderate change. A single control animal had a moderate number of abnormal sperm within the epididymis and cauda epididymis. There was no evidence of change in the epithelium lining the seminiferous tubules of the testes in F_1 eight-to-nine week old interim animals in the 6000 ppm group. There were no abnormal sperm cells observed in F_1 eight-to-nine week old interim animals at 500 ppm and below.

Sexual maturation:

There were no treatment-related findings observed in the F_1 generation on either preputial separation or vaginal patency at any dietary level tested.

Conclusion:

Systemic toxicity was limited to body weight gain decreases in the F_1-generation animals. The target organ

is the testes with progressive effects over successive generations: the F_1 males were affected at a lower dose than the P-generation males. Adverse effects on the testes (sperm) resulted in decreased reproductive performance (fertility).
The parental systemic toxicity LOAEL for spirotetramat in male and female Wistar rats is 6000 ppm (320.1 and 384.1 mg/kg bw/day in males and females, respectively) based on decreased body weight gain (P females) and terminal body weight (F_1 males). The parental systemic NOAEL is 500 ppm (27.8 and 31.4 mg/kg bw/day in males and females, respectively).
The reproductive toxicity LOAEL for spirotetramat is 6000 ppm (approximately 400 mg using a conversion factor of 15) based on decreased sperm motility and progression and increased abnormal sperm cells in the F_1 males. The reproductive toxicity NOAEL is 500 ppm (approximately 33 mg using a conversion factor of 15).
The offspring toxicity LOAEL for spirotetramat is 6000 ppm (approximately 400 mg using a conversion factor of 15) based on decreased body weight and body weight gain during lactation in F_1 pups. The offspring NOAEL is 500 ppm (approximately 33 mg using a conversion factor of 15).

In a ***Two generation reproductive toxicity study in the Wistar rat*** (strain: Wistar Hanover rats Crl:WI(Glx/BRL/Han)IGS BR), dose levels of 0, 250, 1000 and 6000 ppm *(Young, 2006)* were chosen, equivalent to 0, 17, 70 and 419 mg/kg bw (♂) and 0, 20, 82, 485 mg/kg bw (♀).

Study design:

Test Group	Dose in Diet[a] (ppm)	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0 ppm	30	30	30	30
Low (LDT)	250 ppm	30	30	30	30
Mid (MDT)	1,000 ppm	30	30	30	30
High (HDT)	6,000 ppm	30	30	30	30

Findings:

Parental toxicity:

General observations:

There were no treatment-related mortalities or clinical signs observed during the course of the study. Toxicologically significant effects on feed intake were determined at 6000 ppm in females during lactation. Body weight and/or-body weight gain was reduced at 6000 ppm in males and females.

Premating:

A decline in body weight gain (Wk 1-10) was noted in P-generation males (13%), but not females, of the 6000 ppm group. In the F_1-generation, the males of the 6000 ppm group exhibited statistically significant declines in body weight (mean of 6%) from days 14-70 and declines in overall body weight gain (8%). In F_1-generation females of the 6000 ppm group, statistically significant declines in body weight (6%, week 10 of premating) and declines in overall body weight gain (11%) were observed at the end of the premating phase.

Gestation:

A decline in body weight gain (8%) was noted in F_1-generation females of the 6000 ppm group which was accompanied by statistically significantly lower body weight means (7%) from day 0-20 of gestation.

Lactation:
P-generation females of the 6000 ppm group showed statistically significant declines in food consumption on both a g/kg/day (9-14%) and g/animal/day (13.4%) basis. In the F_1-generation, females of the 6000 ppm group exhibited statistically significant body weight declines (6-8%) throughout lactation, as well as significantly declined food consumption (lactation days 7-21) on both a g/kg/day (8-9%) and g/animal/day (13.9%) basis. Statistically significant declines in food consumption were also observed in F1-generation females of the 1000 ppm group (lactation days 14–21) and are not considered toxicologically significant in the absence of changes in body weight or body weight gain.

Gross pathology, organ weights:
No treatment-related macroscopic changes were observed at necropsy in P- and F_1-generation rats. Significant declines in terminal body weight were observed in both F_1 generation males and females of the 6000 ppm group. There were various organ weight changes which were statistically significantly different from controls (testes, epididymis, spleen, thyroid). Those findings were considered to be unrelated to treatment as most of those statistically different organ weights fell within their respective weight ranges from each of the study´s concurrent control groups, or were not correlated with dose, and/or were correlated with a lower terminal body weight with no effects on relative organ weight. Decreases in absolute and/or relative kidney weight at 6000 ppm in F_1 generation males and females were considered toxicologically significant in the presence of histopathology of the kidney.

Histopathology:
Histopathology findings considered to be compound-related were confined to the kidneys of the F_1-generation male and female rats of the 6000-ppm group and the epididymis of a single F_1 male from the 6000-ppm dose group. In F_1-generation male and female rats of the 6000 ppm group, a minimal to moderate multifocal tubular dilatation was observed in the outer portion of the medulla, containing occasional proteinaceous material. In general, both kidneys were affected with the severity and incidence being greater in males.

The epididymis of one single F_1-generation male rat had a moderate number of abnormal sperm within the tubules. Although microscopically there was only one animal with abnormal sperm, the sperm were similar in appearance (amorphous sperm heads) to abnormal sperm observed microscopically in the males of the 6000-ppm F_1 eight-to-nine week old interim animals and the 10000 ppm P males in the Dose Range-Finding Study *(Young, 2006)*.

No treatment-related findings were observed in the reproductive tract of females of either generation (ovaries, oviducts, uterus, cervix, vagina). Ovarian follicles were counted from control and 6000-ppm dose F_1-generation females. There was no difference in the number of primordial follicles, antral follicles, or corpora lutea in the 6000-ppm females, when compared to controls.

Reproductive function:
Estrus cycle staging did not indicate treatment-related effects on mean cycle length and number of normally cycling females in both P- and F_1-generation females at any dietary level tested.

In P-generation males, sperm motility (percent motile and percent progression) and epididymal sperm count were evaluated for the controls and all treatment levels. Morphology and testicular counts were evaluated for the controls and 6000 ppm dose group.

In F_1-generation males, sperm motility (percent motile and percent progression), total sperm count (epididymal and testicular), and morphology were evaluated at all dietary levels. A morphologic effect on sperm, presenting as amorphous sperm heads, was noted and is consistent with what was seen in the one-generation range finding study with spirotetramat *(Young, 2006)*. Variation in susceptibility to this finding was observed as 9/30 males exhibited a minimal effect (one to four amorphous sperm heads were noted out of 200 viewed) but only one outlying male was affected to the extent that it compromised fertilizing

capabilities for this one animal (i.e. the male presented 82% total abnormal sperm with 77% of these sperm presenting as amorphous heads, and the female found sperm positive from this male did not become pregnant). This same male showed abnormal sperm in the epididymis (see histopathology). There were no compound-related effects on sperm motility or total sperm count (epididymal or testicular) observed at any dietary level tested.

Reproductive performance:

Female reproductive performance was not affected for any parameter measured (e.g., mating, fertility or gestation indices, days to insemination, gestation length, or the median number of implants) in either generation at any dietary level tested. However, as stated above, the female found sperm positive from the high-dose male with abnormal sperm did not become pregnant. Comparison of the number of uterine implantation sites at necropsy with the number of delivered pups revealed the absence of any treatment-related effect on post-implantation loss in both generations.

The litter parameters determined at birth (pup weight at birth, total number of pups born, stillborn pups, live birth index, percentages of male pups born, mean litter size at birth) were not affected in both generations.

Neonatal toxicity:

General observations:

There were no treatment-related effects on the viability parameters of the pups (viability index, lactation index), and no clinical signs and no malformations were observed in both F_1 and F_2 pups.

Pup weights at birth were not affected (F_1 and F_2). On lactation day 21, the F_1 pup body weights for both sexes in the 6000 ppm group was significantly lower than control (8.2%) with females also significantly lower on day 14 (6%). The pup body weight gains for 6000-ppm males and females were statistically decreased (9%) during days 4-21 of lactation.

On lactation days 14 and 21, the F_2 pup body weights for both sexes in the 6000-ppm group were significantly less than control (7-8% and 9-11%, respectively). The pup body weight gains for 6000-ppm males and females were statistically decreased (12-14%) during days 4-21 of lactation.

Gross pathology, organ weights:

In F_1 and F_2 weanlings no treatment-related macroscopic alterations were observed at any dietary level tested. Absolute brain weights were statistically significantly decreased (F_2 males, F_2 combined sexes) and relative brain weights were increased (F_1 and F_2 males and females; F_1 and F_2 combined sexes) at 6000 ppm. At 6000 ppm, absolute spleen weights were decreased in combined F_1 weanlings and in F_2 male and female weanlings. F_2 males and females also had a decreased absolute thymus weight at 6000 ppm. The decreases in brain, spleen, and thymus weight were considered secondary to the lower mean body weights of the 6000 ppm pup dose group.

Histopathology:

There were no compound-related microscopic findings observed in F_1 and F_2 weanlings of either sex at any dietary level tested.

Maturation of external sexual organs (balanopreputial separation, vaginal opening) was not affected in F_1 weanlings. Anogenital distance was not triggered to be measured in this study.

Conclusion:

Systemic toxicity in parental animals was evident at 6000 ppm as decreases in body weight and body weight gain in P-generation males and F_1-generation males and females, kidney histopathology, and decreased kidney weight in the F_1 males and females. Reproductive performance was affected in one F_1 male that had an increase in abnormal sperm and reduced fertility at 6000 ppm. Offspring survival was not affected by treatment. However, F_1 and F_2 pup growth was decreased during lactation at 6000 ppm.

The parental systemic LOAEL for spirotetramat in male and female Wistar rats is 6000 ppm (419.3 and 484.7 mg/kg bw/day in males and females, respectively) based on decreases in body weight (F_1 males and females), weight gain (P males, F_1 males and females), and kidney histopathology and decreased kidney weights (F_1 males and females). The parental systemic NOAEL is 1000 ppm (70.7 and 82.5 mg/kg bw/day in males and females, respectively).
The reproductive toxicity LOAEL for spirotetramat in male Wistar rats is 6000 ppm (419.3 mg/kg bw/day in males) based on abnormal sperm cells and decreased fertility in the F_1 males. The reproductive toxicity NOAEL in males is 1000 ppm (70.7 mg/kg bw/day in males). The reproductive toxicity LOAEL was not observed in females; the reproductive NOAEL in females was 6000 ppm (484.7 mg/kg bw/day).
The offspring toxicity LOAEL for spirotetramat in male and female Wistar rats is 6000 ppm (419.3 and 484.7 mg/kg bw/day in males and females, respectively) based on decreased body weight and body weight gain during lactation in both F_1 and F_2 generations. The offspring NOAEL is 1000 ppm (70.7 and 82.5 mg/kg/ bw/day in males and females, respectively).

4.11.1.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

In a ***Developmental toxicity study in rats after oral administration*** *(Klaus, 2004)*, 25 animals of each group (strain: Wistar Rat, Hsd Cpb:WU) received dose levels of 0, 20, 140 and 1000 mg/kg bw (♀).

Findings:
Maternal Toxicity:
Mortality and clinical observations:
Mortality, appearance and behavior were not affected by treatment with spirotetramat.
Body weight:
Transient body weight loss occurred on GD 7 in dams treated at 1000 mg/kg bw/day, and final body weight and weight gain was significantly reduced during gestation by approximately 7% and 20%, respectively at 1000 mg/kg bw/day. Corrected body weight gain at 1000 mg/kg bw/day was reduced significantly by 29 % relative to controls also.
At the 140 mg/kg bw/day dose level body weight loss was not observed, but body weight gain during the treatment (GD 6-19, 14% decrease relative to controls, $p<0.05$) was reduced which resulted in statistically significantly reduced final body weights (5% decrease relative to control, $p<0.05$). These findings were possibly both related to incidentally lower litter size in the 140 mg/kg bw/day dose group and to incidental morbidity of one female (unrelated to treatment), as effects on carcass weight and corrected body weight gain were not evident in the 140 mg/kg bw/day dose group and statistical significance was no longer evident for final body weights when this female was excluded from calculations.
A supplementary oral developmental toxicity study with spirotetramat in Wistar rats *(Klaus, 2004)* at dose levels of 0, 10, 35 and 140 mg/kg bw/day revealed no effects on body weight development at dosages up to and including 140 mg/kg bw/day. Body weight gain during treatment (GD 6-19) (corrected body weight gain from GD 0-20 in parentheses), in grams, in that study was 82.5 (50.8), 85.0 (53.8), 82.5 (49.4), and 81.4 (52.7) in the 0, 10, 35, and 140 mg/kg bw/day dose groups, respectively. Maternal body weight was also

unaffected in the rat developmental toxicity range-finding study at a similar dose. Thus a treatment- related effect on body weight development was not assumed at the 140 mg/kg bw/day dose level in the main study. Necropsy revealed no treatment related findings at a dose level up to and including 1000 mg/kg bw/day.
Body weight and body weight gain were not affected at the 20 mg/kg bw/day dose level.
Food consumption:
Food consumption at 1000 mg/kg bw/day was significantly reduced by 19%, 10%, 9% and 11% relative to controls on gestation days 6–9, GD 9–12, GD 15–18 and GD 18–20, respectively. No treatment-related effect occurred at lower levels. Findings from the supplementary study *(Klaus, 2004)* confirmed no treatment-related effects on food consumption at doses of 10, 35, and 140 mg/kg bw/day.
Gross pathology:
No test-substance related gross pathological findings were ascertained in the females of the dosed groups at dose levels up to and including 1000 mg/kg bw/day.
Cesarean section data:
No effects occurred on gestation rate, postimplantation loss, number of fetuses, sex distribution, and placental weight at a dose up to and including 1000 mg/kg bw/day. A marginal reduction of placental weight (not statistically significant) together with a more distinct reduction of fetal weight (86% of control, $p<0.01$) was observed in the 1000 mg/kg bw/day dose group. The marginally lower fetal weight of the 140 mg/kg bw/day group was considered incidental and not related to treatment. Effects on fetal weight were not evident in the supplementary oral developmental toxicity study with spirotetramat *(Klaus, 2004)*. Here mean fetal weights in the 0, 10, 35 and 140 mg/kg bw/day dose groups were 3.60, 3.61, 3.57 and 3.60 grams, respectively, which renders a treatment related effect at the 140 mg/kg bw/day dose level in the present study unlikely. Effects on fetal weight were not evident at the 20 mg/kg bw/day dose level.
Preimplantation loss was slightly increased in the 140 mg/kg bw/day group, and thus mean number of implantations was marginally lower. However, preimplantation loss and mean number of fetuses available for evaluation lay in the range of historical control data. Furthermore, a treatment related effect was not evident in the supplementary study *(Klaus, 2004)* at dose levels of 10, 35, and 140 mg/kg bw/day. Thus, an impact on the outcome of the study due to unequal distribution of females in the different groups with respect to preimplantation loss and number of implantation sites was excluded.

Developmental Toxicity:

External examination:
Fetal external malformations included: cleft palate (1000 mg/kg bw/day: 1 case), microphthalmia (1 case each in the 0, 20, and 1000 mg/kg bw/day groups), anophthalmia (20 mg/kg bw/day: 1 case), and a combined finding of shortened upper jaw, macroglossia, domed head, dyplasia of limbs, skull and vertebral column (140 mg/kg bw/day: 1 case). Fetal external deviations were not observed in this study, at dose levels up to and including 1000 mg/kg bw/day.
Visceral examination:
Fetal visceral malformations included: co-arctation of aortic arch (1000 mg/kg bw/day: 1 case), atrial septal defect of the heart (1 case each in the 0, 20, and 1000 mg/kg bw/day groups), and absent lobe of the thyroid gland (0 mg/kg bw/day: 3 fetuses in 2 litters; 140 mg/kg bw/day: 1 case). The incidence and type of visceral deviations of fetuses was not affected up to and including 1000 mg/kg bw/day. Common visceral deviations representing the normal range of scattering for the rat strain used included findings at the eyes (slightly folded retina), brain (slight dilation of ventricles; dorsal brain slightly misshapen), cochlea (blood in cochlea), thyroid (reduction in size), arteries (left common carotid artery septated), liver (small additional liver lobe/s), kidneys and ureters (slightly increased renal pelvis cavitation, absence of renal papilla, hydroureter), testes (slightly displaced), bladder (slightly distended), genital tubercle (slightly elongated)

and hemorrhages in different structures.

Table 20: External, Visceral and Skeletal malformations

Observations	Dose (mg/kg bw/day)			
	0	20	140	1000
No. Fetuses(litters) examined	**247 (20)**	**301 (24)**	**253 (23)**	**270 (22)**
No. Fetuses(litters) affected with malformations	7 (4)	5 (4)	2 (2)	12 (9)
% Malformed fetuses (litters) per group	2.83 (20.0)	1.66 (16.7)	0.79 (8.7)	4.4 (40.9)
External malformations				
Cleft palate	0	0	0	1 (1)
Microphthalmia, unilateral	1 (1)	1 (1)	0	1 (1)
Anophthalmia, unilateral	1 (1)	1 (1)	0	0
Upper jaw shortened, macroglossia, domed head, dysplasia fore- and hindlimbs, skull and vertebral column	0	0	1 (1)	0
Visceral malformations				
Lobe of thyroid gland absent	3(2)	0	1 (1)	0
Atrial septal defect of the heart	1 (1)	1 (1)	0	1 (1)
Co-arctation of aortic arch between left carotid and left subclavian arteries, ascending aorta reduced in size, left subclavian artery arises from descending aorta	0	0	0	1 (1)
Skeletal malformations				
Dysplasia of forelimb bones	1 (1)	2 (2)	0 (0)	4 (4)
Altered appearance of sacral vertebral arch; pelvic shift	0	0	0	3 (3)
Supernumerary lumbar vertebra	0	0	0	1 (1)

Skeletal examination:

Table 20 includes fetal skeletal malformations. Skeletal evaluation revealed a slightly increased number of fetuses and litters with generally common unspecific malformations (4.4% and 40.9%, respectively) at the maternally toxic 1000 mg/kg bw/day dose level. Skeletal malformations included dysplastic forelimb bones (0 mg/kg bw/day: 1 case; 20 mg/kg bw/day: 2 fetuses in 2 litters; 1000 mg/kg bw/day: 4 fetuses in 4 litters), a supernumerary lumbar vertebra (1000 mg/kg bw/day: 1 case) and altered appearance of sacral vertebral arches and pelvic shift (1000 mg/kg bw/day: 3 fetuses in 3 litters).

Table 21 lists the skeletal deviations that were statistically significantly different on a fetal and/or litter basis. Statistically significantly retarded ossification in various locations (phalanges, sternebrae, vertebrae, skull bones) were noted at the 1000 mg/kg bw/day dose level in the osseus and/or cartilagineous parts. Increased incidences of skeletal variations (wavy ribs, 14th ribs, combined osseous and cartilagineous findings) were seen as well. All these findings can be ascribed to the observed growth retardation at this dose level and are considered to be an effect of treatment.

Retarded ossification was noted in the 140 mg/kg bw/day dose group and in the 20 mg/kg bw/day dose group on a fetal basis at single bones of the fore- and hindlimbs without clear dose relationship, together with incomplete ossification of the 6th sternal segment and the supraoccipital bone (at 20 mg/kg bw/day only). None of these findings were statistically significantly different on a litter basis and all findings were within the range of historical control values. The changes of the low- and mid-dose groups were poorly correlated with dose. The supplemental study *(Klaus, 2004)* revealed no relevant effects on the degree of ossification at dose levels of 10, 35 and 140 mg/kg bw/day. No statistically significant changes were noted in ossification means of all but two of the respective bones up to and including 140 mg/kg bw/day and this significance was only seen on a fetal basis. Based on the clarifying results of the supplementary study, a

treatment-related effect for retarded ossification in the present study at doses of 20 and 140 mg/kg bw/day is considered unlikely.
The number of individual ribs with wavy appearance and sum of wavy ribs was statistically significantly increased on a fetal basis in the 140 and 20 mg/kg bw/day dose group, but not on a litter basis. The fetal incidences at 20 and 140 mg/kg bw/day for this skeletal variant (17% and 18%, respectively) were outside the historical control incidence ranges of 2.7 – 15.1% (historical control range derived from 22 studies performed between 1996 and 2001). In the supplementary study *(Klaus, 2004)*, no indications for a treatment related increase of wavy ribs at dose levels up to 140 mg/kg bw/day was observed.

Table 21: Skeletal examinations

Observations	Dose (mg/kg bw/day)			
	0	20	140	1000
#Fetuses (litters) examined	127 (20)	159 (24)	135 (23)	146 (22)
#Fetuses (litters) affected	N/A[a]	N/A	N/A	N/A
Incomplete ossification				
Distal phalanx digits, right				
-1st	65 (19)[b]	112** (24)	89 (20)	133** (22)
-2nd	64 (19)	103* (24)	81 (20)	120** (22)
-3rd	20 (12)	45* (15)	29 (10)	78** (18)
-4th	39 (16)	77** (23)	61 (18)	111** (22)
-5th	108 (20)	149 (24)	124 (23)	142** (22)
Distal phalanx digits, left				
-1st	77 (19)	121* (24)	100 (23)	134** (22)
-2nd	74 (19)	112 (24)	90 (22)	126** (22)
-3rd	25 (13)	59** (18)	37 (12)	87** (20)
-4th	50 (18)	85 (23)	76* (20)	118** (22)
-5th	109 (20)	149 (24)	123 (23)	142** (22)
Distal phalanx toes, right				
-1st	57 (19)	101** (23)	87** (23)	111** (22)
-2nd	34 (14)	67* (22)	52 (16)	**95** (22*)**
-3rd	21 (9)	44 (18)	39 (12)	**84** (22**)**
-4th	45 (17)	75 (22)	75** (20)	101** (22)
Distal phalanx toes, left				
-1st	74 (19)	123** (24)	99* (23)	110** (22)
-2nd	41 (16)	75* (22)	62* (19)	93** (22)
-3rd	26 (12)	53 (18)	48* (16)	**82** (22**)**
-4th	45 (16)	84* (22)	75** (20)	101** (22)
Sternebra				
-2nd	28 (16)	35 (16)	29 (16)	97** (21)
-3rd	2 (2)	2 (2)	3 (2)	**14* (10*)**
-4th	2 (2)	5 (4)	4 (3)	**21** (12**)**
-5th	28 (14)	33 (17)	32 (17)	**85** (22*)**
-6th	4 (4)	21** (12)	21** (13)	**56** (16**)**
Thoracic vertebral body, 1st	7 (7)	5 (5)	8 (6)	30** (14)
Sacral vertebral arch(es), right				
-3rd	1 (1)	7 (3)	4 (2)	12* (7)
-4th	20 (11)	35 (14)	37 (18)	**77** (20*)**
Sacral vertebral arch(es), left				
-3rd	1 (1)	7 (3)	4 (2)	13** (8)
-4th	20 (11)	32 (12)	27 (11)	**80** (21**)**
Premaxillary bone, bilateral	0 (0)	1 (1)	0 (0)	8* (4)
Mandible, bilateral	1 (1)	0 (0)	0 (0)	11* (4)
Nasal bone, bilateral	4 (3)	9 (7)	2 (1)	**44** (14**)**
Frontal bone, bilateral	1 (1)	2 (2)	0 (0)	10* (7)
Parietal bone, bilateral	24 (11)	44 (18)	20 (10)	48* (18)
Interparietal bone	30 (13)	54 (20)	32 (16)	70** (20)
Supraoccipital bone	8 (5)	29** (10)	8 (6)	32** (13)
Temporal bone	5 (5)	7 (6)	8 (5)	**41** (18**)**
Zygomatic bone, bilateral	0 (0)	2 (2)	3 (2)	**20** (13**)**

No ossification				
Proximal phalanx digits, right				
-3rd	82 (19)	120 (23)	98 (21)	139** (22)
-4th	87 (19)	132* (24)	107 (22)	144** (22)
Proximal phalanx digits, left				
-3rd	92 (20)	129 (24)	107 (23)	143** (22)
-4th	98 (20)	138 (23)	110 (23)	144** (22)
5th Metacarpal, right	7 (6)	24* (11)	18 (9)	53** (15)
5th Metacarpal, left	10 (8)	27 (12)	21 (8)	61** (15)
Distal phalanx toes, right				
-1st	5 (5)	7 (4)	2 (2)	19* (7)
-2nd	5 (5)	11 (7)	5 (2)	22** (8)
-3rd	5 (5)	11 (7)	4 (2)	23** (8)
-4th	5 (5)	12 (7)	4 (2)	28** (9)
-5th	7 (6)	11 (7)	4 (2)	28** (10)
Distal phalanx toes, left				
-1st	3 (3)	8 (4)	4 (3)	16* (6)
-2nd	4 (4)	11 (7)	3 (2)	23** (9)
-3rd	3 (3)	11 (7)	3 (2)	24** (10)
-4th	5 (5)	12 (7)	4 (2)	29** (10)
-5th	5 (5)	11 (7)	7 (4)	29** (10)
Supraoccipital bone, round area	12 (11)	15 (10)	19 (12)	32* (19)
Wavy ribs, sum of fetal incidence	7 (6)	27** (10)	24** (12)	**84** (21**)**
14th rib, sum of fetal incidence	19 (10)	17 (12)	17 (11)	67** (19)
Thoracic vertebral body, dumbbell shaped				
-11th	28 (14)	21 (12)	19 (12)	69** (20)
-12th	32 (14)	27 (12)	21 (12)	75** (21)
-13th	15 (12)	**5* (5*)**	11 (9)	49** (19)
Fontanelle, slightly enlarged	11 (7)	30 (12)	8 (6)	34** (13)

[a] N/A, total fetal (litter) incidence was not available.

[b] Fetal (litter) incidence; values that are statistically significant on a fetal and litter basis are in **bold** type.

* Statistically different ($p < 0.05$) from the control.

**Statistically different ($p < 0.01$) from the control.

Table 22: Percentage of main skeletal observations (%) in comparison with historical controls

	Dose (mg/kg/d)				% of Historical control data
Observations	0	20	140	1000	
Dysplasia of forelimb bones (%)	0.4	0.7	0.0	1.5**	1.3 (26 out of 1975 animals) [1999 – 2004]
Altered appearance of sacral vertebral arch; pelvic shift (%)	0.0	0.0	0.0	1.1**	0.03 (2 out of 6554 animals) [1999 – 2006]

* $p<0.05$; **$P<0.01$ (Fisher's exact test)

Table 23: Percentage of o**bservations** at the ribs **in comparison with historical controls**

	Dose (mg/kg/d)				Historical control data (range)
Observations	0	20	140	1000	

Wavy ribs	5.5	17**	17.8**	57.5**	(2.7 – 15.1) [1996 – 2001]
14th rib	15	10.7	12.6	45.9**	(0.0 – 13) [1996 – 2000]

Conclusion:

Maternal toxicity:

Mortality, appearance, and behavior were not affected by treatment with spirotetramat at dose levels up to and including 1000 mg/kg bw/day. At 1000 mg/kg bw/day, body weight development was reduced compared to controls. Final body weight was decreased 7% relative to control; weight gain during treatment and throughout gestation was decreased by 20% and 19% relative to control, respectively; and corrected body weight gain was decreased 29% relative to control.

The oral maternal toxicity LOAEL for spirotetramat in rats is 1000 mg/kg bw/day, based on impaired food consumption, transient body weight loss, impaired body weight gain, and reduced final body weight. The maternal NOAEL is 140 mg/kg bw/day.

Developmental toxicity

Decreased food consumption and reduced fetal weight (86% of control) was observed in the 1000 mg/kg bw/day dose group. No effect on fetal weight was evident at doses of 140 mg/kg bw/day and below. At 1000 mg/kg bw/day, developmental variations included incomplete ossification of the distal phalanx digits and toes, 2nd through 5th sternebra, 1st thoracic vertebral body, 3rd and 4th sacral vertebral arches, and several skull bones (e.g., mandible, and premaxillary, nasal, frontal, parietal, interparietal, supraoccipital, temporal, and zygomatic bones). No ossification was evident at the 3rd and 4th proximal phalanx digits, 5th metacarpals, distal phalanx toes, and the round area of the supraoccipital bone. Further, the incidence of the sum of 14th rib deviations, dumbbell shaped 11th, 12th, and 13th thoracic vertebral bodies, and slightly enlarged fontanelle was greater in the 1000 mg/kg bw/day dose group. The total incidence of wavy ribs was increased compared to control values at all dose levels in the main study (20, 140, and 1000 mg/kg bw/day), but was not reproduced in the supplementary study at doses of 10, 35, and 140 mg/kg bw/day *(Klaus, 2004)*. Thus, toxicological relevance was assumed at the 1000 mg/kg bw/day dose level only.

The total incidence of fetal (litter) malformations was higher (not statistically significant) in the 1000 mg/kg bw/day dose group, including two single observations considered rare, cleft palate and co-arctation of the aortic arch.

The oral developmental toxicity LOAEL for spirotetramat in rats is 1000 mg/kg bw/day, based on reduced fetal weight, increased incidence of malformations, and increased incidence of skeletal deviations (retarded ossification of skull bones, fore- and hindlimbs, sternum and vertebral column; wavy ribs, 14th ribs, and combined osseous and cartilaginous findings). The developmental NOAEL is 140 mg/kg bw/day.

In a ***Supplementary Developmental toxicity study in rats after oral administration*** *(Klaus, 2004)*, animals (strain: Wistar Rat, Hsd Cpb:WU) received dose levels of 0, 10, 35 and 140 mg/kg bw (♀) of spirotetramat.

Findings:

Maternal Toxicity:

No treatment related maternal effects were observed at any dose level with respect to mortality, clinical signs, food consumption, body weight development, hematology and clinical chemistry parameters, liver weight, necropsy findings and histopathology of the liver at levels up to and including 140 mg/kg bw/day.

Developmental toxicity:

There were no treatment related effects at any dose level on reproductive parameters, (i.e. gestation rate, postimplantation loss, litter size, placental weight and appearance, fetal weight and fetal sex distribution). The incidence and type of fetal malformations were unaffected by treatment at levels up to and including

140 mg/kg bw/day. Meaningful fetal external or visceral deviations were not evident at a dose level up to and including 140 mg/kg bw/day in this study. Fetal skeletal ossification and incidence of skeletal variations including evaluation of cartilaginous structures revealed no evidence for treatment related effects at a dose level up to and including 140 mg/kg bw/day. The incidence of wavy ribs was not affected at a dose level up to and including 140 mg/kg bw/day. No evidence for a primary embryotoxic or teratogenic potential of spirotetramat was observed.

Conclusion:

The oral maternal toxicity LOAEL for in rats was not established at dose levels up to and including 140 mg/kg bw/day. The maternal and developmental toxicity NOAEL was 140 mg/kg bw/day, based on the absence of effects at 140 mg/kg bw/day.

In a ***Developmental toxicity study in rabbits after oral administration*** *(Klaus, 2004)*, animals (strain: Rabbit, Himalayan CHBB.HM) received dose levels of 0, 10, 40 and 160 mg/kg bw (♀).

Findings:

Maternal Toxicity:

Mortality and clinical observations:

Appearance, behavior and mortality were unaffected at the 10 mg/kg level, while abortion in relation to severe maternal toxicity was seen at the 40 mg/kg and 160 mg/kg dose levels. Death, sacrifice in moribund condition, cold ears, alopecia, altered fecal amount/appearance and reddish excretions occurred at the 160 mg/kg level. One female of the 160 mg/kg group was found dead after having shown reddish excretion and soft feces and 5 females had to be sacrificed in moribund condition after having shown severely reduced to no food consumption, severe body weight loss, cold ears, alopecia, reduced amount or no feces, diarrhea (in one female) and soft and light colored feces, reddish excretion, decreased water intake and urination and discolored urination most probably indicating concentration of urine. Abortion was observed most likely as a consequence of maternal toxicity in two other females of the 160 mg/kg group and in one female of the 40 mg/kg group showing clinical signs, impaired food consumption and body weight loss before abortion. The remaining females of the 160 mg/kg dose group revealed cold ears, alopecia and soft, mucoid and light colored feces.

Body weight:

Marked to severe body weight loss was observed in individual females of the 40 and 160 mg/kg bw/day dose groups which aborted or had to be sacrificed in moribund condition (body weight loss of -257 to -639 grams), while body weight changes in the remaining females did not show meaningful treatment related effects. Statistically significantly reduced body weight gain during treatment (GD 6-29) and the overall gestation period (GD 0-29) was noted in the 10 mg/kg bw/day dose group which resulted in a marginal body weight loss when corrected body weight gain was taken into consideration. Impaired body weight development at the 10 mg/kg bw/day dose level was regarded as an incidental finding and without relation to treatment due to lack of dose dependency. Mortality or early sacrifice at the 40 and 160 mg/kg bw/day dose levels did not contradict the assumed lack of dose dependency since only one female was sacrificed due to abortion in the 40 mg/kg bw/day dose group and a negative impact of mortality on the evaluation of body weight development could thus be excluded at a dose level up to and including 40 mg/kg bw/day.

Gross pathology:

Females of the 160 mg/kg group which had to be sacrificed in moribund condition revealed gross pathological findings of the intestine (fluid and/or gaseous contents in caecum), gall bladder (black or whitish mottled), and the liver (green discolored in the area of the gall bladder/light discoloration). Treatment relationship was assumed for these findings. Incidental findings among all other females included cysts at the oviduct (0 mg/kg: 3 females; 10 mg/kg: 2 females; 160 mg/kg: 3 females); whitish

punctiform areas in the caecum wall (10 mg/kg: 1 female) and gaseous contents in the rectum (40 mg/kg: 1 female).

Cesarean section data:

The gestation rate was decreased by one abortion in the 40 mg/kg dose group and two abortions in the 160 mg/kg group. No effects of treatment were noted in postimplantation loss in the remaining females, the number of fetuses as well as placental and fetal weight and appearance, and fetal sex distribution.

Developmental Toxicity:

External examination:

External malformations included malpositioned forelimbs (one of the most common malformations in the rabbit strain used and most likely due to restriction of fetal movement in the uterus) in all dose groups and single cases of domed head and cleft palate in the 160 mg/kg dose group. No external deviations were evident at doses up to and including 160 mg/kg.

Visceral examination:

Visceral malformations included ventricular septal defects of the heart (3 fetuses in 3 litters in the 10 mg/kg dose group) and single cases of microphthalmia and encephalomeningocele in the 160 mg/kg dose group. Visceral deviations included an increased incidence of fetuses (8 fetuses in 2 litters) with distinct liver lobulation in the 160 mg/kg group; statistical significance was evident on a fetal, but not litter, basis. The toxicological significance of this finding was questioned, since the liver is normally lobed and abnormal liver lobulation was not reported. Other visceral deviations noted (slight dilation of lateral brain ventricles and whitish discoloration of the liver) revealed no dose dependency.

Skeletal examination:

Skeletal malformations were observed in the cartilaginous parts of ribs/cervical ribs/supernumerary ossification centers, scattered among all dose groups. Fetal skeletal evaluations (retardations, variations) including cartilagineous tissues revealed no treatment related effects at a dose level up to and including 160 mg/kg bw/day. Single statistically significant differences were observed at the 10 mg/kg and 40 mg/kg levels, on a fetal basis (retarded ossification of the 5th sternebra in the 10 mg/kg dose group and of the 8th caudal vertebral arches in the 10 mg/kg and 40 mg/kg group). All values regarding retarded ossification at the 10 mg/kg and 40 mg/kg levels lay well within the normal range of scattering for these findings in developmental toxicity studies in the strain of rabbits used. A dose dependency was not evident for these findings and statistical significance was not seen when calculation was done on a litter basis.

Conclusion:

Maternal toxicity:

Appearance, behavior and mortality were unaffected at the 10 mg/kg level. Abortion was observed in the 40 mg/kg bw/day (1 dam) and 160 mg/kg bw/day (2 dams) groups; clinical signs, impaired food consumption and body weight loss were evident before abortion. Death (1 dam), sacrifice in moribund condition (5 dams), cold ears (23 dams), alopecia (13 dams), reddish excretions (4 dams) and altered feces occurred at the 160 mg/kg bw/day level. Females in moribund condition were reported to have severely reduced food consumption, severe body weight loss, cold ears, alopecia, reduced amount/altered appearance of feces, reddish excretion, decreased water intake and decreased/discolored urination. Gross pathology of these females revealed treatment-related findings of the intestine (fluid and/or gaseous contents in caecum), gall bladder (black or whitish mottled), and the liver (green discolored in the area of the gall bladder/light discoloration). Body weight changes in the remaining females did not show meaningful treatment related effects.

The oral maternal toxicity LOAEL for spirotetramat in rabbits is 40 mg/kg bw/day, based on abortion, clinical signs, impaired food and water consumption and body weight loss. The maternal NOAEL is 10 mg/kg bw/day.

Developmental Toxicity:

The total number of fetuses(litters) affected with malformations was 5(5), 8(7), 4(4) and 7(4) in the 0, 10, 40 and 160 mg/kg dose groups, respectively. Thus, malformations were not dose related and/or also were seen in the current control. External malformations included malpositioned forelimbs in 1(1), 3(2), 1(1) and 5(2) fetuses(litters) in the 0, 10, 40 and 160 mg/kg dose groups, respectively, and single cases of domed head and cleft palate in the 160 mg/kg dose group. Visceral malformations included ventricular septal defects of the heart in 3(3) fetuses(litters) in the 10 mg/kg group and single cases of microphthalmia and encephalomeningocele in the 160 mg/kg dose group. A treatment related effect was not assumed for the malformations which appeared only once in the 160 mg/kg dose group (domed head/ encephalomeningocele, cleft palate, and microphthalmia). Skeletal malformations were observed in the cartilaginous parts of ribs/cervical ribs/supernumerary ossification centers, scattered among all dose groups.

The oral developmental toxicity LOAEL for spirotetramat in rabbits was not established. The developmental NOAEL is 160 mg/kg bw/day.

4.11.2.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.11.3 Other relevant information

Mechanistic studies

Spirotetramat: Evaluation of the potential reproductive toxicity in the male rat following daily oral administration by gavage *(Kennel, 2005).*

Groups of 8 Wistar rats (Crl:WI[Glx/BRL/Han]IGS BR strain) were administered spirotetramat by gavage (0.5% methylcellulose vehicle) at a dose of 0 or 1000 mg/kg bw/day for 3, 10, 21, or 41 days.

Findings:

Cross pathological findings:

In treated animals, the absolute weight of the left testis and left epididymis were significantly decreased by 10-11% on day 3; the relative weight (% bw) of the left testis was significantly increased by 12% and the absolute weight of the left epididymis was significantly decreased by 11% on day 10. No significant organ weight changes were observed on day 21, but absolute right and left testes and epididymides were significantly decreased by 22-26% and relative weights were decreased by 11-15% on day 41. No treatment-related effect was observed on prostate weight. The decreased organ weights were due in part to the 12% decrease in terminal body weight in treated animals.

A small testis was observed in one rat treated for 3 days; otherwise, no treatment-related gross findings were observed until day 41. Gross findings on day 41 included small prostate in 2/7 animals, small and/or soft testes in 6/7 animals, and small epididymis in 7/7 animals compared with none in the control group. The small testis observed in one rat after treatment for three days is not considered treatment related. Two of eight rats had increased intraluminal aberrant cells in the epididymis after treatment for 3 but not after 10 days compared with 0/8 controls. No other treatment-related microscopic lesions were observed in the testis or epididymis on day 3 or day 10 or in the prostate at any sacrifice time. Treatment-related changes were observed in the testes (spermatid and Sertoli cell changes) and epididymides (aberrant spermatocytes) on days 21 and 41 days of treatment.

Histopathological findings:

After treatment for 21 days, marked degenerating elongating spermatids were observed in 8/8 treated animals, together with multinucleated giant spermatids in 2/8 rats and moderate to marked degenerating round spermatids in 4/8 rats. The investigators noted that these testicular findings are consistent with a treatment-related effect on round spermatids or late stage spermatocytes. At final sacrifice on day 41, slight to severe degenerating elongating spermatids were observed in 7/7 treated animals, resulting in the loss of elongating spermatids in 5/7 treated rats. Multinucleated giant spermatids and marked degenerating round spermatids were observed in 4/7 and 5/7 rats, respectively. The investigators noted that the microscopic findings in the testes at 41 days also are consistent with a treatment-related effect on round spermatids. Slight to moderate Sertoli cell vacuolation was observed in the testes of 6/7 rats on day 41. There was an increase of intraluminal abnormal aberrant cells in the epididymides of all treated animals after treatment for 21 and 41 days. This finding was slight to moderate on day 21 and had progressed to marked severity by 41 days. Oligospermia was not observed on day 21 but was observed in all treated rats on day 41. The investigators noted that the findings in the epididymides were a consequence of degenerating spermatids in the testis.

Sperm analysis:

Small statistically significant decreases (-13% to -15%) were observed in the absolute and relative (to cauda epididymis weight) sperm count on day 10 and the absolute sperm count on day 21. The absolute and relative sperm counts on 41 days of treatment were markedly decreased ($P<0.01$) by 77% and 68%, respectively, compared with that of controls.

Analysis of sperm morphology showed that the percent abnormal sperm was significantly increased in the treated group on day 21. The greater-than-twofold increase in abnormal sperm on day 21 is considered toxicologically significant and is likely related to the increased incidences in degenerating spermatid in the testis and aberrant cell types in the epididymis on day 21. The percent abnormal sperm in treated rats was markedly increased on day 41; 72% of the sperm were abnormal compared with only 3.8% in the control group.

Conclusion:

In male rats treated with spirotetramat at 1000 mg/kg for up to 41 days, weight loss was observed early in the study followed by decreased body weight gain for the remainder of the study resulting in a 12% decrease in body weight at the end of treatment. Food consumption was significantly reduced during the first week of treatment. The most prevalent clinical observations included soiling of fur along with increased salivation and wasting appearance in several rats. One treated rat died, but this animal was not necropsied to determine the cause of death; therefore, the death cannot be attributed to treatment with the test material.

Postmortem examination showed decreased testis and epididymis weight in animals sacrificed on day 41; these animals also had small testes and epididymides. Microscopic findings confirming an effect of spirotetramat treatment on the testes included degenerating round spermatids, degenerating elongating spermatid and multinucleated giant spermatids in the testes and increase of intraluminal aberrant cells in the epididymis after treatment for 21 days. The effect on the testes progressed to loss of elongating spermatids in the testes and an increase in severity of intraluminal aberrant cells and the presence of oligospermia in the epididymides after treatment for 10 days. Continued treatment with the test material for 41 days also had an effect on Sertoli cells.

Spirotetramat-enol: Investigation of the testicular/sperm toxicity in the rat following 21 days of exposure by gavage *(Tinwell, 2006)*

Groups of five Wistar rats Rj:WI (IOPS HAN) were administered spirotetramat-enol by gavage (0.5% methylcellulose vehicle) at a dose of 0 or 800 mg/kg bw/day for 21 days.

Findings:

Cross pathological findings:

The mean absolute weight of the right and left testis of treated rats was increased by 8% ($p<0.05$ for left testis only) and the relative weight was increased by 17% ($p<0.05$) compared with that of controls. The increase in relative weight was due in part to decreased terminal body weight of treated rats. No significant difference was observed in the weight of the epididymides in treated rats compared with controls. Soiled fur was observed on four treated rats during necropsy compared with none of the controls; this finding was consistent with the clinical observations. No other treatment-related gross changes were observed.

Histopathological findings:

Degenerating round spermatids was observed in 1/5 treated rats, and degenerating elongating spermatids along with diffuse sloughing of germ cells were found in all treated animals. Multinucleated giant spermatids were observed in 2/5 rats, and Sertoli cell vacuolation of minimal degree was noted in 1/5 rats. In the left epididymis, minimal to slight exfoliation of germ cells was found in all treated animals. This finding is considered a consequence of degenerating spermatids observed in the testes.

Sperm analysis:

No statistically significant change in absolute or the relative (based on epididymal weight) numbers of epididymal spermatozoa was observed after 21 days of treatment. The data were highly variable for the control and treated groups; the relative sperm count ranged from 756-1109 x 10^6 spermatozoa/g tissue for controls and 690-929 x 10^6 spermatozoa/g tissue for treated animals.

The percentage of abnormal spermatozoa was significantly increased after 21 days of treatment with spirotetramat-enol when compared with control values (14.9% vs 3.2% in the control group). The majority of the abnormalities consisted of isolated heads with normal morphology (60%) and spermatozoa with a normal head and an abnormal mid-piece (33.3%).

Conclusion:

This study was conducted to determine whether the acyl or enol metabolite of spirotetramat caused the testicular/sperm toxicity in male rats administered the parent compound. It was stated that the acyl chain was highly unstable; therefore, this study was conducted with the enol. Male rats were administered spirotetramat-enol at 800 mg/kg bw by gavage for 21 days; this dose is equivalent to 1000 mg/kg of the parent compound. Male rats administered the enol showed effects similar to those of male rats administered the parent compound, spirotetramat, for 21 or 41 days. Effects in rats administered the enol consisted primarily of soiled fur and salivation (clinical observations), a marked decrease in body weight gain, a slight decrease in testes weight, and microscopic changes in the testes (degeneration of round and elongating spermatids, sloughing of germ cells, the presence of multinucleated giant spermatids, and Sertoli cell vacuolation) and epididymis (exfoliation of germ cells). There was an increase in the percentage in abnormal spermatozoa consisting primarily of isolated normal heads and spermatozoa with an abnormal mid-piece but no treatment-related change in sperm count.

In conclusion, testicular/sperm toxicity in male rats administered spirotetramat is due to the enol and is unlikely due to the acyl chain alone.

4.11.4 Summary and discussion of reproductive toxicity

In addition to testicular histopathology observed following subchronic and chronic exposure of male rats to spirotetramat, evidence of male reproductive toxicity was provided in the 2-generation reproductive toxicity study. Abnormal sperm cells were reported in F_1-generation male rats treated with 6000 ppm (419 mg/kg bw/day) spirotetramat in the diet, and decreased reproductive performance was also observed in one of these males. Similar results were obtained in the 1-generation reproductive toxicity range-finding

study, in which decreased sperm motility and progression and increased abnormal sperm cells in the epididymides were observed in F_1males at ≥6000 ppm (400 mg/kg bw/day). The highest dose level of 10000 ppm, equivalent to 538 mg/kg bw/d, was associated with no fertility in parental generation animals. There were no implantation sites noted in the females due to treatment-related effects on sperm cells of males at this dose level (increased numbers of abnormal sperms, reduced epididymal sperm counts, decline in both motility and progression of epididymal sperm cells). Absolute and relative weight of the cauda epididymis was decreased in parental males. Histopathology showed abnormal sperm cells of minimal to moderate severity in the epididymis and the cauda epididymis.
Offspring toxicity was limited to decreased body weights in both studies, observed in F_1 and F_2 pups respectively of both sexes during lactation at 6000 ppm (400 and 419.3 mg/kg bw/day respectively). Decreased body weights were also observed in parental animals at the same dose.
Development of the sexual organs of offspring (balano-preputial separation, vaginal opening) was unaffected in both studies. Developmental toxicity in the absence of maternal toxicity was not observed in either the rat or rabbit.
In February 2008 the notifier submitted a position paper (*High dose reproductive effects in male rats and their relevance to humans; Temerowski M., 2008*). It was stated that the effects on testicular spermatogenesis were attributed to spirotetramat-enol, which is the main metabolite in the rat. Spirotetramat-enol is further metabolised by oxidation reactions to spirotetramat-desmethylenol, spirotetramat-enol-alcohol and spirotetramat-ketohydroxy. Oxidation products accounted for approximately 14%. Conjugation was not detected. In the mouse, conjugation of spirotetramat-enol with glucuronic acid accounted for approximately 30%. In human liver cells, conjugation to spirotetramat-enol-glucuronic acid was 6%. The in vitro conjugation rate is dependent on the concentration used and declined in mice from 30% to 9% and in humans from 6% to 2% at liver concentrations of 19 µg/g and 190 µg/g spirotetramat, respectively. Glucuronidation of the spirotetramat-enol in mice leads to much lower systemic levels of free spirotetramat-enol when compared to the rat. The conjugation enables the mouse to utilize separate active transport systems in the kidneys, thus avoiding a saturation of the elimination process. Thus, the utilization of different transport systems renders the mouse less sensitive to spirotetramat-mediated testicular toxicity when compared to the rat. Based on the metabolic similarity between mice and humans, it is likely that humans are also less sensitive to spirotetramat-mediated testicular toxicity than rats.
This statement of the notifier can not be agreed to. In contrast to mice, for humans the ability to conjugate spirotetramat-enol with glucuronic acid is fivefold lower than for the mouse (dependent on the concentration, in humans 6% respectively 2%, in mice 30% respectively 9%). Therefore a similarity in the metabolic pathway can not be followed. As for humans conjugation is only 2% at high doses, it can not be assumed that humans are less sensitive to spirotetramat than rats.

In the developmental toxicity study in rats, toxicity to the offspring was observed in the presence of maternal toxicity, including decreased food consumption and body weight/gain, at 1000 mg/kg bw/day. Reduced fetal weight and increased incidences of skeletal malformations and skeletal deviations were observed at 1000 mg/kg bw/day. Malformations at the high dose included one case of supernumerary lumbar vertebra, one case of cleft palate and one case of co-arctation of aortic arch. One case of atrial septal defect of the heart and microphthalmia were observed in the control, low and high dose each, but not at the mid dose.
Four cases of dysplastic forelimb bones (1.5 %) and three cases of malformed sacral vertebral arches with pelvic shift (1.1 %) were observed in the high dose. Historical control data of the performing laboratory (Bayer HealthCare AG) for dysplastic forelimb bones in studies conducted in the years 1999 – 2004 showed

26 affected animals out of 1975 animals, a percentage of 1.3 % [range 0.4 – 4.3% due to one study conducted in the year 2000, were 10 animals out of
232 were affected (4.3%)]. An incidence of 1.5 % in the study with spirotetramat is therefore outside the concurrent control (0.4%) and the historical control data (1.3%).
Statistically significantly increased incidences of sacral vertebral alterations (1.1 %) were observed at a dose level of 1000 mg/kg bw spirotetramat in comparison to the concurrent controls (0.0 %). The incidence in historical controls in studies conducted 1999 – 2006 showed 2 affected animals out of 6554 animals, a percentage of 0.03 % (range 0 – 0.4%).
Statistically significantly increased incidences of wavy ribs were observed at all dose levels compared to concurrent and historical control values. Statistically significantly increased numbers of fetuses with 14th ribs were observed at a dose level of 1000 mg/kg/d spirotetramat.
The maternal and the developmental NOAEL for this study was set at 140 mg/kg bw/d.

In the developmental toxicity study in rabbits, offspring toxicity was not observed at any dose up to 160 mg/kg bw/day. However, maternal toxicity was observed at ≥40 mg/kg bw/day, including a dose-dependent increase in abortion and clinical signs of toxicity in affected animals (severely reduced food consumption, body weight loss, alopecia, altered appearance of feces, discoloured urination). Gross pathology revealed treatment-related fluid/gaseous contents in caecum, mottled gall bladder, discolouration of the liver. The maternal NOAEL was set at 10 mg/kg bw/d.

In a mechanistic study designed to explore the time of onset of testicular toxicity of spirotetramat in rats (*Kennel, 2005*), decreased epididymal sperm counts were recorded ≥10 days of treatment with 1000 mg/kg bw/day by gavage. Therefore, the author of the study concluded that repeated dosing is necessary to produce male reproductive toxicity in rats, not considering the relatively prolonged period of spermatogenesis (about 60 days in man, 55 in rat). A single high dose at one critical point in development may be required, but the effects would not be seen until some time later.
In a second mechanistic study, male rats were treated by gavage with the enol metabolite of spirotetramat for 21 days at a dose of 800 mg/kg bw/day (*Tinwell, 2006*). Spermatotoxicity, abnormal sperm, and Sertoli cell vacuolation were observed in the testes-epididymides of treated animals. Therefore, male reproductive toxicity in rats is likely due to the enol metabolite of spirotetramat.

4.11.5 Conclusions on classification and labelling

Due to abnormal sperm cells, decreased sperm motility and progression and decreased reproductive performance, spirotetramat should be classified to "category 3 of reproductive substances" and labelled with the risk phrase "R 62 – Possible risk of impaired fertility" according to Annex VI of the EC Council Directive 67/548/EEC.

According to Regulation EC 1272/2008, fluazinam should be classified in hazard category 2 for reproductive toxicity and labeled with signal word "Warning" and hazard statement H361f (Suspected of damaging fertility).

Due to increased incidences of skeletal malformations and skeletal deviations and according to Annex VI of the EC Council Directive 67/548/EEC, spirotetramat should be classified to "category 3 of reproductive substances" and labelled with the risk phrase "R 63 – Possible risk of harm to the unborn child".

According to Regulation EC 1272/2008, fluazinam should be classified in hazard category 2 for reproductive toxicity and labeled with signal word "Warning" and hazard statement H361d (Suspected of damaging the unborn child).

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Fertility

In addition to testicular histopathology observed following subchronic and chronic exposure of male rats to Spirotetramat, evidence of male reproductive toxicity was provided in the 2-generation reproductive toxicity study. Abnormal sperm cells were reported in F1-generation male rats treated with 6000 ppm Spirotetramat in the diet (equivalent to 419 mg/kg bw/day), and decreased reproductive performance was also observed in one of these males. Similar results were obtained in the 1-generation reproductive toxicity range-finding study, in which decreased sperm motility and progression and increased abnormal sperm cells in the epididymides were observed in F_1 males at ≥6000 ppm (400 mg/kg bw/day). The highest dose of 10000 ppm, equivalent to 538 mg/kg bw/day, was associated with complete infertility in the parental generation animals. There were no implantation sites noted in the females due to treatment-related effects on sperm cells of males at this dose (increased numbers of abnormal sperms, reduced epididymal sperm counts, reduction in both motility and progression of epididymal sperm cells). Absolute and relative weights of the cauda epididymis were decreased in parental males. Histopathology showed abnormal sperm cells of minimal to moderate severity in the epididymis and the cauda epididymis. Toxicity in the offspring was limited to decreased body weights in both studies, observed in F1 and F2 pups, respectively, of both sexes during lactation at 6000 ppm (400 and 419.3 mg/kg bw/day, respectively). Decreased body weights were also observed in parental animals at the same dose. Development of the sexual organs of offspring (balano-preputial separation, vaginal opening) was unaffected in both studies.

In February 2008, the notifier of the substance prepared a position paper (Temerowski M., 2008. High dose reproductive effects in male rats and their relevance to humans). In this paper it was stated that the effects on testicular spermatogenesis were attributed to Spirotetramat-enol, which is the main metabolite in the rat. Spirotetramat-enol is further metabolised by oxidation reactions to Spirotetramat desmethyl-enol (DME), Spirotetramat-enol-alcohol and Spirotetramat-ketohydroxy. Oxidation products accounted for approximately 14% of total metabolites. Conjugation was not detected. In the mouse liver cells, conjugation of Spirotetramat-enol with glucuronic acid accounted for approximately 30%. In human liver cells, conjugation to Spirotetramat-enol-glucuronic acid was 6%. The *in vitro* conjugation rate is dependent on the concentration used and declined in mice liver cells from 30% to 9% and in human liver cells from 6% to 2% at concentrations of 19 µg/g and 190 µg/g Spirotetramat, respectively. Glucuronidation of the Spirotetramat-enol in mice leads to much lower systemic levels of free Spirotetramat-enol when compared to the rat. The conjugation enables the mouse to utilise separate active transport systems in the kidneys, thus avoiding saturation of the elimination process. The notifier hypothesised that the utilisation of different transport systems renders the mouse less sensitive to Spirotetramat-mediated testicular toxicity when compared to the rat. The position paper argued that based on the metabolic similarity between mice and humans, it is likely that humans are also less sensitive to Spirotetramat-mediated testicular toxicity than rats.

The DS however did not agree with the conclusions of the position paper from the notifier.

For human liver cells the ability to conjugate Spirotetramat-enol with glucuronic acid is five-fold lower than for the mouse liver cells (depending on the concentration, 6% and 2% in human liver cells; and 30% and 9% in mice liver cells, at 19 and 190 µg/g Spirotetramat, respectively). Therefore the DS considered that an argument based on the similarity in the metabolic pathways between mice and humans cannot be sustained. Since for human liver cells conjugation is only 2% at high doses, it cannot be assumed that humans are less sensitive to Spirotetramat than rats.

In a mechanistic study designed to explore the time of onset of testicular toxicity of Spirotetramat in rats (Kennel, 2005), decreased epididymal sperm counts were recorded after ≥10 days of treatment with 1000 mg/kg bw/day by gavage. Therefore, the author of the study concluded that repeated dosing is necessary to produce male reproductive toxicity in rats; however, the author did not consider the relatively long period of spermatogenesis (about 60 days in man, 55 in rat). Hence, a single high dose at one critical point in development may be required, but the effects would not be seen until later.

In a second mechanistic study, male rats were treated by gavage with the enol metabolite of Spirotetramat for 21 days at a dose of 800 mg/kg bw/day (Tinwell, 2006). Spermatotoxicity, abnormal sperm, and Sertoli cell vacuolation were observed in the testes-epididymides of treated animals. Therefore, the author concluded that male reproductive toxicity in rats is likely to be due to the enol metabolite of Spirotetramat.

From the above mechanistic investigations, the DS concluded that classification for reproductive toxicity in category 2 according to the CLP Regulation for fertility would be appropriate.

<u>Developmental toxicity</u>

In the developmental toxicity study in rats, toxicity to the offspring was observed in the presence of maternal toxicity, including decreased food consumption and body weight or body weight gain, at 1000 mg/kg bw/day. Reduced fetal weight and increased incidences of skeletal malformations and skeletal deviations were observed at 1000 mg/kg bw/day. Malformations at the high dose included one case of supernumerary lumbar vertebra, one case of cleft palate and one case of co-arctation of aortic arch. One case of atrial septal defect of the heart and microphthalmia were observed in the control, low and high dose groups, but not at the mid-dose.

Four cases of dysplastic forelimb bones (1.5%) and three cases of malformed sacral vertebral arches with pelvic shift (1.1%) were observed at the high dose. Historical control data of the performing laboratory (Bayer HealthCare AG) for dysplastic forelimb bones in studies conducted in the years 1999 to 2004 showed 26 affected animals out of 1975 animals, (1.3 %) [range 0.4 – 4.3% due to one study conducted in the year 2000, were 10 animals out of 232 were affected (4.3%)]. An incidence of 1.5 % in the study with Spirotetramat is therefore outside the concurrent control (0.4%) and the historical control data (1.3%). Statistically significantly increased incidences of sacral vertebral alterations (1.1 %) were observed at a dose of 1000 mg/kg bw/day Spirotetramat in comparison with the concurrent controls (0.0 %). The incidence in historical controls in studies conducted from 1999 to 2006 showed 2 affected animals out of 6554 animals, i.e. 0.03 % (range 0 – 0.4%). Statistically significantly increased incidences of wavy ribs were observed at all doses compared to concurrent and historical control values. Statistically significantly increased numbers of fetuses with 14^{th} ribs were observed at a dose of 1000 mg/kg bw/day

Spirotetramat. The NOAELs for both maternal and developmental toxicity in this study were set at 140 mg/kg bw/day.

In the developmental toxicity study in rabbits, offspring toxicity was not observed at any dose up to 160 mg/kg bw/day. However, maternal toxicity was observed at ≥40 mg/kg bw/day, including a dose-dependent increase in abortions and clinical signs of toxicity in affected animals (severely reduced food consumption, body weight loss, alopecia, altered appearance of faeces, discoloured urination). Gross pathology revealed treatment-related fluid/gaseous contents in the caecum, mottled gall bladder and discolouration of the liver. The maternal NOAEL was set at 10 mg/kg bw/day.

The DS therefore concluded that Repr. 2 for developmental effects would be appropriate.

Comments received during public consultation

Fertility

Two MSCA supported the proposal to classify as Repr. 2 for fertility without further comment. Two other MSCAs requested a clear rationale why category 2 is more appropriate than category 1B, although one of them expressed their support for a category 2. One MSCA supported classification as Repr. 1B for fertility due to robust findings of testis toxicity in rats and the absence of information that casts doubts on the human relevance of this finding.

One industry comment recognised the testicular toxicity in rats but considered this to be a high dose phenomenon unlikely to occur in humans on the basis of risk assessment considerations. Interspecies differences in toxicokinetics and metabolism were also pointed out and it was proposed that reproductive effects occur in rats at doses which were above those resulting in saturation of elimination mechanisms, which is not expected in humans, leading them to conclude that no classification for fertility is warranted.

Developmental toxicity

Three MSCA supported the proposal to classify as Repr. 2 for developmental effects. Another MSCA requested a clear rationale why category 2 would be more appropriate than category 1B for reproductive toxicity in general (not specifying developmental toxicity). One other MSCA requested further discussion on the incidence of malformations and deviations with regards to historical control data to decide whether it was sufficient for classification in category 2.

Industry considered that classification of Spirotetramat as a reproductive toxicant is not justified and commented more specifically on the historical control data relating to the findings in rats.

Additional key elements

Fertility

No effect on male reproductive organs or sperm parameters were detected in the available repeated toxicity studies in mice up to 1022 mg/kg bw/day in an 18-month study, and in dogs up to 81 mg/kg bw/day in a 90-day study and 55 mg/kg bw/day in a 1-year study.
In rats, by the oral route, the following effects were reported in repeated-dose toxicity

studies:

- at 616 mg/kg bw/day in the 90-day study: 6% decrease in absolute testis weight, incidence of 9/10 animals with minimal to severe abnormal spermatozoa (multi-nucleated giant cells, immature sloughed germ cells) and 5/10 animals with hypospermia in the epididymis, 5/10 animals with minimal to moderate tubular degeneration in the testis. Tubular degeneration and vacuolisation was generally multifocal in distribution and occurred in the germinal layer of seminiferous tubules with degeneration and loss of epithelial cells.
- at 414 mg/kg bw/day in the one-year study: incidence of 3/25 males with exfoliated germ cells/debris in the epididymis and 2/25 with abnormal spermatozoa (none in the controls for both findings).
- At 373 mg/kg bw/day in the two-year study: slight morphological testicular change in the testis characterised by depletion, asynchrony and degeneration of the latter stage spermatid in 9/55 animals (none in controls and at lower doses); increased incidence of immature/exfoliated germ cells/debris in the epididymis (observed in 6, 10, 6 and 31 animals in the controls, low, mid and high dose groups, respectively).

In a range-finding reproductive toxicity study Wistar rats were exposed through diet to 200, 500, 6000 or 10000 ppm (Young, 2006) (corresponding to 10/13, 28/31, 320/384 or 538/646 mg/kg bw/day in premating males/females, respectively). In the P-generation, absolute and relative weights of the cauda epididymis were decreased by 29 and 27%, respectively, in high dose males. Minimal to moderate presence of abnormal sperm was reported in the epididymis and in the cauda epididymis in 10/10 and 9/9 high dose males, respectively. It was described as retention of the residual body of the tail of the spermatozoa. At this dose, all males were infertile and mating resulted in no gestation. Reductions in motility and sperm counts in the epididymis and increases in abnormal sperm were also reported (see table 6 below). Other effects in the high dose males consisted in a reduction in body weight gain of 10% although body weight was lower by only 2% compared to controls.
In the F1-generation, 8-9-week old males showed a slight alteration of sperm parameters (see table 6) at 6000 ppm and minimal to moderate presence of abnormal sperm was reported in the epididymis and in the cauda epididymis of 4/15 and 4/14 dose males, respectively (versus 1 control animal). Other effects in this group were restricted to an 8% reduction in body weight.

Table 6 – mean sperm parameters in the range finding reproductive toxicity study, Wistar rat

Sperm Analysis	Dose Group (ppm)				
	0	200	500	6,000	10,000 [a]
P generation males (n≈10/group)					
Motility: % motile	74.1	74.0	79.0	75.2	30.6
Motility: % progressive	57.4	53.7	58.3	52.4	14.1
Sperm counts: sperm/g testis	98.8	128.4	109.7	111.0	97.7
Sperm counts: sperm/g epididymis	985.6	732.8	798.1	944.2	464.0
Morphology: number of normal cells	199.2	199.3	199.5	199.0	105.0
Morphol.: number of amorphous heads	0.7	0.7	0.5	0.8	95.5
Morphology: number of small heads	0.1	0.0	0.0	0.2	0.0
F1 eight-to-nine week old interim animals (n≈15/group)					
Motility: % motile	89.5	89.7	88.3	75.5	-
Motility: % progressive	69.2	63.5	62.5	55.7	-
Sperm counts: sperm/g testis	81.8	75.1	73.1	73.0	-
Sperm counts: sperm/g epididymis	503.6	485.7	507.1	463.4	-
Morphol.: number of normal cells	197.2	198.5	198.7	179.6	-
Morphol.: number of amorphous heads	2.8	1.5	1.3	20.2	-
Morphol.: number of small heads	0.1	0.0	0.0	0.2	-

Data taken from Table 22, pp. 98-99, Report No. 201300-1
a = no pups at this dietary level

In a two-generation study conducted in accordance to OECD TG 416 (Young, 2006), Wistar rats were exposed through the diet to 250, 1000 or 6000 ppm (corresponding to 17/20, 71/82 or 419/485 mg/kg bw/day in premating P-generation males/females, respectively).
In the P-generation, the only effect on sperm parameters was a dose-related decrease in epididymal sperm count from 1000 ppm (see table 7 below). Reproductive performance was not affected. Other effects in the high dose males consisted in a reduction of body weight gain of 13% although body weight was lower by only 3% compared to controls. Historical control data from this study reports an epididymis sperm count range between 280 and 986 $x10^6$ sperm/g (mean: 506 $x10^6$ sperm/g) from parental males in 4 two-generation studies. In a later study from the same laboratory, historical control data from parental males in 11 two-generation studies (including the Spirotetramat study) ranges from 177 to 986 $x10^6$ sperm/g (mean: 374 $x10^6$ sperm/g). Epididymis sperm count at 1000 and 6000 ppm are therefore within the historical control range and the link with treatment is uncertain.

In F1 males of the 6000 ppm group (corresponding to 487 mg/kg bw/day), one animal had a moderate number of abnormal sperm within the tubules in epididymis at histopathology with similar appearance (amorphous sperm heads) to observation in the dose-finding study. Sperm count and morphology were affected (see table 7 below). 9/30 animals exhibited a minimal effect on abnormal sperm but one animal was affected to the extent that it compromised its fertilisation capabilities. Other effects in this group were a 6% reduction in body weight, a decrease in relative kidney weight (4-6%) and minimal to moderate tubular dilatation in the kidneys in 23/30 animals.

Table 7 - mean sperm measures in the two-generation reproductive toxicity study, Wistar rat

Sperm Analysis	Dose Group (ppm)			
	0	250	1,000	6,000
P generation males				
Motility: % motile	83.7	82.8	83.9	85.0
Motility: % progressive	58.8	59.0	60.6	61.7
Sperm counts: sperm/g testis	89.95	N/A	N/A	87.43
Sperm counts: sperm/g epididymis	622.95	562.85	481.82*	472.27*
Morphol.: number of normal sperm cells	197.3	N/A	N/A	196.0
Morphol.: number of abnormal sperm cells	2.0	N/A	N/A	2.8
Morphol: number with detached heads	0.8	N/A	N/A	1.2
F_1 generation males				
Motility: % motile	81.1	82.8	83.9	79.9
Motility: % progressive	56.6	59.7	59.3	55.7
Sperm counts: sperm/g testis	87.8	90.3	89.9	79.3
Sperm counts: sperm/g epididymis	517.2	490.3	520.1	496.5
Morphol.: number of normal sperm cells	196.3	196.8	197.3	189.9
Morphol.: number of abnormal sperm cells	2.9	2.6	1.9	8.9
Morphol: number with detached heads	0.8	0.6	0.9	1.3

Data taken from Table 22, pp. 137-138, Report No. 201426-1
* = $p < 0.05$

A mechanistic study (Kennel, 2005) investigated the time of onset of effect on the reproductive function in rats (n=8/group) exposed by gavage to 1000 mg/kg bw/day Spirotetramat for 3, 10, 21 or 41 days. Reductions in testis and epididymis weight and gross findings (small/soft testis, small epididymis and/or small prostate) were observed only at day 41. Microscopic findings confirming an effect of Spirotetramat treatment on the testes included degenerating round spermatids, degenerating elongating spermatid and multinucleated giant spermatids in the testes and increase in intraluminal aberrant cells in the epididymis after treatment for 21 days. The effect on the testes progressed to loss of elongating spermatids in the testes and an increase in severity of intraluminal aberrant cells (see table 8 below). Oligospermia in the epididymides was also reported with significant decreases observed at all time-points, although the sperm count at day 10 was similar to the control value at day 3 and the time-response relationship is not clear (see table 9 below). The toxicity of Spirotetramat on the testis, epididymis and sperm parameters is therefore apparent from day 21. Continued treatment with the test material for 41 days also had an effect on Sertoli cells.

Table 8 – incidence of microscopic findings in the testes and epididymides, rat

Parameter	Day 21		Day 41	
Dose Group (mg/kg)	0 mg/kg	1,000 mg/kg	0 mg/kg	1,000 mg/kg
Number of animals examined	8	8	8	7
Testes				
Degenerating round spermatids (steps 7-8)	0	4 (2.50)[a]	0	5 (3.00)
Multinucleated giant spermatids	0	2 (2.50)	0	4 (1.25)
Degenerating elongating spermatids (steps 9-14)	0	8 (3.0)	0	7 (2.86)
Loss of elongating spermatids (steps 9-14)	0	0	0	5 (3.00)
Sertoli cell vacuolation	0	0	0	6 (1.17)
Epididymis				
Increase of intraluminal aberrant cell types	0	8 (1.75)	0	7 (3.00)
Oligospermia	0	0	0	7 (2.86)

[a]numbers in parentheses are average severity grade in affected animals : 1=slight, 2=moderate, 3=marked, 4=severe

Table 9 – Sperm count and morphology

Day 3		Day 10		Day 21		Day 41	
Control	1000 mg/kg	Control	1000 mg/kg	Control	1000 mg/kg	Control	1000 mg/kg
Epididymal sperm count (absolute) x 10^6							
184.4 ± 25.5	160.6 ± 33.9 (-13%)[a]	218.4 ± 31.5	188.5 ± 19.5* (-14%)	199.1 ± 26.9	169.6 ± 24.1* (-15%)	214.3 ± 11.6	50.3 ± 9.5** (-77%)
Relative sperm count to caudal epididymal weight x 10^6							
849.6 ± 81.0	801.7 ± 121.3 (-6%)	940.6 ± 84.3	818.0 ± 102.4* (-13%)	871.5 ± 82.5	808.0 ± 95.4 (-7%)	878.1 ± 63.7	281.4 ± 37.6** (-68%)
Sperm morphology (% abnormal cells)							
4.1 ± 0.8	2.8 ± 1.7	3.2 ± 1.3	3.7 ± 0.5	2.5 ± 1.5	6.4 ± 2.6**	3.8 ± 1.4	72.0 ± 19.4**

[a] percent different from control
*p<0.05; **p<0.01

In a further mechanistic study (Tinwell, 2006), male rats (n=5/group) were exposed by gavage to 800 mg/kg bw/day Spirotetramat-enol for 21 days. The mean absolute weight of the right and left testis of treated rats was increased by 8% ($p<0.05$ for left testis only) and the relative weight was increased by 17% ($p<0.05$ for both testis) compared with that of controls. The increase in relative weight was due in part to decreased terminal body weight of treated rats (8%). Degenerating round spermatids were observed in 1/5 treated rats, and degenerating elongating spermatids along with diffuse sloughing of germ cells were found in all treated animals. Multinucleated giant spermatids were observed in 2/5 rats, and Sertoli cell vacuolation of minimal degree was noted in 1/5 rats. In the left epididymis, minimal to slight exfoliation of germ cells was found in all treated animals. The percentage of abnormal spermatozoa was significantly increased after 21 days of treatment with Spirotetramat-enol when compared with control values (14.9% vs 3.2% in the control group). The majority of the abnormalities consisted of isolated heads with normal morphology (60%) and spermatozoa with a normal head and an abnormal mid-piece (33.3%).

Developmental toxicity

In the range-finding reproductive toxicity study Wistar rats were exposed through diet to 200, 500, 6000 or 10000 ppm (Young, 2006) (corresponding to 13/27, 32/74, 394/831 mg/kg bw/day in pregnant/lactating females up to 6000 ppm, respectively). No litters were produced at 10000 ppm due to the infertility of males. At lower doses, no malformations and no effect on body weight at birth were observed. At 6000 ppm, a 14% decrease in pup body weight was observed at lactation day 21 with a 15% reduction in body weight gain. In the lactating dams a body weight reduction of 15% was observed.

In the two-generation study (Young, 2006), Wistar rats were exposed through diet to 250, 1000 or 6000 ppm (corresponding to 18/39, 77/163 or 467/896 mg/kg bw/day in pregnant/lactating P-generation females, respectively). No effect on body weight at birth was observed. Neonatal effects were restricted to a decrease in pup body weight during lactation at 6000 ppm, that reached 8% in F1 pups and 9-11% in F2 pups at lactation day 21. In the lactating F1 dams, a body weight reduction of 6% was observed.

In a rat prenatal toxicity study (Klaus, 2004), Wistar rats were exposed to 20, 140 or 1000 mg/kg bw/day Spirotetramat by gavage from gestation day 6 to 19. Maternal toxicity consisted of a significant decrease in corrected body weight gain of 29% at 1000 mg/kg

bw/day with decreased food consumption from gestation day 6. An increased incidence in pre-implantation loss was observed at 140 mg/kg bw/day but this was considered incidental as it was in the historical control range and not confirmed at the highest dose (10.0, 15.6, 20.2* and 11.2% at 0, 20, 140 and 1000 mg/kg bw/day, respectively). A decrease of 14% in fetal body weight was reported at 1000 mg/kg bw/day. Malformations are summarised in Table 9 below.

Table 9: External, Visceral and Skeletal malformations in rats (Klaus, 2004)

Observations	**Dose (mg/kg bw/day)**				**Historical control fetal mean (range)**
	0	**20**	**140**	**1000**	
No. fetuses (litters) examined	247 (20)	301 (24)	253 (23)	270 (22)	
No. fetuses (litters) affected by malformations	7 (4)	5 (4)	2 (2)	12 (9)	
% malformed fetuses (litters) per group	2.83 (20.0)	1.66 (16.7)	0.79 (8.7)	4.4 (40.9)	
External malformations					
Cleft palate	0	0	0	1 (1)	1 fetus with cleft palate/lip in each of 2 studies (out of 10 studies)
Microphthalmia, unilateral	1 (1)	1 (1)	0	1 (1)	No data
Anophthalmia, unilateral	1 (1)	1 (1)	0	0	No data
Upper jaw shortened, macroglossia, domed head, dysplasia fore- and hindlimbs, skull and vertebral column	0	0	1 (1)	0	No data
Visceral malformations					
Lobe of thyroid gland absent	3 (2)	0	1 (1)	0	No data
Atrial septal defect of the heart	1 (1)	1 (1)	0	1 (1)	No data
Co-arctation of aortic arch between left carotid and left subclavian arteries, ascending aorta reduced in size, left subclavian artery arises from descending aorta	0	0	0	1 (1)	1 fetus with a defect of the great vessel in 1 out of 10 studies.
Skeletal malformations					
Dysplasia of forelimb bones	1 (1)	2 (2)	0 (0)	4 (4)	1.3% (0.4-4.3%)
Altered appearance of sacral vertebral arch;	0	0	0	3 (3)	0.03% (0-0.4%)

pelvic shift					
Supernumerary lumbar vertebra	0	0	0	1 (1)	No data

Only the incidence of 1% of malformed sacral vertebral at 1000 mg/kg bw/day exceeded study controls (as well as historical control data, when available). In the absence of historical control data on supernumerary lumbar vertebra, it is not possible to conclude on the single occurrence of this finding at the high dose.
Statistically significantly retarded ossification in various locations (phalanges, sternebrae, vertebrae, skull bones) were noted at the 1000 mg/kg bw/day dose in the osseus and/or cartilagineous parts. Retarded ossification was noted in the 140 mg/kg bw/day dose group and in the 20 mg/kg bw/day dose group on a fetal basis at single bones of the fore- and hind limbs without a clear dose relationship, together with incomplete ossification of the 6th sternal segment and the supraoccipital bone (at 20 mg/kg bw/day only). None of these findings were statistically significantly different on a litter basis and all findings at 20 or 140 mg/kg bw/day were within the range of historical control values according to the CLH report. The changes of the low- and mid-dose groups were poorly correlated with dose.

Increased incidences of other skeletal deviations were seen at 1000 mg/kg bw/day (see Table 10 below) and consisted in wavy ribs, 14th rib, dumbbell shaped thoracic vertebral bodies, slightly enlarged fontanelles. All these findings are considered to affect the structure of the bone and not only its ossification (Makris *et al.*, 2009). They are observed together with growth retardation at this dose. It is noted that supernumerary ribs are likely to be permanent (Makris *et al*., 2009), but as an outcome of a harmonization workshop on terminology and classification of fetal abnormalities, there is a good agreement to consider it as a variation (Solecki *et al.*, 2001) and not a malformation. Involvement (or not) of the cartilage in dumbbell shaped vertebral bodies was not documented in the CLH report. Solecki *et al*. (2001) distinguish between "dumbbell" and "dumbbell ossification", with the former being a malformation and the latter a variation. It is stated that 'The term dumbbell implies that the bone precursor is affected as well as the ossification site and the change is likely to be permanent. Therefore the condition would classify it as a malformation. Dumbbell ossification would suggest that only the ossification site is abnormally shaped and, as with bipartite ossification, this alteration would be classified as a variation.' Thus RAC concluded that the effect described as "dumbbell-shaped thoracic vertebral bodies" without reference to ossification is a malformation. Solecki *et al*. (2001) also reports that there is a good agreement that enlarged fontanelles is a variation and not a malformation.
An increased incidence of wavy ribs was also observed in the groups exposed to 20 and 140 mg/kg bw/day, in addition to the high dose group.

Table 10 – Incidence of skeletal deviations (except incomplete or absent ossification) in the rat prenatal toxicity study (Klaus, 2004).

Observations	**Dose (mg/kg bw/day)**				**Historical control fetal range**
	0	**20**	**140**	**1000**	
#Fetuses (litters) examined	127 (20)	159 (24)	135 (23)	146 (22)	
Wavy ribs, sum of	7 (6)	27**	24**	**84** (21**)**	2.7-15.1%

fetal incidence		(10)	(12)		
14th rib, sum of fetal incidence	19 (10)	17 (12)	17 (11)	67** (19)	0-13%
Thoracic vertebral body, dumbbell shaped -11th -12th -13th	28 (14) 32 (14) 15 (12)	21 (12) 27 (12) **5* (5*)**	19 (12) 21 (12) 11 (9)	69** (20) 75** (21) 49** (19)	
Fontanelle, slightly enlarged	11 (7)	30 (12)	8 (6)	34** (13)	

*statistically different ($p < 0.05$) from the control. **statistically different ($p < 0.01$) from the control.

In a supplementary rat prenatal toxicity study (Klaus, 2004), Wistar rats were exposed to 10, 35 or 140 mg/kg bw/day Spirotetramat by gavage from gestation day 6 to 19. No maternal toxicity was observed. Foetal weight and the incidence and type of malformations was unaffected by treatment. Significantly increased incidence in ossification retardation in phalanges, in the 4th sacral vertebral arch and in the sphenoid bone was observed at 140 mg/kg bw/day. Some significant incidences were also reported at 10 and/or 35 mg/kg bw/day. The dose-response relationship was generally weak (except for the incomplete ossification of the 3rd distal left phalanx digit and absence of ossification of the 5th left phalanx toes). At all doses, incidences were within the historical controls values according to the CLH report.
On other skeletal deviations, the incidence of wavy ribs was not significantly increased at any dose (see Table 11 below). Significant increases in the incidence of punctiform or comma-shaped 14th rib was observed at 140 mg/kg bw/day, but the overall incidence of supplementary 14th rib was not significantly elevated at any dose.

Table 11 – Incidence of wavy ribs in the supplemental rat study.

Observations	**Dose (mg/kg bw/day)**			
	0	**10**	**35**	**140**
#Fetuses (litters) examined	140 (22)	136 (22)	144 (22)	118 (19)
Wavy ribs, sum of fetal incidence	13 (8)	12 (5)	19 (6)	11 (4)

In a rabbit prenatal toxicity study (Klaus, 2004) Himalayan rabbits were exposed to 10, 40 or 160 mg/kg bw/day Spirotetramat by gavage from gestation day 6 to 28.
Maternal mortality was observed at 160 mg/kg bw/day (5 dams sacrificed moribund and one found dead). Abortions were reported in two high dose females and one mid-dose female. Affected females showed clinical signs, impaired food consumption and body weight loss and it was therefore considered to be a consequence of maternal toxicity. Clinical signs consisting mainly of cold ears, alopecia, reduced amount of faeces, soft faeces and decreased water consumption were observed at 160 mg/kg bw/day and with a lower incidence at 40 mg/kg bw/day. When females which aborted or were sacrificed in moribund condition were excluded, no effect on food consumption was observed at 40 or 160 mg/kg

bw/day and corrected body weight gain was not affected. A decrease in these two parameters was observed at 10 mg/kg bw/day but was considered incidental due to lack of dose-response and agreement with historical control ranges.
Observed malformations are summarised in Table 12. Malpositioned forelimbs were reported as one of the most common malformations in the rabbit strain used. Other malformations consisted of isolated findings without a dose-response relationship, therefore all malformations were considered as unrelated to treatment.

Table 12 – Malformations in the rabbit prenatal toxicity study

Parameters	**Dose group (mg/kg bw/day)**			
	0	**10**	**40**	**160**
Eyeball reduced in size (microphthalmia)	-	-	-	1
Domed head/encephalomeningocele	-	-	-	1
Cleft palate	-	-	-	1
Malpositioned forelimbs	1	3 (2)	1	5 (2)
Ventricular septal defect of the heart	1	3 (3)	-	-
Fusion, bifurcation in the cartilaginous part, or slight thickening of ribs with/without supernumerary ossification center above the 1st	1	3 (3)	1	1
Sternal segment or at 8th rib cervical rib, supernumerary ossification center (with cartilage) above the 1st sternal segment fused with it	-	-	1	-
Missing presacral vertebra	2 (2)	-	-	-
Supernumerary presacral vertebra	-	-	1	-
Number of fetuses per group	170	152	172	132
Number of fetuses with malformations	5	8	4	7
Malformed fetuses per group (%)	2,9	5,3	2,3	5,3
Number of litters per group	22	22	21	19
Number of litters with malformations	5	7	4	4
Malformed litters per group (%)	22,7	31,8	19,0	21,1

Visceral deviations included an increased incidence in foetuses (8 foetuses in 2 litters, representing a foetal incidence of 6%) with distinct liver lobulation in the 160 mg/kg bw/day group. Statistical significance was evident on a foetal, but not litter basis and incidence was

slightly above historical control data (foetal incidence of 0 to 4.4%). The toxicological significance of this finding was questionable, since the liver is normally lobed and abnormal liver lobulation was not reported.

An *in vitro* study (Totis, 2006) indicates that species differences occur in metabolism of Spirotetramat (see Table 13 below).

Table 13 – Comparison of metabolites detected *in vitro* in hepatocytes from various species

	Rat	**Mouse**	**Human**
Spirotetramat	ND	ND	ND
Spirotetramat-enol	87%	66%	92%
Spirotetramat-enol glucuronide	ND	30%	6%
Spirotetramat-enol metabolites* including desmethyl-enol (DME)	14% 7%	4% 1%	1% 1%

ND: not detected; *sum of Spirotetramat-enol alcohol, Spirotetramat-desmethyl-enol, Spirotetramat-ketohydroxy formed from further oxidation and/or demethylation of the Spirotetramat-enol.

In all species tested, metabolism is complete and Spirotetramat-enol is the main metabolite obtained after incubation with rat, mouse and human liver cells (no information is available from studies in dogs). However, further conjugation with glucuronic acid was prominent in the mouse (30% of total metabolites) compared to rat (not detected). Conjugation in human liver cells was intermediate between rat and mouse with conjugated enol amounting to 6% of total metabolites. In rats, further metabolism of the enol derivative occurs through oxidation and demethylation so that 14% of total metabolites are metabolites of Spirotetramat-enol while it represents only 4% in mice and 1% in humans. Due to low conjugation and very limited metabolism, humans are therefore the species with the highest observed enol derivative level *in vitro* (92% versus 87% in rats and 66% in mice). It is suggested that glucuronidation in mice may lead to lower systemic levels of the enol by avoiding saturation of the process by which the metabolites are eliminated. However, it is noted that data on comparative systemic levels of Spirotetramat, Spirotetramat-enol and its metabolites *in vivo* are not available from mice or humans, therefore the hypothesis that conjugation allows a more efficient elimination of Spirotetramat metabolites than its oxidation and demethylation has not been confirmed *in vivo*.

The study investigating the effect of Spirotetramat-enol on the reproductive system reports effects that were to some extent similar to the adverse effects of Spirotetramat. It is therefore likely that effects of Spirotetramat on the reproductive system are mediated through its enol derivative. It is, however, not known whether it is the enol derivative, or some of its metabolites which are more specific to the rat, that are actually involved.

RAC, as well as the further documentation provided by industry, have also raised the potential role of Spirotetramat desmethyl-enol (DME) in inter-species differences. Both DME and the enol are expected to be actively removed from the kidney by the same Organic Anion Transporter (OAT) that may become saturated at higher doses. PBPK modelling (Schmitt, 2006) has shown that DME affinity for OAT is greater than affinity for the enol and industry concludes that the presence of DME leads to a competitive inhibition of the elimination of the enol. *In vitro,* DME is produced at higher rates in rat hepatocytes (7%) than in mouse or human hepatocytes (1%) and would contribute to accumulation of the enol toxic metabolite in rats together with an absence of glucuronidation pathway.

A study has been performed to test interactions of enol and DME with human OAT1, OAT3

and OAT4 in transfected Human Embryonic Kidney cells. It shows that the enol inhibits substrate uptake via OAT1 by a maximum value of 36%, via OAT3 by 76% and via OAT4 by 73% and DME inhibits substrate uptake via OAT3 by 24% and via OAT4 by 62%. The higher affinity of DME for OAT transporter compared to enol has therefore not been confirmed experimentally.

Overall, the presence of DME in rats may slow down the uptake of the enol by competition with some transporters but is not expected to block the transporters completely and totally inhibit excretion of metabolites (enol and DME). On the other hand, lower formation of DME in humans contributes to a higher enol concentration in humans based on the *in vitro* findings and the overall consequences of the species specificity is difficult to evaluate in the absence of *in vivo* data in other species than in rats.

Assessment and comparison with the classification criteria

Fertility

Spirotetramat has no effect on male reproductive organs and spermatogenesis in the mouse or the dog, although it is noted that a lower range of doses has been tested in the dog. Available data provide clear and consistent evidence that Spirotetramat alters spermatogenesis in the rat. Fertility was ultimately affected in all male rats exposed to 645 mg/kg bw/day in the range-finding reproductive toxicity study as well as in one F1 animal exposed to 487 mg/kg bw/day in the two-generation study.

Considering the occurrence of other toxic effects together with toxicity on the reproductive system and their potential relationship, RAC noted that effects on the reproductive system were generally observed at doses inducing a decrease in body weight compared to controls. However, decreases in body weight were generally limited (always equal to or less than 10%) and restricted to 2% in high dose parental males that were infertile in the range-finding reproductive study. The most sensitive effect was on sperm count and morphology. Reproductive organ weight was not systematically affected (i.e., in the 1-year rat study, 2-year rat study, and F1 generation of the range-finding reproductive toxicity study). Effects on sperm count and morphology cannot be explained by the limited decreases in body weight and are not considered to be secondary to other toxic effects.

It was commented during the public consultation that effects on spermatogenesis were observed in rats at doses where, due to the saturation of active transport mechanisms in the kidney elimination of Spirotetramat may be restricted. The existence of a conjugation pathway for Spirotetramat in mice and to a lesser extent in humans may prevent saturation of the elimination mechanisms and consequent exposure to high systemic levels of Spirotetramat metabolites in humans.

No information is available on the mechanism of toxicity of Spirotetramat on the reproductive system and the relevance of the observed effects for humans was not challenged based on mechanistic considerations.

RAC indeed noted that effects on the male reproductive system were observed only at doses that may exceed saturation of elimination. Toxicokinetic data *in vivo* shows that excretion into urine in rats was lower after a single dose of 1000 mg/kg (27% excreted in urine after 24h) than after a single dose of 2 mg/kg (88-95% excreted in urine after 24h). This indicates that saturation of active transport mechanisms occurs at the high dose and

physiologically based pharmacokinetic (PBPK) simulations (assuming Spirotetramat enters the systemic simulation as an enol) suggests that repeated daily doses of ≥ 300 mg/kg may lead to non-linear elimination kinetics in rats.

On the other hand, the following uncertainties were identified:

- It cannot be excluded that mechanistic elements other that toxicokinetics may explain the species specific sensitivity;
- The existence of a glucuronidation pathway has been identified in mice in an *in vitro* study and such a pathway is not present in rats. Although, it is not known whether this would result in lower *in vivo* systemic levels of Spirotetramat and its metabolites at high doses in the mice, it may indicate that elimination patways other than direct renal excretion of Spirotetramat exist in the mouse and in humans;
- *In vitro* data shows that the glucuronidation pathway is five-fold lower in humans than in mouse hepatocytes;
- In human hepatocytes, due to limited metabolism of the enol metabolite into further metabolites and limited conjugation, the level of enol metabolite formed *in vitro* is greater than in mouse or rat hepatocytes. This metabolite is likely to be involved in the reproductive toxicity of Spirotetramat;
- DME is formed in significant amounts in rats and this leads to lower levels of enol than in humans. DME and enol both have an affinity for OAT transporters and DME is likely to compete with enol for excretion into urine. But considering the lower level of the enol due to it being metabolised into DME, it is not known whether this difference in metabolism will lead to differences in systemic level of enol between rats and humans;
- It is noted that only one donor was used as the source for human hepatocytes in the *in vitro* metabolism study and the interpretation of the quantitative species differences is therefore not robust;
- Overall, the most appropriate model for humans between rats and mice is not established with certainty.

In conclusion, RAC considered that while the available data provided clear evidence of male reproductive toxicity of Spirotetramat in rats that is not secondary to other toxic effects, the absence of similar effects in mice and to some extent in dogs (although only a lower dose range was tested in dogs) suggests that this may be a species-specific mode of action. Effects on spermatogenesis were observed in rats at doses that may exceed doses above which the elimination rate is limited by saturation of active transport mechanisms. Metabolism in mouse and human hepatocytes also involves a conjugation pathway with glucuronic acid that is very limited in rats (up to 0.8% of metabolites *in vivo*). Such a pathway may prevent saturation of elimination mechanisms and exposure to high systemic levels of Spirotetramat metabolites.

Uncertainties exist in relation to the *in vivo* impact of the existence of the glucuronidation pathway in mice and the absence of formation of DME on the systemic level of Spirotetramat and its metabolites as well as in relation to the impact of the quantitative differences in glucuronidation between humans and mice. Besides, it is not known with certainty whether the sensitivity of rats can be explained by mechanistic factors and not (or not only) by toxicokinetic differences. On this basis, the relevance for humans cannot be excluded and a classification of Spirotetramat for fertility is justified.

However, the existence of a glucuronidation pathway in humans that is not present in rats indicates that humans may eliminate the Spirotetramat metabolites more efficiently and introduces doubt as to the relevance for humans of the effect observed in the rats. Therefore, on balance and having weighed all the available evidence, RAC concluded that

classification as Repr. 2 according to the CLP Regulation and Repr. Cat. 3; R62 according to DSD was justified for fertility.

SCL assessment

The most sensitive effects on the rat reproductive function were observed as follows:

- from 373 mg/kg bw/day in the rat two-year study, 16% of animals showed degeneration of latter stage spermatids versus none in the controls
- from 6000 ppm in F1 males in the range-finding reproductive toxicity study (equivalent to 320 mg/kg bw/day in premating P-generation males, intake in F1 males not stated in the DAR) showed an increase in the number of abnormal sperm presenting as amorphous sperm head to 20.2 versus 2.8 in controls.

In both studies, these doses therefore exceed the effective dose with a 10% effect level above the background (ED_{10}). According to the CLP guidance, ED_{10} is the relevant parameter for a preliminary assessment of potency. Since the ED_{10} value of Spirotetramat is between 4 and 400 mg/kg bw/day, it is considered of medium potency.

Toxicokinetic differences may be a relevant modifying factor to consider when concluding on potency. However, the uncertainties regarding the metabolite involved in the reproductive toxicity of Spirotetramat and their comparative *in vivo* kinetics between species, do not allow interpretation in the context of a potency assessment. It can be concluded that Spirotetramat induces effects on the male reproductive function with a moderate potency and the setting of specific concentration limits is therefore not justified.

Developmental toxicity

In the rabbit, an increased incidence of distinct liver lobulation was observed at the highest dose where severe maternal toxicity was observed (maternal deaths). Due to the unknown toxicological significance of this variation and its incidence being comparable to the historical control range, RAC did not consider this finding as relevant to justify classification for development.

In rats, the following findings were noted:

- Elevated incidence of retardation of ossification was observed, in particular in the phalanges. These findings were observed in the supplemental study in the absence of any maternal toxicity or foetal weight retardation, therefore a relationship with general growth retardation was not observed up to 140 mg/kg bw/day. However, in both studies, only a weak dose-response relationship was observed and incidences were within the historical control values; there is therefore only slight evidence that Spirotetramat may delay skeletal ossification.
- An increased incidence of wavy ribs was observed from 20 mg/kg bw/day in the initial study but this was not reproduced up to 140 mg/kg bw/day in the supplementary study and therefore only the high incidence observed at 1000 mg/kg bw/day was attributed to treatment. At this dose, this finding was present together with decreased maternal and foetal weight and may have been associated with general growth retardation. This finding is most often considered as reversible (Solecki, 2001) and as a variation. The increased incidence of two other skeletal variations, i.e. supernumerary ribs and enlarged fontanelles, was reported at this dose in the presence of general growth retardation. As variations, these findings are not considered sufficient to justify a classification for development.
- Dumbbell shaped thoracic vertebral bodies and pelvic shift in sacral vertebrae were observed at 1000 mg/kg bw/day in the presence of maternal toxicity. However, RAC considers that the decrease in maternal and foetal body weights observed may not explain these malformations and that this provides some evidence of developmental

toxicity of spirotretramat in the rat.

Based on this rationale, RAC considered that a classification as Repr. 2 according to the CLP Regulation and Repr. Cat. 3; R63 according to DSD was justified for developmental toxicity of Spirotetramat.

Supplemental information - In depth analyses by RAC
None

4.12 Other effects

No data evaluable.

4.12.1.1 Neurotoxicity

Spirotetramat has been assessed for potential neurotoxicity in two acute neurotoxicity studies in the rat (*Gilmore, 2005*) and was shown to have no neurotoxic potential in these studies.

In the first acute neurotoxicity study, rats received single oral doses of 0, 200, 500 or 2000 mg/kg bw spirotetramat via gavage. Evidence of acute oral toxicity was observed in both sexes at all dose levels but was limited to clinical signs (urine and perianal stain) and decreased activity in the figure-eight maze beginning on the day of treatment and with complete recovery by day 7.
In a follow-up study, rats received single oral doses of 0, 50, 100 or 500 mg/kg bw spirotetramat via gavage. The findings from the first study were confirmed at a dose level of 500 mg/kg bw. No compound-related effects were observed at 50 and 100 mg/kg bw.
The NOAEL for systemic effects in this study was established at 100 mg/kg bw spirotetramat. Neurotoxic effects were not observed up to a limit dose of 2000 mg/kg bw.

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

No data available.

4.12.1.4 Human information

No data available.

RAC evaluation of aspiration toxicity

Summary of the Dossier submitter's proposal
The DS concluded that no classification for aspiration toxicity was warranted for Spirotetramat but did not provide any further details.

Comments received during public consultation

No specific comments were received.

Additional key elements

No human data were reported which would raise concerns for a potential aspiration hazard.

The viscosity of Spirotetramat was not reported in the CLH report, but as the substance is not a hydrocarbon, this is not relevant in this case.

Assessment and comparison with the classification criteria

The substance does not fulfil the criteria for human evidence of an aspiration hazard, nor is it a hydrocarbon with a kinematic viscosity of 20.5 mm^2/s or less. No classification was therefore proposed by the RAC.

Supplemental information - In depth analyses by RAC

None.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 24: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis Guideline: 94/37/EC, 95/36/EC, OECD Guideline 111 (2002)	Spirotetramat, [3-^{14}C] and [5-^{14}C]-label: DT50 (pH 4, 25 °C): 32.5 d (SFO, r^2 = 1) DT50 (pH 7, 25 °C): 8.6 d (SFO, r^2 = 1) DT50 (pH 9, 25 °C): 0.32 d (SFO, r^2 = 1)	radiochemical purity: > 99 % and > 98 % (HPLC)	Heinemann, O. (2004a) Document No: M-09312401-2, MEF-04/176
Photolysis Guideline: 95/36/EC, 94/37/EC, SETAC (1995)	Spirotetramat, [3-^{14}C] and [5-^{14}C]-label: **Sterilized buffer solutions** DT50 (pH 5.0, 25 °C, irradiated): 2.7 d (SFO) DT50 (pH 5.0, 25 °C, dark): 26.2 d (SFO) DT50 (pH 5.0, 25 °C, net): 3.0 d (SFO) Converted to natural summer light: Athens (Greece, EU): DT50 22.2 days Phoenix (AZ, USA): DT50 14.4 days Edmonton (Alberta, Canada): DT50 20.2 days Spirotetramat, [3-^{14}C] and [5-^{14}C]-label: **Sterilized natural water** DT50 (pH 7.9, 25 °C, irradiated): 0.19 d (SFO) DT50 (pH 7.9, 25 °C, dark): 1.54 d (SFO) DT50 (pH 7.9, 25 °C): 0.22 d (SFO) Converted to natural summer light: Athens (Greece, EU): DT50 1.15 days Phoenix (AZ, USA): DT50 0.74 days Edmonton (Alberta, Canada): DT50 1.05 days	radiochemical purity: > 99 % and > 98 % (HPLC)	Stupp, H.-P., (2005a) Document No: M-266695-01-2, MEF-05/206
Biological degradation Guideline: ECCD 92/69/EEC C.4-D, OECD 301 F	Not ready biodegradable	purity 97.4 %	Weyers, A. (2005) Document No. M-263287-01-1, 2005/0077/01

Method	Results	Remarks	Reference
Water/Sediment Study Guideline: 95/36/EC, OECD 308 (2002)	Water: DT_{50}: 1.02 d DT_{90}: 3.4 d Whole system: DT_{50}: 1.06 d DT_{90}: 3.52 d	radiochemical purity: > 99 % and > 98 % (HPLC)	Menke, U. (2006c) Document No: M-269307-01-2, MEF-04/511
Kinetic Evaluation of the Aerobic Aquatic metabolism (Menke, 2006c) Guideline: Recommendations of FOCUS working group on degradation kinetics (FOCUS, 2006)	Water: DT_{50}: 0.78 d (geometric mean) DT_{90}: 2.58 d (geometric mean) Whole system: DT_{50}: 0.78 d (geometric mean) DT_{90}: 2.59 d (geometric mean)		Röpke, B., 2006b M-277415-01-1, MEF-06/279

5.1.1 Stability

Hydrolysis

Reference:	**[Azaspirodecenyl-3-^{14}C]- and [Azaspirodecenyl-5-^{14}C]BYI08330: Hydrolytic degradation**
Author(s), year:	Heinemann, O., 2004a
Study/report number:	M-09312401-2, MEF-04/176
Guideline(s):	94/37/EC, 95/36/EC, OECD Guideline 111 (2002), US-EPA N 161-1 (1982), Canada PMRA DACO 8.2.3.2 (1987), Japan MAFF New Test Guideline 12 Nousan 8147 (2001)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

MATERIAL AND METHODS:

Test substance:
- [Azaspirodecenyl-3-^{14}C]-spirotetramat, 3.71 MBq mg^{-1}, > 99 % radiochemical purity (HPLC), batch BECH 0980 and BECH 1518
- [Azaspirodecenyl-5-^{14}C]-spirotetramat, 4.03 MBq mg^{-1}, > 98 % radiochemical purity (HPLC), batch BECH 0981 and BECH 1519

Labels regarded as replicates

Reference substances: Spirotetramat (unlabelled), Spirotetramat-enol, Spirotetramat-ketohydroxy

Test systems:
- pH 4: 0.01 M sodium acetate/acetic acid buffer (adjusted with acetic acid)
- pH 7: 0.01 M tris(hydroxymethyl)aminomethane buffer (adjusted with 0.1 M HCl and/or 0.1 M NaOH)
- pH 9: 0.01 M borate buffer (adjusted with 0.4 M NaOH and/or boric acid)

All buffers sterilized (steam pressure sterilization) and checked for sterility throughout the test duration. Oxygen content reduced by nitrogen bubbling.

Volatile traps: No volatile traps (no volatiles expected – confirmed by complete material balance)

Test temperature: 50 °C (pre-test), 30 °C (pre-test, pH 9 only), 25 °C (main test) and 20 °C (optional test, pH 4 and 7 only)

Test duration: From 7 to 48 days in the dark

Sample conc.:	1.0 mg L^{-1}
Co-solvent:	Acetonitrile (< 0.5 %, v/v)
Analysis:	LSC, HPLC-UV/RAD, TLC, HPLC-MS/MS (ESI)
	LOQ < 1 % of AR
Kinetic evaluation:	Simple first order (SFO) kinetics, ModelManager 1.1, curve fit based on mean values of both labels

FINDINGS:

Mean material balances of all (*n* = 8) experiments were in a range of 97.2 – 104.4 % of AR. Owing to the complete mass balance $^{14}CO_2$ formation is considered to be negligible. Spirotetramat showed distinct hydrolysis at all temperatures and pH value tested, hydrolysis increased with increasing temperature and increasing alkalinity. No distinct differences between labels tested occurred. At 25 °C calculated half-life times were 32.5, 8.6 and 0.32 days (all SFO, R^2 > 0.99) at a pH of 4, 7 and 9. The only major metabolite formed in all tests was the common hydrolysis product spirotetramat-enol, which can be considered to be stable under the experimental conditions. Maximum of one unknown peak area was 8.8 % of AR (only using 5-^{14}C labelled parent at 50 °C and at a pH of 4). However, this peak area is considered as artefact, probably due to sample autoxidation prior to chromatography. Unidentified radioactivity did not exceed 2.7 % of AR.

Table 25: Hydrolysis of spirotetramat in sterile water buffered at pH 4, 7 and 9 [% of AR].

pH	4									7									9					
Tempera-ture	50 ℃			25 ℃			20 ℃			50 ℃			25 ℃			20 ℃			30 ℃			25 ℃		
Com-pound/ Label	DAT	[3-^{14}C]	[5-^{14}C]	DAT	[3-^{14}C]	[5-^{14}C]	DAT	[3-^{14}C]	[5-^{14}C]	HAT[a]	[3-^{14}C]	[5-^{14}C]	DAT	[3-^{14}C]	[5-^{14}C]	DAT	[3-^{14}C]	[5-^{14}C]	HAT	[3-^{14}C]	[5-^{14}C]	HAT	[3-^{14}C]	[5-^{14}C]
Spiro-tetramat	**0**	100.0	100.0	**0**	100.0	100.0	**0**	100.0	99.2	**0**	100.0	100.0	**0**	100.0	99.5	**0**	100.0	100.0	**0**	97.0	97.3	**0**	98.0	97.6
	0.25	99.9	98.0	**3**	96.4	94.1	**5**	91.5	91.7	**2**	90.4	88.8	**1**	89.8	90.9	**5**	77.5	75.2	**1**	75.9	79.4	**1**	87.2	87.7
	3	71.3	69.6	**7**	86.4	85.7	**8**	88.3	89.1	**4**	82.8	81.9	**3**	77.4	79.5	**8**	65.5	65.3	**2**	61.4	65.5	**2**	79.0	81.0
	4	62.6	60.3	**10**	79.6	79.4	**13**	82.3	83.4	**8**	68.9	69.2	**7**	55.7	56.0	**13**	50.2	48.5	**3**	50.6	53.0	**3**	72.4	73.3
	5	54.7	52.3	**14**	74.7	74.0	**16**	78.6	78.9	**10**	61.8	63.4	**13**	35.8	35.3	**16**	42.0	44.5	**4**	41.0	41.7	**4**	64.9	65.4
	6	51.0	48.6	**17**	69.8	68.0	**19**	76.0	75.5	**24**	32.1	32.6	**20**	19.2	19.8	**19**	36.6	35.8	**5**	33.4	31.5	**6**	55.6	58.1
	7	44.0	43.4	**21**	65.4	64.4	**23**	69.8	71.7	**30**	25.0	24.9	**24**	15.0	14.0	**23**	28.7	30.3	**6**	28.3	26.3	**8**	47.3	45.9
	10	30.2	29.8	**24**	60.7	59.4	**26**	67.2	68.7	**48**	10.2	11.0	**29**	10.3	8.9	**26**	26.4	25.0	**7**	21.3	22.3	**10**	na	38.7
	12	23.2	22.4	**31**	52.9	51.0	**30**	63.5	64.8							**30**	20.4	20.5				**24**	9.9	12.5
																						30	na	7.1
Spiro-tetramat-enol	**0**	nd	nd	**0**	nd	nd	**0**	nd	nd	**0**	nd	nd	**0**	nd	nd	**0**	nd	nd	**0**	3.0	2.3	**0**	2.0	2.4
	0.25	3.0	3.5	**3**	6.3	6.9	**5**	7.2	7.0	**2**	9.6	11.2	**1**	9.6	9.3	**5**	24.0	25.0	**1**	21.6	17.5	**1**	11.7	11.8
	3	29.1	30.0	**7**	14.0	14.0	**8**	10.9	10.8	**4**	18.6	18.7	**3**	23.0	23.1	**8**	36.3	35.8	**2**	36.6	31.9	**2**	20.6	20.3
	4	39.8	38.1	**10**	19.2	18.9	**13**	17.6	16.7	**8**	33.5	32.4	**7**	44.5	45.4	**13**	51.8	53.1	**3**	47.8	42.9	**3**	27.8	27.9
	5	43.5	43.9	**14**	26.1	25.4	**16**	21.0	20.3	**10**	40.5	38.5	**13**	65.2	66.5	**16**	60.4	57.2	**4**	57.5	56.9	**4**	35.0	34.6
	6	51.5	52.6	**17**	30.4	30.3	**19**	23.9	24.8	**24**	72.1	70.1	**20**	82.8	83.0	**19**	65.5	64.0	**5**	65.2	64.5	**6**	43.5	44.2
	7	58.7	52.9	**21**	36.0	36.5	**23**	27.4	27.6	**30**	79.5	77.8	**24**	84.1	87.5	**23**	72.3	71.4	**6**	70.8	68.1	**8**	52.3	55.8
	10	71.4	52.2	**24**	38.3	38.8	**26**	31.0	30.6	**48**	**93.4**	**93.4**	**29**	**91.4**	**92.2**	**26**	75.4	73.8	**7**	**75.4**	**75.9**	**10**	na	64.1
	12	**75.4**	**69.3**	**31**	**49.8**	**47.8**	**30**	**35.0**	**33.9**							**30**	**81.1**	**79.9**				**24**	**90.0**	89.6
																						30	na	**92.6**

nd denotes not determined
na denotes not analysed
[a] HAT denotes hours after treatment.

Table 26: Calculated hydrolytic DT_{50} and DT_{90} of spirotetramat in sterile water buffered at pH 4, 7 and 9 using SFO kinetics (mean of both labels).

pH	Temp [°C]	DT_{50} [days]	DT_{90} [days]	R^2	Kinetics
4	50	5.69	18.9	1.00	SFO
	25	32.5	108	1.00	SFO
	20	47.6	158	1.00	SFO
7	50	0.62	2.08	1.00	SFO
	25	8.6	28.7	1.00	SFO
	20	13.1	43.5	1.00	SFO
9	30	0.14	0.45	1.00	SFO
	25	0.32	1.04	1.00	SFO

CONCLUSION:

At environmental relevant conditions spirotetramat is degrading with a half-life of 32.5, 8.6 and 0.32 days at pH 4, 7 and 9 and 25 °C. It is concluded that hydrolysis is relevant for the overall elimination of spirotetramat in aquatic systems, especially under neutral and alkaline conditions. The only major metabolite deriving from hydrolysis of spirotetramat is spirotetramat-enol which is considered to be stable under the experimental conditions.

Photolysis:

Studies on the photolytic degradation were conducted with spirotetramat in

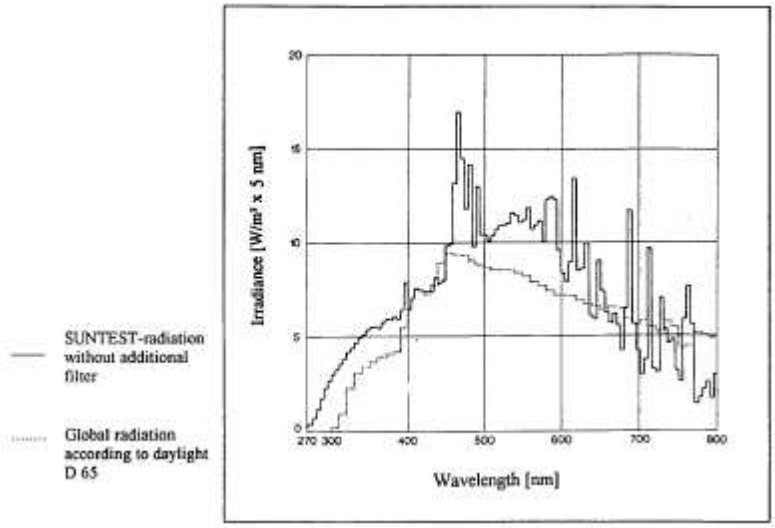
- sterile buffered water (pH 5.0), [3-^{14}C] and [5-^{14}C] label
- sterile natural water (pH 7.9), [3-^{14}C] and [5-^{14}C] label

Reference:	**BYI08330: Phototransformation of BYI08330 in sterile water**
Author(s), year:	Stupp, H.-P., 2005a
Study/report number:	M-266695-01-2, MEF-05/206
Guideline(s):	95/36/EC, 94/37/EC, SETAC (1995), US-EPA N 162-1 (1982), Canada PMRA DACO 8.2.3.3.2 (1987)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

MATERIAL AND METHODS:

Test substances:	• [Azaspirodecenyl-3-^{14}C]-spirotetramat, 3.67 MBq mg^{-1}, > 98 % radiochemical purity (HPLC), batch BECH 0950 • [Azaspirodecenyl-5-^{14}C]-spirotetramat, 4.03 MBq mg^{-1}, ≥ 98 % radiochemical purity (HPLC), batch BECH 0952 Labels regarded as replicates
Reference substances:	Spirotetramat (unlabelled), Spirotetramat-enol
Test system:	Sterile pH 5.0 buffer (0.1 M acetate buffer, autoclaved), adjusted with 0.1 M NaOH, sterility was checked throughout the experiment

Test temperature:	25 ± 1 °C
Test duration:	7 days continuous irradiation (equivalent to 51.7, 33.3 and 47.0 solar midsummer days in at Athens (Greece, EU, 38 °N), Phoenix (AZ, USA, 33 °N) and Edmonton (Alberta, CAN, 53 °N), respectively) or dark incubation
Sample conc.:	1.0 mg L^{-1}
Co-solvent:	Acetonitrile (0.1 %, v/v)
Test system:	Xenon arc lamp (Xenotest), cut-off < 290 nm, 990 W m^{-2} (300 – 800 nm), 22 cm above test vessel, light intensity measured by an irradiance sensor



Spectrum of experimental radiation is qualitatively similar to solar irradiation.

Volatile traps:	1 x soda lime and 1 x polyurethane foam
Analysis:	LSC, HPLC-UV/RAD, TLC, HPLC-MS/MS (ESI), LC-NMR LOD = 1 % of AR
Kinetic evaluation:	Simple first order (SFO) kinetics, ModelManager 1.1, curve fit based on mean values of both labels

FINDINGS:

Mass balance was in a range of 93.8 to 108.4 % of AR for all experiments. Under the impact of light small amounts (maximum 2.6 % of AR) of $^{14}CO_2$ were released from the [3-^{14}C] labelled parent indicating mineralization at this part of the molecule. Almost no ^{14}C was released from [5-^{14}C] labelled spirotetramat. Formation of organic volatiles was negligible. Under irradiation spirotetramat was subjected to extensive photolytic rearrangement procedures, resulting in 4 major metabolites: spirotetramat-photo-cyclopentyl (maximum 42.9 % of AR), spirotetramat-photo-hydroxymethyl (22.9 % of AR), spirotetramat-photo-formyl (11.5 % of AR) and spirotetramat-photomethyl carbonate (19.3 % of AR). These major transformation products did not decline significantly. Unknown compounds did not exceed 5.5 % of AR individually. Without irradiation only the common hydrolysis product spirotetramat-enol was formed.

Table 27: Calculated photolytic DT_{50} and DT_{90} [days] of spirotetramat in 0.01 M acetate buffer at pH 5.0 using SFO kinetics (mean value of both labels).

Conditions	Experimental			Natural conditions			Kinetics
	DT_{50} [days]	DT_{90} [days]	R^2	DT_{50} [days], Athens, Greece, EU	DT_{50} [days], Phoenix, AZ, USA	DT_{50} [days], Edmonton, Alberta, Canada	
Irradiated	**2.7**	**9.0**	**0.98**	**12.0[a]**	**9.3[a]**	**11.4[a]**	**SFO**
Dark	26.2	86.9	0.89	26.2	26.2	26.2	SFO
Net[b]	3.0	10.0	-	22.2	14.4	20.2	SFO

[a] Re-calculated by the reviewer (see comments)
[b] Calculated by the reviewer

CONCLUSION:
Under irradiated experimental conditions, spirotetramat degraded with a half-life of 2.7 days at a pH of 5 and 25 °C (SFO kinetics, R^2 = 0.98) owing to photolysis and hydrolysis. This experimental half-life corresponds to 12.0, 9.3 and 11.4 days under environmental midday summer light in Athens (Greece, EU), Phoenix (AZ, USA) and Edmonton (Alberta, Canada). In the dark control DT_{50} was 26.2 days (owing to hydrolysis) indicating that photodegradation of spirotetramat can be considered a significant route for the elimination of the compound from the environment. Under the sterile experimental conditions and the impact of light, spirotetramat is transformed to the major metabolites spirotetramat-photo-cyclopentyl, spirotetramat-photo-hydroxymethyl, spirotetramat-photo-formyl and spirotetramat-photomethyl carbonate (all these metabolites considered as photo-rearrangement products). Maximum amounts formed were 42.9, 22.9, 11.5 and 19.3 % of AR, respectively. Under dark conditions only the common hydrolysis product spirotetramat-enol was observed.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

As measured data are available estimation is not relevant for this dossier.

5.1.2.2 Screening tests

Readily biodegradability

Reference:	**BYI 08330 Biodegradation**
Author(s), year:	Weyers, A., 2005
Study/Report number:	M-263287-01-1, 2005/0077/01
Guideline(s):	ECCD 92/69/EEC C.4-D, OECD 301 F
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

MATERIAL AND METHODS:

Test substance:	Spirotetramat (unlabelled), purity 97.4, batch MIX-Batch 08045/0014
Reference substance:	Na-benzoate
Inoculum:	Aeration tank of a waste water plant treating predominately domestic sewage

	(30 mg L^{-1})
Treatments:	• Blank control • Reference substance: Na-benzoate (100 mg L^{-1}) • Test substance: Spirotetramat (100 mg L^{-1}) • Toxicity control: Spirotetramat (100 mg L^{-1}) and Na-benzoate (100 mg L^{-1}) pH of the test vessel at the end of test: 7.3
Analysis:	Biochemical oxygen demand (corrected for oxygen consumption by nitrification)
Incubation conditions:	22 ± 2 °C, 28 days

FINDINGS:

Table 28: Biodegradation of spirotetramat and reference compound [% of theoretically possible degradation].

DAT	Reference substance	Test substance	Toxicity control
4	73	0	29
12	88	1	37
20	91	0	38
28	91	1	40

Within 28 days almost no degradation (maximum 1 %) was determined for spirotetramat. The reference substance (Na-benzoate) has reached level for ready biodegradability by 14 days.

CONCLUSION:

Spirotetramat is considered to be not readily biodegradable.

5.1.2.3 Simulation tests

Biodegradation in water/sediment systems

Reference:	**BYI08330: Aerobic Aquatic Metabolism**
Author(s), year:	Menke, U., 2006c
Study/report number:	M-269307-01-2, MEF-04/511
Guideline(s):	95/36/EC, OECD Guideline 308 (2002), US-EPA N 162-4 (1982)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

MATERIAL AND METHODS:

Test substances:	• [Azaspirodecenyl-3-^{14}C]-spirotetramat, 3.67 MBq mg^{-1}, > 98 % radiochemical purity (HPLC), batch BECH 0955 • [Azaspirodecenyl-5-^{14}C]-spirotetramat, 4.03 MBq mg^{-1}, > 98 % radiochemical purity (HPLC), batch BECH 0956 Labels regarded as replicates
Reference substances:	Spirotetramat (unlabelled), spirotetramat-enol, spirotetramat-ketohydroxy, spirotetramat-MA-amide, spirotetramat-di-hydroxy, spirotetramat-oxo-enol, benzoic acid
Application rate:	29 µg L^{-1} (under the assumption of 288 g ai ha^{-1}, 100 cm water depth)
Co-solvent:	Acetonitrile (0.015 %, v/v)
Incubation set-up:	102.4 g (‘Hönniger Weiher’) and 182.1 g (‘Anglerweiher’) dry sediment (2 mm sieved), 519 mL of water (sediment/water, 1/3, w/v), equivalent to 2 cm sediment and 6 cm water column, water phase kept in motion by stirring, sediment stagnant
Acclimatization period:	Approx. 7 days
Test duration:	120 days
Incubation cond.:	20 ± 1 °C in darkness

Volatile traps:	Polyurethane foam (organic volatiles), soda lime (CO_2), identity of $^{14}CO_2$ confirmed by Grignard Reaction
Monitoring:	pH, O_2, redox potential
Analysis:	Water phase radio-assayed by LSC and concentrated using a speedvac concentrator. Sediment phase extracted 3 times with 100 ml of acetonitrile/water/formic acid (50/50/0.5, v/v/v). All three extracts were combined (**'organic extract'**). Then the sediment was extracted 2 times with 100 ml of acetonitrile/1 M HCl (50/50, v/v) and once with 100 ml of pure acetonitrile (all three combined to **'acid extract'**). Organic extracts were separately concentrated using a speedvac concentrator. Aliquots of the acidic extracted soils from 120 DAT samples were separated into humic acids, fulvic acids and humins. Non extractable residues were radio-assayed by combustion LSC. LOQ(HPLC) approx. 0.5 – 0.8 % of AR
Analytical techniques:	LSC, HPLC-UV/RAD, TLC, LC-MS/MS (ESI)
Kinetic evaluation:	Simple first order (SFO) kinetics, MatLab

FINDINGS:

The oxygen content of the water phase of the samples was in a range of 7.2 – 8.5 mg L^{-1} ('Hönniger Weiher') and 7.7 – 8.3 mg L^{-1} ('Anglerweiher'), indicating aerobic conditions throughout both experiments. The pH in the water phase was slightly increasing from 6.8 by 0 DAT to approx. 7.5 by 120 DAT ('Hönniger Weiher') and from 7.9 – 8.2 ('Anglerweiher'). In the sediment the pH stayed more or less unchanged with values about 6.8 ('Hönniger Weiher') and 7.3 ('Anglerweiher'). The redox potential in the water phase was fairly constant in a range of +353 – +428 mV ('Hönniger Weiher') and +351 – +397 mV ('Anglerweiher'), in the sediment the redox potential was in a range of +42 – +189 mV ('Hönniger Weiher') and +154 – +360 mV ('Anglerweiher') with a steady decrease in the initial time of the latter test system. In summery, in both systems water and sediment stayed aerobically throughout the study period.

Total mass balance was in a range of 94.4 – 102.6 % of AR for both systems and both labels. Formation of $^{14}CO_2$ using 3-^{14}C accounted for maximum of 11.0 % ('Hönniger Weiher) and 24.0 % ('Anglerweiher') of AR at study termination. Formation of $^{14}CO_2$ from 5-^{14}C labelled spirotetramat was approx. half indicating that this part of the molecule is less available to biodegradation than the 3-^{14}C position. Formation of NER steadily increased in both systems towards study termination with a maximum of 40.7 % of AR by 91 DAT.

Spirotetramat quickly degraded in both water/sediment systems, by 7 DAT no spirotetramat was detectable in the water or in the sediment phase. Transfer of the test item into the sediment phase was almost negligible with a maximum occurrence of 3.4 % of AR by 1 DAT. No distinct differences between labels used were observed. Degradation of spirotetramat led to extinct formation of the common hydrolysis product spirotetramat-enol (maximum 99.0 % of AR by 14 DAT in the entire system of 'Hönniger Weiher' and 92.1 % of AR by 7 DAT in 'Anglerweiher'). Maximum amounts of spirotetramat-enol found in the sediment were 41.2 % and 15.7 % of AR ('Hönniger Weiher' and 'Anglerweiher'). After reaching maximum, amounts of spirotetramat-enol significantly decreased thereafter in both, water and sediment. As a second major metabolite spirotetramat-ketohydroxy was formed > 10 % of AR in the entire system with a more or less increasing trend towards study termination (maximum in water, sediment, entire system: 17.4 %, 42.8 % and 50.8 % of AR, respectively). Three further identified metabolites (spirotetramat-

MA-amide, spirotetramat-oxo-enol isomer and spirotetramat-di-hydroxy) did not exceed 9.2 %, 6.0 % and 9.8 % of AR, respectively (all significantly decreasing thereafter). Unidentified radioactivity (sum) was below 8.1 % of AR in the entire system of both test systems.

Table 29: Calculated half-lives [days] of spirotetramat in the aerobic water/sediment systems 'Hönniger Weiher' and 'Anglerweiher' based on SFO kinetics.

System	Label	Water			Total system			Kinetics
		Dissipation			Degradation			
		DT_{50} [days]	DT_{90} [days]	R^2	DT_{50} [days]	DT_{90} [days]	R^2	
'Hönniger Weiher'	Labels used as replicates	1.00	3.31	0.99	1.06	3.52	1.00	SFO
'Anglerweiher'	Labels used as replicates	1.02	3.40	0.99	1.05	3.50	0.99	SFO

CONCLUSION:

Spirotetramat quickly degraded in two contrasting aerobic water/sediment systems with a degradation half-life in the entire system of 1.0 day (both systems, SFO, $R^2 \geq 0.99$). Dissipation from the water layer was fast as well. No distinct differences between labels used were observed. As major metabolites spirotetramat-enol (maximum in the total system 99.0 % of AR by 14 DAT, decreasing thereafter) and spirotetramat-ketohydroxy (maximum in the total system 50.8 % of AR at study termination) were observed. Other identified substances (spirotetramat-MA-amide, spirotetramat-oxo-enol isomer and spirotetramat-di-hydroxy) did not exceed 9.8 % of AR in the total system individually, non identified radioactivity accounted for maximum of 8.1 % of AR in sum. Formation of $^{14}CO_2$ (maximum 24.0 % of AR in one system by study termination) indicate significant mineralization of the test item, NER accounted for maximum 40.7 % of AR by 91 DAT.

Supplemental studies: Kinetic evaluation of the water/sediment study

Reference:	**Kinetic Evaluation of the Aerobic Aquatic metabolism of BYI08330, BYI08330-enol and BYI08330-ketohydroxy in water sediment systems**
Author(s), year:	Röpke, B., 2006b
Study/Report number:	M-277415-01-1, MEF-06/279
Guideline(s):	Recommendations of FOCUS working group on degradation kinetics (FOCUS, 2006)
GLP:	Not applicable
Deviations:	Not applicable
Validity:	Study considered acceptable

MATERIAL AND METHODS:

The behaviour of spirotetramat and its major water/sediment metabolites spirotetramat-enol and spirotetramat-ketohydroxy in an aquatic environment was investigated by kinetic evaluation of the aerobic water/sediment studies conducted with the two test systems 'Hönniger Weiher' and 'Anglerweiher'.

Simple first order (SFO) dissipation half-life of spirotetramat in the water phase is based on SFO kinetics. Additionally, overall SFO degradation of spirotetramat and spirotetramat-enol and spirotetramat-

ketohydroxy in the entire system were derived using a four-compartment model (no additional pathways to sink).

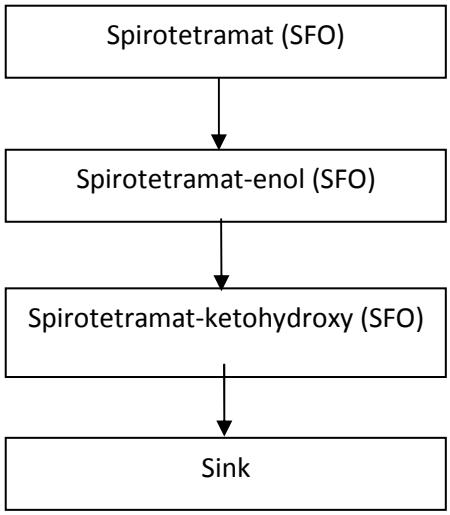


Figure 5.1.2.3-1: Multi-compartment model used for fitting residues of spirotetramat and metabolites in water/sediment studies (entire system).

The goodness of fit was assessed by visual inspection, R^2, χ^2 error and t-test statistics. Calculations were performed with MatLab 7.0.4.365 (MatLab, 2005). Different equations were integrated by a Runge-Kutta method, and the Levenberg-Marquardt algorithm was used for non-linear parameter optimisation.

In case of spirotetramat-ketohydroxy, the 3 DAT sampling of 'Hönninger Weiher' was considered an outlier, amounts of spirotetramat-ketohydroxy were set to < LOQ.

FINDINGS:

Table 30: Calculated dissipation (water phase) and degradation (entire system) half-life of spirotetramat and the metabolites spirotetramat-enol and spirotetramat-ketohydroxy in two aerobic water/sediment systems based on SFO kinetics (both labels handled as replicates).

Substance	Compartment	Test system	DT_{50} [days]	DT_{90} [days]	R^2	χ^2 error [%]	Kinetics
Spirotetramat	Water (Dissipation)	'Hönniger Weiher'	0.82	2.73	0.99	6.9	SFO
		'Anglerweiher'	0.74	2.45	0.97	12.3	SFO
	Entire system (Degradation)	'Hönniger Weiher'	0.86	2.85	0.99	6.2	SFO
		'Anglerweiher'	0.70	2.34	0.97	11.9	SFO
Spirotetramat-enol	Entire system (Degradation)	'Hönniger Weiher'	59.0	196	0.93	13.4	$P_{SFO} \rightarrow M_{SFO}$
		'Anglerweiher'	37.9	126	0.93	15.6	$P_{SFO} \rightarrow M_{SFO}$
Spirotetra	Entire	'Hönniger	Not evaluable, no decline				$P_{SFO} \rightarrow M1_{SFO} \rightarrow$

Substance	Compartment	Test system	DT_{50} [days]	DT_{90} [days]	R^2	χ^2 error [%]	Kinetics
mat-ketohydroxy	system (Degradation)	Weiher'					$M2_{SFO}$
		'Anglerweiher'	Not evaluable, no decline				$P_{SFO} \rightarrow M1_{SFO} \rightarrow M2_{SFO}$

CONCLUSION:

Degradation of spirotetramat in the entire system was calculated to be 0.86 and 0.70 days for the 'Hönniger Weiher' and 'Anglerweiher' test system, respectively (following SFO kinetics, $R^2 \geq 0.97$, $\chi^2 \leq 6.9$). Based on multi-compartment modelling, metabolite spirotetramat-enol degraded with a DT_{50} in the range of 37.9 – 59.0 days in the total system (all SFO, $R^2 > 0.93$). Metabolite spirotetramat-ketohydroxy was considered to be stable in both systems.

5.1.3 Summary and discussion of degradation

Hydrolysis: Spirotetramat was shown to hydrolyse in strong dependence of the pH value and temperature, $DegT_{50}$ values at 25 °C were 32.5, 8.6 and 0.32 days at pH 4, 7 and 9, respectively. Hydrolysis rates decreased with decreasing temperature.

Photolysis: Under irradiation in sterile buffer solutions at pH 5.0, spirotetramat degraded with a half-life of 2.7 days (following SFO kinetics, $R^2 = 0.98$, net photolysis half-life corrected for hydrolysis was 3.0 days). The experimental half-life (including photolysis and hydrolysis) corresponds to 12.0, 9.3 and 11.4 days under environmental midday summer light in Athens (Greece, EU), Phoenix (AZ, USA) and Edmonton (Alberta, Canada). In the dark control, $DegT_{50}$ was 26.2 days (owing to hydrolysis, only) indicating that photodegradation of spirotetramat can be considered a significant route for the elimination of the compound from the environment.

Ready biodegradability: Results of a readily biodegradability study indicate that spirotetramat is not readily biodegradable.

Water/sediment study: Dark aerobic water/sediment studies were conducted with two contrasting (pH, texture) natural systems, 'Hönniger Weiher' and 'Anglerweiher', using [3-^{14}C] and [5-^{14}C] labelled spirotetramat. The 'Hönniger Weiher' test system represents a loam sediment with an organic carbon content of 4.4 %, a microbial activity of 36 mg CO_2 h^{-1} kg^{-1} and a pH of 5.6 ($CaCl_2$). The 'Anglerweiher' test system is characterized by a loamy sand sediment with a pH of 6.8 ($CaCl_2$), with an organic carbon content of 0.99 % and a lower microbial activity (10 mg CO_2 h^{-1} kg^{-1}). In both systems, water and sediment stayed aerobically throughout the test period.

Mineralization of spirotetramat to $^{14}CO_2$ was low in the 'Hönniger Weiher' test system, accounting for 11.0 % and 5.9 % of AR by study termination (120 DAT) using [3-^{14}C] and [5-^{14}C] labelled parent, respectively. Formation of $^{14}CO_2$ from [5-^{14}C] labelled spirotetramat was approx. half indicating that this part of the molecule is less accessible to biodegradation than the [3-^{14}C] position. Formation of $^{14}CO_2$ was higher in the 'Anglerweiher' test system accounting for 24.0 % and 13.5 % of AR using [3-^{14}C] and [5-^{14}C] labelled parent, respectively.

Maximum formation of NER (mean value of both labels) accounted for 36.4 % and 33.1 % of AR in the 'Hönniger Weiher' and Anglerweiher', respectively. In the first test system, maximum of NER formation was observed by study termination (120 days), in the latter system, maximum formation of NER was found by

91 DAT, slightly decreasing thereafter to 34.6 % of AR by study termination (120 days). Differences between labels used were small in case of the 'Hönninger Weiher' and insignificant in case of the 'Angler Weiher'.

In the total system, a rapid decline of spirotetramat was observed with $DegT_{50}$ of 0.9 and 0.7 days in the 'Hönninger Weiher' and 'Anglerweiher' test system (following SFO kinetics), respective $DegT_{90}$ values were 2.9 and 2.3 days. Dissipation in the water phase was calculated to be 0.8 and 0.7 in the 'Hönninger Weiher' and 'Anglerweiher' test system. No distinct differences between labels used were observed.

By using a **kinetic evaluation** method, the degradation of spirotetramat in the entire system was calculated to be 0.86 and 0.70 days for the 'Hönniger Weiher' and 'Anglerweiher' test system, respectively (following SFO kinetics, $R^2 \geq 0.97$, $\chi^2 \leq 6.9$).

5.2 Environmental distribution

Route of degradation in soil

The route of degradation of spirotetramat was established on 3 EU soils and one US soil in two studies using either spirotetramat or spirotetramat-enol (which is rapidly released from spirotetramat) as parent.

Under standard aerobic conditions (20 °C), the metabolism of spirotetramat ([3-^{14}C] label) was shown to proceed via three pathways which all start with the hydrolytic cleavage of the spirotetramat ester into the spirotetramat-enol. Following the major degradation pathway, spirotetramat-enol is rapidly oxidized at the benzylic carbon position into spirotetramat-ketohydroxy which is hydrolytically opened into spirotetramat-MA-amide and finally mineralized into CO_2. In the second (minor) pathway demethylation of spirotetramat-enol into spirotetramat-desmethyl-enol and oxidation of this degradate into spirotetramat-oxo-enol occurred. In a third degradation pathway spirotetramat-enol-dimer 1 and spirotetramat-enol-dimer 2 were pretended to be built through oxidative enol dimerization. However, this pathway is regarded as non relevant under environmental conditions, because the formation of dimers is considered an artificial process mainly caused by the hot-spot application in this laboratory tests.

Formation of CO_2 in the spirotetramat degradation study was in a range of 9.7 – 19.4 % of AR by 50 DAT (i.e. the study termination in case of the EU soils), in the US soil, CO_2 further increased from 9.7 % of AR by 50 DAT to 15.3 % of AR by study termination (360 DAT). No volatile organics could be detected. The soil with the highest content of microbial biomass showed the highest mineralization rate. Formation of CO_2 in the spirotetramat-enol degradation study was in a range of 16.7 – 43.0 % of AR by study termination (119 DAT). Significant higher $^{14}CO_2$ amounts were released from spirotetramat-enol labelled in the [3-^{14}C] position. No volatile organics could be detected.

In the spirotetramat study, the portion of non-extractable radioactivity (NER) rapidly increased (by 1 – 3 DAT), reaching more or less a plateau concentration in the range of approx. 22 – 35 % of AR, hardly decreasing thereafter. A similar but even more pronounced formation of NER was determined in the spirotetramat-enol study using a slightly alkaline extraction procedure. Directly after application (0-DAT sampling) already 4.2 – 28.4 % of AR was bound to the soil matrix. Amounts of NER rapidly increased further to reach a plateau concentration by 1 DAT and thereafter. Plateau concentration was in a range of 40 – 60 % of AR. No significant decrease of NER was found with time.

In an additional outdoor study, the degradation of [3-^{14}C] labelled spirotetramat (spirotetramat formulated as an OD 100) was investigated under more realistic environmental conditions including natural sunlight. In

comparison to the laboratory degradation study, beside the already mentioned metabolites three new metabolites were identified within this study: spirotetramat-glyoxylic amide (maximum 2.3 % of AR by 7 DAT), dimethyl-benzoic acid (3.3 % of AR by 7 DAT) and spirotetramat-ketohydroxy-carboxy (1.3 % of AR). Further, it has to be concluded that the formation of the spirotetramat-enol dimers within the outdoor study with another application technique (broadcast application) was about ≤ 1.5 % of AR. Maximum formation of NER was distinct lower than observed in the laboratory studies with 16.8 % of AR by 63 DAT decreasing thereafter indicated an enhanced overall degradation of the parent and its metabolites in soil under real outdoor conditions in comparison to the laboratory degradation studies.

Under anaerobic conditions the degradation pathway is almost identical to the degradation pathway obtained in aerobic soil. It was shown that spirotetramat declines quickly in a subsequently flooded anaerobic soil situation. The detected metabolites were almost identical to those formed upon the aerobic transformation in the aerobic metabolism study, except of the minor metabolite spirotetramat-di-hydroxy. But spirotetramat-di-hydroxy was formed in the aerobic aquatic metabolism study. Therefore it can be excluded that this metabolite will be specially formed under anaerobic conditions.

It was not possible to assess the rate of photodegradation of spirotetramat on moist soil because most degradation was due to microbial activity (which was more active under dark conditions). Under dry conditions microbial activity was reduced and spirotetramat photodegraded, but the (supplemental) study had too few data points to calculate a half-life. In general, phototransformation on moist soil surfaces is not considered to significantly contribute to the overall dissipation of spirotetramat from the environment owing to the extremely rapid degradation under moist conditions.

Rate of degradation in laboratory studies

The laboratory soil degradation rate of spirotetramat and metabolites was investigated in 3 EU soils and 1 US soil with the following range of soil properties (pH, organic C, clay content) using [3-^{14}C] labelled spirotetramat or [5-^{14}C] and [3-^{14}C] labelled spirotetramat-enol as parent.

- pH ($CaCl_2$) 5.4 – 6.7
- organic C 0.93 – 2.1 %
- clay content 5.0 – 12.0 %

No degradation rates are available for more alkaline soil (with a pH > 7). However, degradation of spirotetramat was extremely fast in the pH range tested and no dependency on soil pH was observed. From hydrolysis behaviour it can be deduced that degradation of spirotetramat is likely to be even faster at higher pH values. In field studies conducted on 4 US soils with a pH range of 7.0 – 8.1 (pH measured in water, equivalent to approx. pH 5.5 – 7.3 in $CaCl_2$), no dependence on soil pH was observed. In this respect, it is highly unlikely that degradation of spirotetramat under more alkaline conditions will significantly differ from degradation rates observed under more acidic conditions.

Under aerobic conditions, spirotetramat was found to be a very fast degrading compound following biphasic degradation. Following best fit kinetics (i.e. double first order in parallel kinetics, DFOP), non-normalized degradation half-life ($DegT_{50}$) values of spirotetramat were in a range of 0.09 – 0.30 days (chi^2 error ≤ 6.1 %); respective $DegT_{90}$ was in a range of 0.34 – 1.26 days. Since most environmental models are not capable to handle DFOP kinetics, a conservative SFO-$DegT_{50}$ for modelling may be derived from the DFOP-$DegT_{90}$ divided by 3.32. This procedure results in a $DegT_{50recalc}$ in the range of 0.10 – 0.38 days.

Applying SFO kinetics (chi^2 error ≤ 21.8 % for one soil, for three soils chi^2 error ≤ 9.5 %), non-normalized $DegT_{50}$ values of spirotetramat were in a range of 0.08 – 0.33 days, respective $DegT_{90}$ values were in a range of 0.28 – 1.09 days. Following normalization to 20 °C and pF2, obtained $DegT_{50norm}$ values were in a range of 0.05 – 0.23 days.

The biodegradation of spirotetramat was additionally investigated in two soils under outdoor climatic conditions considered more realistic for the indented use (metabolism studies). In close agreement to the lab results, the parent compound was quickly degraded with a DT_{50} of 1.2 and 2.9 days ('Laacherhof AIIIa' and 'Molino' soil, respectively, both SFO kinetics, $R^2 \geq 0.98$).

Table 31: Summary on laboratory non-normalized, recalculated and normalized recalculated DegT50 and DegT90 values [days] of spirotetramat in soil according to DFOP (best fit for both compounds), FOMC and SFO kinetics.

Compound	Soil	DFOP (best fit kinetics)				FOMC				SFO		
		Deg T_{50} [d]	Deg T_{90} [d]	Deg $T_{50\ recalc}$[a] [d]	Norm. $DegT_{50\ recalc}$ [d]	Deg T_{50} [d]	Deg T_{90} [d]	Deg $T_{50\ recalc}$[a] [d]	Norm. Deg $T_{50\ recalc}$ [d]	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Norm. $DegT_{50}$ [d]
Spirotetramat (parent in study)	'Laacherhof AXXa'	0.24	0.89	0.27	0.21	0.23	1.04	0.31	0.24	0.21	0.69	0.17
	'Laacherhof AIII'	0.26	0.97	0.29	0.21	0.25	1.09	0.33	0.23	0.23	0.77	0.16
	'Hoefchen'	0.09	0.34	0.10	0.07	0.01	0.44	0.13	0.09	0.08	0.28	0.05
	'Molino'	0.30	1.26	0.38	0.26	0.26	2.61	0.79	0.55	0.33	1.09	0.23
	Geometric mean (EU)	nc	nc	0.23	0.17	nc	nc	0.32	0.23	0.19	0.63	0.13
	80th %ile (PMRA)	nc	nc	0.33	nc	nc	nc	0.51	nc	0.27	0.90	nc
	90th %ile C.I. on mean[b] (US-EPA)	nc	nc	0.36	nc	nc	nc	0.62	nc	0.30	0.98	nc

[a] Conservative SFO-DT_{50} recalculated from DFOP-DT_{90} and FOMC-DT_{90}, respectively, divided by 3.32.
[b] Calculated by [arithmetic mean + ($t_{90,n-1}$ × SD) / $n^{0.5}$], whereby $t_{90,n-1}$ = one-sided students *t* value at α = 0.1, SD = standard deviation and n = number of data available

Field dissipation studies

Four field dissipation studies (including bare and cropped soils) were conducted with spirotetramat on a range of soil types in the USA, representing rather worst-case scenarios in respect to degradation (low amounts of organic C) and leaching behaviour (sandy soils with low contents of clay). Spirotetramat was applied once at 439 g ai ha^{-1} (single application) formulated as the oil dispersion BYI08330 100 OD.

Dissipation of spirotetramat in field trials was rapid with a dissipation half-life (DT_{50}) in a range of 0.3 – 1.0 day (SFO kinetics), respective DT_{90} values were in a range of 1.1 – 3.5 days. Since almost no transfer of spirotetramat into soil layers below 15 cm was observed and volatilization is considered to be minimal (vapour pressure 5.6 × 10^{-9} Pa), dissipation of spirotetramat is considered more or less consistent with degradation. No differences were observed between bare ground and cropped plots. Owing to the short half-life of spirotetramat in soil, potential of accumulation and carry over of spirotetramat from one year to the other year is considered negligible.

Residues of spirotetramat did not move below the surface layer (0 – 15 cm) in all sites, except Florida where residues of spirotetramat-enol and spirotetramat-ketohydroxy were detected above the LOQ (i.e. 5 µg kg^{-1}) at 15 – 30 cm layer between 1 and 7 DAT. Thereafter, residues completely degraded to less than LOQ and LOD. It should be noted that the Florida test site represents worst-case conditions with heavy rainfall, very light soil (95 % sand in the top layer) and low organic matter (0.2 %). Based on field dissipation data, leaching and groundwater contamination is not likely to occur after application of spirotetramat according to GAP.

Table 32: Summary on dissipation half-lives of spirotetramat in four US dissipation field trials (non-normalized data).

Site	Crop	Texture	pH ($CaCl_2$)	pH (soil/water, 1/1, m/v)	Organic C [%]	Clay [%]	DT_{50} [days]	DT_{90} [days]	R^2	χ^2 error [%]	Kinetics
		0 – 15 cm									
'New York', US	Bare ground	Loamy sand	5.5	6.3	0.70	9	0.5	1.6	0.94	5.1	SFO
'Florida', US	Bare ground	Sand	-	7.1	0.23	2	0.9	2.9	0.96	2.2	SFO
	Bush beans	Sand	-	7.1	0.23	2	1.0	3.3	0.96	0.4	SFO
'California', US	Bare ground	Sandy loam	-	7.2	0.29	9	1.0	3.5	0.98	12.1	SFO
	Tomatoes	Sandy loam	-	6.8	0.29	7	1.0	3.4	0.97	12.6	SFO
'Washington', US	Bare ground	Sandy loam	-	8.1	0.41	5	0.4	1.4	0.99	1.6	SFO
	Onions	Loamy sand	-	8.0	0.35	5	0.3	1.1	0.99	0.4	SFO
Geometric mean (EU)							0.7	2.2			
80th %ile (PMRA)							1.0	3.4			
90th %ile of C.I. on mean[a] (US-EPA)							0.9	3.0			

[a] Calculated by [arithmetic mean + ($t_{90,n-1}$ × SD) / $n^{0.5}$], whereby $t_{90,n-1}$ = one-sided students *t* value at α = 0.1, SD = standard deviation and n = number of data available

5.2.1 Adsorption/Desorption

Reliable adsorption constants according to Freundlich isotherms (equilibrium batch experiments) could be achieved for spirotetramat using 5 soils from the EU, USA and Canada with a representative set of soil properties:

Soil pH ($CaCl_2$): 4.7 – 6.8

Organic carbon: 0.7 – 2.4 %

Clay: 7 – 28 %

No adsorption/desorption constants are available for spirotetramat in more alkaline soils (pH ≥ 7.0 in $CaCl_2$). However, no dependency of the adsorption behaviour onto the soil pH could be stated in the pH range tested. In this respect, it is unlikely that adsorption/desorption under more alkaline conditions will significantly differ from values obtained in the tested range. This assumption is also confirmed by modelling results from the US field trials, showing a topsoil pH in the range of 6.3 – 8.1 (in water). These field trials

were fully re-modelled with the (uncalibrated) parameter set used for spirotetramat and metabolites, based on K_{OC} values obtained from the tested pH range of 4.7 – 6.8 (for details refer to Röpke and Ramanarayanan, 2007). Modelled and measured data were highly consistent also in more alkaline soils.

Linear adsorption constants (K_d) and Freundlich adsorption constants (K_F) of spirotetramat have been determined in batch equilibrium experiments with five different soils using the [3-^{14}C] labelled test substance. Since significant degradation of the test item was observed in a pre-test, the main test was performed with sterilized soil (using mercuric(II)chloride) and the equilibrium time was restricted to 3 hrs. Based on organic carbon content, K_{FOC} values for the different soils were in a range of 159 – 435 L kg^{-1} (arithmetic mean 1/n = 0.94). Linear adsorption constants (K_{OC}) were close to the Freundlich adsorption constants with a range of 184 – 437 L kg^{-1}. Based on these values, spirotetramat is classified as medium mobile according to the classification scheme of McCall et al. (1981)[1] and moderately mobile according to FAO[2] classification.

5.2.2 Volatilisation

Based on the low vapour pressure of spirotetramat (< 10^{-9} Pa) no significant transfer into the atmosphere is expected to occur. In the eventuality that spirotetramat enters the atmosphere, the compound is expected to rapidly degradate owing to reaction with OH radicals (atmospheric half-life < 2 hrs based on Atkinson calculation).

5.2.3 Distribution modelling

No information available.

5.3 Aquatic Bioaccumulation

Table 33: Summary of relevant information on aquatic bioaccumulation

Method	Results				Remarks	Reference
Partition coefficient n-octanol/water Guideline: EC A.8, OECD 117 (HPLC-method)	pH $logP_{OW}$	4.0 (20 °C) 2.51	7.0 (20 °C) 2.51	9.0 (20 °C) 2.50	Test substance: BYI 08330, mix-batch 08045/0003, purity 99.1 %	Lemke, G., Muehlberger, B.; 2003 M-103244-01-1

[1] McCall, J.P., D.A. Laskowsky, R.L. Swann, and H.J. Dishburger. 1981. Measurement of sorption coefficients of organic chemicals and their use in environmental fate analysis. Pages 89-109 In *Test protocols for environmental fate & movement of toxicants.* Proceedings of a symposium. Association of Official Analytical Chemists. 94th Annual Meeting, October 21-22, 1980. Washington, DC.

[2] Food and Agriculture Organization of the United Nations. FAO PESTICIDE DISPOSAL SERIES 8. Assessing Soil Contamination: A Reference Manual. Appendix 2. Parameters of pesticides that influence processes in the soil. Editorial Group, FAO Information Division: Rome, 2000.

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

No estimations are available.

5.3.1.2 Measured bioaccumulation data

A study on bioaccumulation in fish is not available. A test is not required, because spirotetramat has a $logP_{OW}$ 2.51 and a bioaccumulation study is not triggered according the criteria in directive 91/414/EC.

5.3.2 Summary and discussion of aquatic bioaccumulation

Based on the measured log P_{ow} values ($logP_{ow}$ = 2.51 at pH 7) spirotetramat has a low potential of bioaccumulation in aquatic system.

5.4 Aquatic toxicity

Standard toxicity studies on fish, aquatic invertebrates, algae and higher plants with Spirotetramat were performed. Spirotetramat is toxic (EC50 is ≥ 1 mg and < 10 mg/L) to the used standard fresh water test species. Additional, aquatic tests with marine organisms (fish, marine invertebrates, algae) are available and the most sensitive species is the algae *Skeletoneam costatum* (E_bC50: 0.32 mg/L).

Table 34: Summary of relevant information on aquatic toxicity

Method	test organism	test condition	exp. time	test conc.	Results			Reference
					endpoint	NOEC (mg ai/L)	EC50/LC50 (mg ai/L)	
OECD 203, EPA 72-1, OPPTS 850.1075, EU Directive 92/69/EEC C.1	*Oncorhynchus mykiss* Rainbow trout	semi static	96 hr	mm	mortality	0.825	2.54	Dorgerloh, 2004a Document: DOM 24025, Edition Number: M-182649-01-2, 16.12.2004
OECD 203, EPA 72-1, OPPTS 850.1075, EU Directive 92/69/EEC C.1	*Cyprinus carpio* Common carp	semi static	96 hr	mm	mortality	1.02	2.59	Dorgerloh, 2004b Document: DOM24022, Edition Number: M-128667-01-2, 25.11.2004
OECD 203, EPA 72-1, OPPTS 850.1075, EU Directive 92/69/EEC C.1	*Lepomis macrochirus* Bluegill sunfish	semi static	96 hr	nom	mortality	0.5	2.2	Dorgerloh, 2005a Document: DOM24056, Edition Number: M-242689-01-2, 12.01.2005
OECD 203, US EPA OPPTS 850.1075, FIFRA 72-2	*Cyprinodon variegatus* Sheephead minnow	flow through	96 hr	mm	mortality	0.52	1.96	Banman& Lam, 2005 Document: EBFNX006, Edition Number: M-255363-01-1, 27.07.2005
EPA 72-4a, EPA OPPTS 850.1400, OECD 210	*Pimephales promelas* Fathead minnow	flow-through	33 d	mm	hatchability fry survival growth	1.16 0.534 1.16	> 1.16 1.16 > 1.16	Dorgerloh 2005b EBFN0305, Edition Number: M-260676-01-2, 16.11.2005
OECD 202, EPA 72-2, OPPTS 850.1010, EU Directive 92/69/EEC C.2	*Daphnia magna* Waterflea	static	48 hr	mm	immobility	20.3	> 42.7	Dorgerloh, 2005c Document: DOM 24004, Edition Number: M-242683-01-2, 14.01.2005
OECD 211, EEC Directive C.20, US EPA 72-4, OPPTS 850.1300	*Daphnia magna* Waterflea	semi static	21 d	nom	mortality adults reproduction growth	2.0 5.0 5.0	5.0 12.5 12.5	Dorgerloh, 2005deEBFN0245, Edition Number: M-251843-01-2, 13.05.2005
No specified guideline, the study is following the procedure of OECD 202	*Chironomus riparius* Midge	static (water-only)	48 hr	mm	mortality	<0.53	1.30	Dorgerloh, 2005g Document : EBFNX072, Edition Number: M-262632-01-2, 08.12.2005

Method	test organism	test condition	exp. time	test conc.	Results			Reference
					endpoint	NOEC (mg ai/L)	EC50/LC50 (mg ai/L)	
OECD 219	*Chironomus riparius* Midge	Static (spiked water)	28 d	nom	emergence development	0.1 0.8	0.2 1.6	Dorgerloh, 2005i M-248099-02-2, 16.03.2005, Amended: 29.06.2006
EPA OPPTS 850.1035, FIFRA 72-3	*Americamysis bahia* Saltwater mysid	flow through	96 hr	mm	mortality	< 0.73	4.27	Cafarelle, 2005a Document: EBFNX010, Edition Number: M-270200-01-1, 06.01.2005
EPA OPPTS 850.1025, FIFRA 72-3	*Crassostrea virginica* Eastern oyster	flow through	96 hr	mm	shell deposition	0.33	0.85	Cafarelle, 2005b Document: EBFNX011, Edition Number: M-257677-01-1, 15.06.2005
Draft proposal for updating OECD 201, OPPTS 850.5400, JMAFF guideline (12 Nousan No 8147)	*P. subcapitata* Green alga	static	72 hr	mm	biomass growth rate	1.46	6.58 8.15	Dorgerloh, 2004c Document: DOM 23092, Edition Number: M-128874-01-2, 29.11.2004
Draft proposal for updating OECD 201 (2004), OPPTS 850.5400, FIFRA 123-2	*Navicular pellicullosa* Diatom (freshwater)	static	96 hr	mm	biomass growth rate	0.19 1.00	4.05 15.0	Kern&Lam, 2005 Document: EBFNX008, Edition Number: M-252794-01-1, 15.06.2005
Draft proposal for updating OECD 201 (2004), OPPTS 850.5400, FIFRA 123-2	*Anabaena flos-aqua* Blue-green alga	static	96 hr	mm	biomass growth rate	5.68 15.1	15.2 > 15.1	Kern&Lam 2006 Document: EBFNX007, Edition Number: M-264055-01-1, 12.01.2006
Draft proposal for updating OECD 201 (2004), OPPTS 850.5400, FIFRA 123-2	*Skeletonema costatum* Diatom (marine)	static	96 hr	mm	biomass growth rate	0.124	0.36 0.96	Banman& Lam, 2006 Document: EBFNX009-1, Edition Number: M-271037-02-1, 16.06.2006
OECD 221, EPA OPPTS 850.4400	*Lemna gibba* Duckweed	semi static	7 d	mm	yield growth rate	1.54	4.49 6.21	Dorgerloh, 2005j Document: DOM 24019, Edition Number: M-255296-

Method	test organism	test condition	exp. time	test conc.	Results			Reference
					endpoint	NOEC (mg ai/L)	EC50/LC50 (mg ai/L)	
								01-2, 29.07.2005

Test conc.: test concentration based on mean measured (mm) or nominal (nom) concentration

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Acute toxicity to fish (IIA 8.2.1)

Reference:	**Acute toxicity of BYI 08330 (tech.) to fish (*Oncorhynchus mykiss*)**
Author(s), year:	Dorgerloh, M., 2004 a
Report/Doc. number:	DOM 24025, Edition Number: M-182649-01-2, 16.12.2004
Guideline(s):	OECD 203, EPA 72-1, OPPTS 850.1075, EU Directive 92/69/EEC C.1
GLP:	Yes
Deviations:	Measured test concentration dropped below 80 % of nominal concentration.
Validity:	Acceptable

Test substance:	Spirotetramat (BYI 08330), purity: 97.4 %, batch: 08045/0014
Material and methods:	
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number organisms:	10 fish per concentration and control
Weight, length:	2.3 ± 1.1 g (mean ± SD) and 5.8 ± 0.9 cm (mean ± SD)
Loading:	0.58 g fish/L test medium
Type of test, duration:	Semi static test (renewal of test media every 24 hours), 96 hours
Applied conc.:	
Nominal:	0 (control and solvent control), 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L
Measured (mean):	-- (control and solvent control), 0.409,0.825, 1.5, 3.14 and 6.44 mg/L
Solvent	Aceton 0.2 mL/L
Test conditions:	
Water quality:	Reconstituted water (according to ISO), hardness: 40 - 60 mg/L as $CaCO_3$
Temperature:	11.3 – 12.3 °C
pH:	7.0 – 7.1 (0 h, new medium), 7.0 (96 h, aged medium)
O_2 content:	89 – 99 % saturation
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 4, 24, 48, 72 and 96 hours. For chemical analysis (HPLC-MS/MS and HPLC-UV) of BYI 08330 in test solutions samples were taken daily from new and aged test media. Test samples were directly injected into the HPLC-UV without centrifugation.
Statistics:	EC50: Probit analysis, NOEC: directly from raw data
Findings:	
Analytical data:	Over the whole test period the mean measured concentrations were in the range from 77 to 85 % of nominal.
Behavioural effects:	Controls and concentration levels up to 0.825 mg/L: No sublethal effects were reported over the whole test period. At test concentration ≥ 1.5 mg/L: After 96 hours following symptoms were noted: Remained on the bottom of aquarium, loss of equilibrium, labored respiration,

remained at water surface, turned in a vertical position.
Thus the NOEC was 0.825 mg/L based on sublethal effects.

Mortality:

Table 35: Effects on rainbow trout (O. mykiss) exposed to spirotetramat

Spirotetramat [mg a.s./L] (mean measured)	Cumulative mortality (%)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
Solvent control	0	0	0	0
0.409	0	0	0	0
0.825	0	0	0	0
1.5	0	0	0	0
3.14	0	30	30	80
6.44	60	100	100	100
NOLC: 1.5 mg/L				
LC_{50} (96 h): 2.54 mg/L (95 % C.I. 2.11 – 3.05 mg/L)				

Details of the Probit: Slope b: 8.4
95 %CI of b: not stated
Variance of b: 3.85
Intercept a: 1.6
Goodness of fit, Chi^2: 1.25
Degrees of freedom: 3
p (Chi^2): 0.74

Conclusion: LC_{50} (96 h): 2.54 mg/L
NOEC (96 h): 0.825 mg/L
based on mean measured concentration

Reference:	**Acute toxicity of BYI 08330 (tech.) to fish (*Cyprinus carpio*)**
Author(s), year:	Dorgerloh, M., 2004 b
Report/Doc. number:	DOM24022, Edition Number: M-128667-01-2, 25.11.2004
Guideline(s):	OECD 203, EPA 72-1, OPPTS 850.1075, EU Directive 92/69/EEC C.1
GLP:	Yes
Deviations:	Measured test concentration dropped below 80 % of nominal concentration.
Validity:	Acceptable

Test substance: Spirotetramat (BYI 08330), purity: 97.4 %, batch: 08045/0014

Material and methods:

Test species: Common carp (*Cyprinus carpio*)
Number organisms: 10 fish per concentration and control
Weight, length: 0.9 ± 0.3 g (mean ± SD) and 4.0 ± 0.5 cm (mean ± SD)
Loading: 0.23 g fish/L test medium

Type of test, duration:	Semi static test (renewal of test media every 24 hours), 96 hours
Applied conc.:	
Nominal:	0 (control and solvent control), 1.25, 2.5, 5.0, 10.0 and 20.0 mg/L
Measured (mean):	-- (control and solvent control), 1.02, 1.71, 3.65, 8.13 and 13.4 mg/L
Solvent	Aceton 0.2 mL/L
Test conditions:	
Water quality:	Reconstituted water (according to ISO), hardness: 40 - 60 mg/L as $CaCO_3$
Temperature:	20.5 – 23.2 °C
pH:	7.1 – 7.2 (0 h, new medium), 7.1 (96 h, aged medium)
O_2 content:	97 – 103 % saturation
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 4, 24, 48, 72 and 96 hours. For chemical analysis (HPLC-MS/MS and HPLC-UV) of BYI 08330 in test solutions samples were taken daily from new and aged test media. Test samples were directly injected into the HPLC-UV without centrifugation.
Statistics:	EC50: Probit analysis, NOEC: directly from raw data
Findings:	
Analytical data:	Over the whole test period the mean measured concentrations were in the range 69 – 83 % of nominal.
Behavioural effects:	Controls and at 1.02 mg/L: No sublethal effects were observed over the whole test period. At concentration levels ≥ 1.71 mg/L: After 96 hours following symptoms were noted: Remained on the bottom of aquarium, loss of equilibrium, labored respiration, dark coloration, laying on the sides or backs, turned in a vertical position. Thus the NOEC was 1.02 mg/L based on sublethal effects.
Mortality:	

Table 36: Effects on common carp (C. carpio) exposed to spirotetramat

Spirotetramat [mg a.s./L] (mean measured)	**Cumulative mortality (%)**			
	24 hours	**48 hours**	**72 hours**	**96 hours**
Control	0	0	0	0
Solvent control	0	0	0	0
1.02	0	0	0	0
1.71	0	0	0	0
3.65	0	10	50	100
8.13	100	100	100	100
13.4	100	100	100	100
NOLC: 1.71 mg/L				
LC_{50} (96 hours): 2.59 mg/L (95 % C.I.: not determined due to mathematical reasons)				

Details of the Probit:	Slope b: 16.09 95 %CI of b: not stated

	Variance of b: 29.9
	Intercept a: -1.66
	Goodness of fit, Chi^2: 17.69
	Degrees of freedom: 3
	p (Chi^2): < 0.001
Conclusion:	LC_{50} (96 h): 2.59 mg/L
	NOEC (96 h): 1.02 mg/L; based on mean measured concentration

Reference:	**Acute toxicity of BYI 08330 (tech.) to fish (*Lepomis macrochirus*)**
Author(s), year:	Dorgerloh, M., 2005 a
Report/Doc. number:	DOM24056, Edition Number: M-242689-01-2, 12.01.2005
Guideline(s):	OECD 203, EPA 72-1, OPPTS 850.1075, EU Directive 92/69/EEC C.1
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	Spirotetramat (BYI 08330), purity: 97.2 %, batch: 08045/0014
Material and methods:	
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Number organisms:	10 fish per concentration and control
Weight, length:	1.7 ± 0.7 g (mean ± SD) and 4.9 ± 0.6 cm (mean ± SD)
Loading:	0.43 g fish/L test medium
Type of test, duration:	Semi static test (renewal of test media every 24 hours), 96 hours
Applied conc.:	
Nominal:	0 (control and solvent control), 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L
Measured (mean):	-- (control and solvent control), 0.48, 0.95, 1.90, 3.56 and 7.61 mg/L
Solvent	Aceton 0.2 mL/L
Test conditions:	
Water quality:	Reconstituted water (according to ISO), hardness: 40 – 60 mg/L as $CaCO_3$
Temperature:	17.9 – 20.5 °C
pH:	7.4 – 7.5 (0 h, new medium), 7.3 (96 h, aged medium)
O_2 content:	96 – 105 % saturation
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 4, 24, 48, 72 and 96 hours. For chemical analysis (HPLC-MS/MS and HPLC-UV) of BYI 08330 in test solutions samples were taken daily from new and aged test media. Test samples were directly injected into the HPLC-UV without centrifugation.
Statistics:	EC50: Probit analysis, NOEC: directly from raw data
Findings:	
Analytical data:	Over the whole test period the mean measured concentrations were in the range 91 – 99 % of nominal.
Behavioural effects:	Controls and at 0.5 mg/L: No sublethal effects were observed over the whole test period. At concentration levels ≥ 1.0 mg/L: Transient effects like remaining on the

bottom of aquarium, laying on the sides or backs, labored respiration, hyperactivity, inactivity, convulsions and remaining on the water surface were observed.
Thus the NOEC was 0.5 mg/L based on sublethal effects.

Mortality:

Table 37: Effects on bluegill sunfish (*L. macrochirus*) exposed to spirotetramat

Spirotetramat [mg a.s./L] (nominal)	**Cumulative mortality (%)**			
	24 hours	**48 hours**	**72 hours**	**96 hours**
Control	0	0	0	0
Solvent control	0	0	0	0
0.5	0	0	0	0
1.0	0	20	20	20
2.0	0	10	10	10
4.0	20	50	100	100
8.0	80	100	100	100
NOLC: 0.5 mg/L				
LC_{50} (96 hours): 2.2 mg/L (95 % C.I.: not determined due to mathematical reasons)				

Details of the Probit: Slope b: 4.44
95 %CI of b: not stated
Variance of b: 1.21
Intercept a: 3.48
Goodness of fit, Chi^2: 8.93
Degrees of freedom: 3
p (Chi^2): 0.0302

Conclusion: LC_{50} (96 h): 2.2 mg/L
NOEC (96 h): 0.5 mg/L
based on nominal concentration

Reference:	**Acute toxicity of BYI 08330 technical to the sheepshead minnow (*Cyprinodon variegatus*) under flow-trough conditions**
Author(s), year:	Banman, C. S.; Lam, C. V., 2005
Report/Doc. number:	EBFNX006, Edition Number: M-255363-01-1, 27.07.2005
Guideline(s):	OECD 203, US EPA OPPTS 850.1075, FIFRA 72-2
GLP:	Yes
Deviations:	OECD: None of relevance USEPA: Based on EFED's guidance document, "Estuarine Fish 96-Hour Acute Toxicity Standard Evaluation Procedure's the recommendation of fish weight is between 0.5 and 5 g.
Validity:	Acceptable.

Test substance:	Spirotetramat (BYI 08330), purity: 97.99 %, batch: 08045/0014
Material and methods:	
Test species:	Sheepshead minnow (*Cyprinodon variegatus*)
Number organisms:	20 fish per concentration and control
Weight, length:	0.13 ± 0.03 g (mean ± SD), 1.72 ± 0.14 cm (mean ± SD)
Loading:	0.03 g fish/L test material passing through tank each day
Type of test, duration:	Flow-through test (renewals approx. 5.3 x per day), 96 hours
Applied conc.:	
Nominal:	0 (control and solvent control), 0.63, 1.25, 2.50, 5.0, 10 mg/L
Measured (mean):	-- (control and solvent control), 0.52, 1.17, 2.52, 4.54, 9.77 mg/L
Solvent	0.1 mL/L Aceton
Test conditions:	
Water quality:	Artificial sea salts mixed with reverse osmosis water or blended soft water to produce a salinity of 17 ‰
Temperature:	21.0 – 22.5 °C
pH:	8.1 – 8.4 (0 h), 7.7 - 8.1 (96 h)
O_2 content:	5.3 – 6.9 mg O_2/L (67 – 87 %)
Light regime:	16 hours light / 8 hours darkness, 30 min light/dark transition period
Test parameters:	Mortality and sublethal effects were assessed after 4, 24, 48, 72 and 96 hours. For chemical analysis (HPLC method) of BYI 08330 in test solutions samples were taken at 0, 24 and 96 hours. Test samples were analysed by direct injection into an HPLC instrument without centrifugation.
Statistics:	LC_{50}: Moving average method, NOEC: directly from raw data
Findings:	
Analytical data:	Mean measured concentrations were in the range of 92 – 115 % of nominal concentrations.
Behavioural effects	None in controls and at 0.52 mg/L concentration level. After 96 hours at ≥ 1.17 mg/L effects like loss of equilibrium and lying on the bottom of the aquarium were observed. Thus the NOEC is 0.52 mg/L.
Mortality:	

Table 38: Cumulative mortality of sheepshead minnow exposed to spirotetramat

Spirotetramat (mean measured) [mg/L]	**Cumulative mortality (%)**				
	4 hours	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Blank control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.52	0	0	0	0	0
1.17	0	0	0	0	10
2.52	0	0	0	35	60
4.54	0	10	100	100	100
9.77	0	100	100	100	100
NOEC = 0.52 mg/L					
LC_{50} = 1.96 mg/L (95%C.I. 1.64 – 2.37) based on moving average method					

Spirotetramat (mean measured) [mg/L]	Cumulative mortality (%)				
	4 hours	24 hours	48 hours	72 hours	96 hours
LC_{50} = 2.10 mg/L (95%C.I. 1.73 – 2.52) based on Probit analysis					

Details of the Probit:	Slope b: 5.696
	95 % C.I. of b: 3.495 – 7.897
	Variance of b: 1.12
	Intercept a: -1.838
	Goodness of fit, Chi^2: 1.282
	Degrees of freedom: 3
	p (Chi^2): 0.733
Conclusion:	LC_{50} (96 h): 1.96 mg/L
	NOEC: 0.52 mg/L
	based on mean measured concentration

5.4.1.2 Long-term toxicity to fish

Chronic toxicity to fish (IIA 8.2.2)

Prolonged toxicity (21 day exposure) to fish (IIA 8.2.2.1)

No study submitted. Not required, because a fish early life stage toxicity test has been carried out.

Fish early life stage toxicity test (IIA 8.2.2.2)

Reference:	Early-life stage toxicity of BYI 08330 tech. to fish (*Pimephales promelas*)
Author(s), year:	Dorgerloh, M., 2005 b
Report/Doc. number:	EBFN0305, Edition Number: M-260676-01-2, 16.11.2005
Guideline(s):	EPA 72-4a, EPA OPPTS 850.1400, OECD 210
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	Spirotetramat (BYI 08330), purity: 97.1 %, batch: 08045/0014
Material and methods:	
Test species:	Fathead minnow (*Pimephales promelas*)
Number organisms:	4 replicates x 25 eggs per test concentration and control, after completion of hatch larvae were thinned to 15 individuals per egg cup
Age:	Freshly fertilized eggs < 24 hours old
Type of test, duration:	Flow-through test, 33 days (28 days post hatch)
Applied conc.:	
Nominal:	0 (control and solvent control), 0.0625, 0.125, 0.250, 0.500, 1.00 mg/L
Measured (mean):	-- (control and solvent control), 0.0607, 0.121, 2.243, 0.486, 0.971 mg/L
Solvent	Dimethylformamide (DMF) 0.1 mL/L
Test conditions:	

Water quality:	Reconstituted water (according to ISO), hardness: 45 – 52 mg/L as $CaCO_3$
Temperature:	24.5 (23 – 26) °C
pH:	7.0 – 7.3 during the total test period
O_2 content:	98 – 100 % saturation
Light regime:	16 hours light / 8 hours darkness, 30 min transient period
Feeding	Larvae were fed 5 x daily (except on weekends/holidays: 2 x daily) with live newly hatched shrimp nauplii *ad libidum*. Feeding was started shortly after hatching. Uneaten food an faeces were siphoned in order to minimise microbiological growth and biodegradation of test substance.
Test parameters:	Embryo mortality, hatched larvae, fry mortality and abnormal appearance or behaviour were assessed weekdays. At test termination the length and the weight were determined. Determined endpoints were: Time to hatch, hatching success, overall fry survival (fry survival before and after thinning), standard length and dry weight. For chemical analysis (HPLC-MS/MS and HPLC-UV) of BYI 08330 in test solutions samples were taken at -1, 0, 7, 14, 21, 28 and 33 days
Statistics:	If control and solvent control can be pooled: T-test Testing for normal distribution: R/s procedure Time to hatch, hatching success, fry survival: Williams test with previous acrisine transformation Growth data: Williams test without previous data transformation

Findings:

Analytical data:	Overall mean measured concentrations in test media were 93 – 119 % of nominal.
Biological observation:	Time to hatch: In control and all treatment levels hatching began on day 4 and continued until day 5. Over the total test period no morphological and behavioural effects were observed.

Effects:

Table 39: Hatching success and fry survival

Spirotetramat [mg/L] (mean measured)	**Egg hatch [%] phd 0 Mean (SD)**	**Fry survival [%] phd 0 Mean (SD)**	**Fry survival [%] phd 28 Mean (SD)**	**Overall fry survival [%][a)] Mean (SD)**
pooled control [b)]	85 (8.8)	98 (2.6)	88 (11.1)	87 (11.1)
0.0566	85 (11.5)	94 (9.0)	87 (16.3)	82 (18.4)
0.115	84 (8.0)	95 (5.9)	85 (6.4)	81 (6.0)
0.247	88 (5.7)	100 (0)	85 (17.5)	85 (17.5)
0.534	91 (5.0)	92 (4.7)	78 (19.9)	73 (21.7)
1.16	84 (8.0)	83 (7.1)	78 (14.8)	65 (11.5)*
NOEC	1.16 mg/l			0.534 mg/L
LOEC	> 1.16 mg/L			1.16 mg/l

[a)] calculated as follows: (fry survival before thinning/number of fry hatched)*(fry survival/number of fry after thinning)*100

phd – post hatch day

[b)] no statistically significant difference between dilution control and solvent control

* significant difference when compared to pooled control (alpha = 0.05)

Table 40: Length and Weight

Spirotetramat [mg/L] (mean measured)	Length [mm] Test termination (phd 28) Mean (SD)	Dry weight [mg] Test termination (phd 28) Mean (SD)
pooled control [a)]	18.0 (3.5)	20.4 (7.4)
0.0566	18.4(3.4)	21.8 (11.4)
0.115	18.3 (3.4)	21.4 (11.3)
0.247	18.3 (3.9)	21.5 (11.4)
0.534	19.1 (2.2)	22.1 (7.5)
1.16	18.6 (3.4)	21.9 (10.4)
NOEC	1.16 mg/l	
LOEC	> 1.16 mg/L	

[a)] no statistically significant difference between dilution control and solvent control
* significant difference when compared to pooled control (alpha = 0.05)

Conclusion: Fry survival: NOEC: 0.534 mg/L, LOEC: 1.16 mg/L
Hatchability and growth: NOEC : 1.16 mg/L, LOEC > 1.16 mg/L
based on mean measured concentrations

Fish life cycle test (IIA 8.2.2.3)

No study submitted. The $logP_{OW}$ of spirotetramat is 2.51 and the potential of bioaccumulation is expected to be low. Furthermore spirotetramat is not persistent in water or sediment of aquatic systems and therefore a fish life-cycle test is not required.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Reference:	**Acute toxicity of BYI 08330 (tech.) to the waterflea *Daphnia magna* under static conditions**
Author(s), year:	Dorgerloh, M., 2005 c
Report/Doc. number:	DOM 24004, Edition Number: M-242683-01-2, 14.01.2005
Guideline(s):	OECD 202, EPA 72-2, OPPTS 850.1010, EU Directive 92/69/EEC C.2
GLP:	Yes
Deviations:	Minor deviation from EPA guideline: The pH is lower and the hardness is higher than EPA-recommended range.
Validity:	Acceptable

Test substance: Spirotetramat (BYI 08330), purity: 97.2 %, batch: 08045/0014
Material and methods:
Test species: Waterflea (*Daphnia magna*)
Number organisms: 6 replicates each with 5 daphnids per treatment and control
Age: First instar < 24 hours old
Type of test, duration: Static test, 48 h
Applied conc.:
Nominal: 0 (control and solvent control), 6.25, 12.5, 25.0, 50, 100 mg/L
Measured (mean): -- (control and solvent control), 4.7, 8.77, 20.3, 38.8, 42.7 mg/L

Solvent	0.4 mL/L Aceton
Test conditions:	
Water quality:	M7 medium (according to "original draft" of an EEC *Daphnia magna* pilot ring test), hardness: 196 mg/L as $CaCO_3$
Temperature	20.5 – 20.6 °C
pH	6.3 – 6.4 (0 h), 6.6 – 6.7 (48 h)
O_2 content:	96 – 102 %, 8.5 – 9.0 mg O_2/L
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Immobility and sublethal effects were assessed after 24 and 48 hours. For chemical analysis (HPLC-UV) of BYI 08330 in the test media samples were taken at test initiation (0 h) and termination (48 h). The water samples were analysed by direct injection into the HPLC-UV instrument without centrifugation.
Statistics:	EC50: None, because at highest test concentration (100 mg/L nominal) the immobilisation were < 50 %, NOEC: directly from the raw data
Findings:	
Analytical data:	The mean measured concentrations at the start and end of the test were in the range of 22 – 90 %, overall mean measured concentration ranged from 42.7 – 81.0 % of nominal.
Effects:	After 48 hours no immobility was observed in the control, solvent control and in test concentrations up to 20.3 mg/L. At 38.8 and 42.7 mg/L the immobilisation was 7 and 23 %. No sublethal effects were noted during the whole study. Thus the NOEC was determined to be 20.3 mg/L and the EC50 was > 42.7 mg/L.
Conclusion:	EC50 (48 h): > 42.7 mg/L NOEC: 20.3 mg/L based on mean measured concentration

Reference:	**Acute toxicity of BYI 08330 (tech.) to larvae of *Chironomus riparius* in a 48 h water-only study**
Author(s), year:	Dorgerloh, M., 2005 g
Report/Doc. number:	EBFNX072, Edition Number: M-262632-01-2, 08.12.2005
Guideline(s):	No specified guideline, the study is following the procedure of OECD 202
GLP:	Yes
Deviations:	None of relevance considering OECD 202
Validity:	Acceptable

Test substance:	Spirotetramat (BYI 08330), purity: 97.4 %, batch: 08045/0014
Material and methods:	
Test species:	Midge (*Chironomus riparius*)
Number organisms:	4 replicates each with 10 larvae per treatment and control
Age:	L1 (first instar) larvae
Type of test, duration:	Static (water-only) test, 48 h
Applied conc.:	
Nominal:	0 (control and solvent control), 0.56, 1.00, 1.80, 3.20, 5.60, 10.0 mg/L
Measured (mean):	-- (control and solvent control), 0.53, 0.96, 1.72, 3.04, 5.37, 9.47 mg/L

Solvent	0.1 mL/L Aceton
Test conditions:	
Water quality:	M7 medium (according to "original draft" of an EEC *Daphnia magna* pilot ring test), hardness: not stated
Temperature	20.4 – 21 °C
pH	8.2 – 8.6 (0 h), 8.2 – 8.9 (48 h)
O_2 content:	> 80 %, (8.6 – 8.9 mg O_2/L)
Light regime:	16 hours light / 8 hours darkness
Feeding	Only on time 0.01 mL aqueous fish food solution directly after insertion of the larvae
Test parameters:	Mortality and sublethal effects were assessed after 48 hours. For chemical analysis (HPLC-MS/MS and HPLC-UV) of BYI 08330 in the test media samples were taken at test initiation (0 h) and termination (48 h).
Statistics:	Correction of mortality: Abbott's formula, LC10 and LC50: Probit analysis, NOEC: not determined, comparison of controls: Student-t test for homogeneous variances. All statistical analyses are based on data of pooled control.
Findings:	
Analytical data:	The mean measured concentrations at the start and end of the test were in the range of 106 – 109 % and 82 – 83 % of nominal, respectively. Overall mean measured concentrations ranged from 95 to 96 % of nominal.
Effects:	After 48 hours the mortality in pooled controls was 8.7 %, thus the validity criteria of maximum control mortality of 10 % is met, further information see table:

Table 41: Effects on midge (C. riparius) exposed to spirotetramat

Spirotetramat [mg a.s./L] mean measured	**Exposed chironomids (= 100 %)**	**Mortality after 48 hours**	
		[n]	**[%]**
Control*	40	3	7.5
Solvent control*	40	4	10.0
0.528	40	4	10.0
0.958	40	13	32.5
1.715	40	29	72.5
3.035	40	39	97.5
5.365	40	40	100
9.465	40	40	100
LC10 (48 h): 0.699 mg/L (95% C.I. 0.536 – 0.913 mg/L)			
LC50 (48 h): 1.299 mg/L (95% C.I. 1.120 – 1.506 mg/L)			

* Result of t-test is indicated no significant differences between control and solvent control, thus pooled control are used for statistical analysis.

Details of the Probit:	Slope b: 4.76 95 % CI of b: not stated Variance of b: 0.54

	Intercept a: 4.46
	Goodness of fit, Chi^2: 6.62
	Degrees of freedom: 4
	p (Chi^2): 0.158
Conclusion:	LC_{10} (48 h): 0.699 mg/L
	LC_{50} (48 h): 1.299 mg/L
	NOEC (48 h): < 0.528 mg/L
	based on mean measured concentration
Comments:	While the study not deviated from recommendations given in OECD 202 guideline, significant deviation from USEPA guidelines are notable:
	-Age of the test organisms is 2nd or 3rd instar, 1st instars were used here.
	-Test organisms should not be fed.
	-For invertebrates other than Daphnia, the test duration should be 96-hours.
	-Mortality and sublethal effects should be measured every 24 hours.

Reference:	**BYI 08330 - Acute toxicity to mysids (*Americamysis bahia*) under flow-through conditions**
Author(s), year:	Cafarelle, M. A., 2005 a
Report/Doc. number:	EBFNX010, Edition Number: M-270200-01-1, 06.01.2005
Guideline(s):	EPA OPPTS 850.1035, FIFRA 72-3
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable.

Test substance:	Spirotetramat (BYI 08330), purity: 97.2 %, batch: 08045/0014
Material and methods:	
Test species:	Saltwater mysid (*Americamysis bahia*)
Number organisms:	2 replicates each with 10 mysids per treatment and control
Age:	5- to 6-days old
Type of test, duration:	Flow-through test, 96 h
Applied conc.:	
Nominal:	0 (control and solvent control) 0.63, 1.3, 2.5, 5.0, 10.0 mg/L
Measured (mean):	-- (control and solvent control), 0.73, 1.2, 2.6, 4.6, 9.0 mg/L
Solvent	0.1 mL/L Dimethylformamide (DMF)
Test conditions:	
Water quality:	Filtered natural seawater diluted with well water, salinity: 21 – 20 ‰
Temperature	24 – 25 °C
pH	8.1 (0 h), 7.8 – 8.1 (96 h)
O_2 content:	> 60 % saturation, 4.9 – 7.4 mg O_2/L
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 24, 48, 72 and 96 hours. For chemical analysis (HPLC method) of spirotetramat in the test solution samples were taken at 0 and 96 hours. Test samples were analysed by direct

injection into an HPLC instrument without centrifugation.

Statistics: EC_{50}: Binominal probability and probit analysis, NOEC: directly from the raw data

Findings:

Analytical data: Overall mean measured concentrations were in the range of 90 – 120 % of nominal concentrations.

Behavioural effects At 2.6 mg/L and higher concentrations sublethal effects like erratic swimming, lethargy and laying on the bottom of test vessel were observed. Thus the NOEC was determined to be 1.2 mg/L.

Mortality:

Table 42: Cumulative mortality of saltwater mysids exposed to spirotetramat

Spirotetramat (BYI 08330		**Cumulative mortality (%)**			
Nominal [mg/L]	**Mean measured [mg/L]**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Control		0	0	0	0
Solvent control		0	0	0	0
0.63	0.73	5	5	5	5
1.3	1.2	0	0	5	5
2.5	2.6	10	20	20	30
5.0	4.6	15	20	25	25
10	9.0	60	100	100	100
NOLC (96 hours) < 0.73 mg/L					
LC_{50} (96 h) = 5.5 mg/L (95%C.I. 1.2 – 9.0 mg/L) based on binominal probability LC_{50} (96 h) = 4.27 mg/L (95%C.I. not calculated due to mathematical reasons) based on probit analysis					

Details of the Probit: Slope b: 2.926
95%C.I. of b: 0.793 and 5.059
Intercept a: -1.845
Goodness of fit, Chi^2: 13.25
Degrees of freedom: 3
p (Chi^2): 0.0041

Conclusion: LC_{50} (96 h): 5.5 mg/L
NOLC: < 0.73 mg/L
based on mean measured concentration

Reference:	**BYI 08330 - Acute toxicity to eastern oysters (*Crassostrea virginica*) under flow-through conditions**
Author(s), year:	Cafarella, M. A., 2005 b
Report/Doc. number:	EBFNX011, Edition Number: M-257677-01-1, 15.06.2005
Guideline(s):	EPA OPPTS 850.1025, FIFRA 72-3
GLP:	Yes
Deviations:	• During the final 24 hours of exposure the oxygen saturation drop below 60 % (ranged from 52 – 96 %). • The measured concentrations drop below 59% of nominal concentration.
Validity:	Acceptable

Test substance:	Spirotetramat (BYI 08330), purity: 97.1 %, batch: 08045/0014
Material and methods:	
Test species:	Eastern Oyster (*Crassostrea virginica*)
Number organisms:	20 oysters per treatment and control
Valve height:	37 ± 5 mm (mean ± SD)
Type of test, duration:	Flow-through test, 96 h
Applied conc.:	
Nominal:	0 (control and solvent control), 0.19, 0.38, 0.75, 1.5, 3.0 mg/L
Measured (mean):	-- (control and solvent control), 0.20, 0.33, 0.55, 0.89, 2.1 mg/L
Solvent	0.1 mL/L Dimethylformamide (DMF)
Test conditions:	
Water quality:	Filtered natural seawater, salinity: 31 – 32 ‰
Temperature	20 – 22 °C
pH	7.6 – 8.0
O_2 content:	3.8 – 7.3 mg O_2/L (52 – 96 % saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Observations of mortalities and sublethal effects were made at 0, 24, 48, 72 and 96 hours and the shell growth was determined at test termination. For chemical analysis (HPLC method) of spirotetramat in the test solution samples were taken at the beginning and at the end of the test. Test samples were analysed by direct injection into an HPLC instrument without centrifugation.
Statistics:	EC_{50}: Linear regression analysis, NOEC: Williams test, comparison of control groups: T-test. All statistical analyses are based on data of pooled control.
Findings:	
Analytical data:	Overall mean measured concentrations were in the range of 59 – 100 % of nominal concentrations.
Mortality:	None
Shell Deposition:	

Table 43: Mean shell deposition of eastern oysters (C. virginica) exposed to spirotetramat

Spirotetramat [mg/L] (mean measured)	Mean shell deposition (mm ± SD)	Inhibition of shell growth [%]
Pooled control [a)]	3.5 ± 1.2	--
0.20	3.8 ± 1.2	+7.9
0.33	3.4 ± 1.1	3
0.55	2.4 ± 1.0	31*
0.89	1.6 ± 0.9	54*
2.1	0.4 ± 0.7	89*
NOEC = 0.33 mg/L		
EC_{50} (96 h)= 0.85 mg/L (95%C.I. 0.59 – 1.3 mg/L)		

[a)] no statistically significant difference between dilution control and solvent control
* statistically significant difference from the pooled control (p ≤ 0.05)

Conclusion: EC_{50} (48 h): 0.85 mg/L
NOEC: 0.33 mg/L
based on mean measured concentration

5.4.2.2 Long-term toxicity to aquatic invertebrates

Reference:	**Influence of BYI 08330 (tech.) on development and reproductive output of the waterflea *Daphnia magna* in a static renewal laboratory test system**
Author(s), year:	Dorgerloh, M., 2005 e
Report/Doc. number:	EBFN0245, Edition Number: M-251843-01-2, 13.05.2005
Guideline(s):	OECD 211, EEC Directive C.20, US EPA 72-4, OPPTS 850.1300
GLP:	Yes
Deviations:	The concentraton of the solvent (acetone) is slightly higher than recommended in OPPTS 850.1300 (0.2 ml/L instated of 0.1 ml/L). However an additional solvent control was added to make sure that the solvent was not initiating any toxic response, thus no negative impact on the results can be concluded.
Validity:	Acceptable

Test substance:	Spirotetramat (BYI 08330), purity: 97.1 %, batch: 08045/0014
Material and methods:	
Test species:	Waterflea (*Daphnia magna*)
Number organisms:	12 replicates each with one daphnid per treatment and control
Age:	First instar < 24 hours old
Type of test, duration:	Semi static test, 21 d, renewals of test solutions: 0, 2, 5, 7, 9, 12, 14, 16, 19 days
Applied conc.:	
Nominal:	0 (control and solvent control), 0.05, 0.13, 0.32, 0.8, 2.0, 5.0, 12.5 mg/L
Measured (mean):	-- (control and solvent control), 0.055, 0.129, 0.322, 0.796, 1.84, 4.41, 10.9 mg/L
Solvent	0.2 mL/L Aceton
Test conditions:	
Water quality:	M7 medium (according to “original draft” of an EEC *Daphnia magna* pilot ring

	test), hardness: 196 mg/L as $CaCO_3$
Temperature	20 – 20.9 °C
pH	7.0 – 7.3 (new test solution) 7.4 – 8.1(aged test solution)
O_2 content:	6.3 – 10.8 mg O_2/L (> 60 % saturation)
Light regime:	16 hours light / 8 hours darkness
Feeding	Daily with *Desmodesmus subspicatus* suspension (1 x 10^8 cells/L), one exception was noted: On day 2 the three fold amount was fed for the first weekend
Test parameters:	Parent mobility, reproduction (mean time to first brood, age at first brood, offspring per surviving parental) and sublethal effects were observed daily (with exception of the first weekend after test initiation), at study termination body length and parental body mass (dry weight) were reported. For chemical analysis (HPLC/UV) of spirotetramat in test media duplicate samples were taken from fresh (on days 0, 9, 19) and old solutions (on days 2, 12, 21) from each test concentration.
Statistics:	In general data for parent mobility were acrisine transformed before further evaluation. Variance homogeneity and normal distribution were analysed by Bartletts test and Kolmogorroff-Smirnov test. If control groups can be pooled a t- test was performed. All NOEC were derived by comparing each treatment group with pooled controls: Parent mortality: Fishers Exact Binominal Test with Bonferroni Correction Reproduction: T-test Body length: Dunnett's Test Parental body mass (dry weight): U-test after Bonferroni-Holm Statistical analyses concidering nominal concentrations are based on data of pooled controls. Statistical analyses concidering mean measured concentrations are using only negative control.
<u>Findings:</u>	
Analytical data:	The mean measured concentrations ranged from 87.2 – 110.7 %
Effects:	

Table 44: Summary of effects of long-term exposure of spirotetramat on *Daphnia magna*

Spirotetramat [mg/L] (nominal)	**Spirotetramat [mg/L] (mean measured)**	**Parent mortality at day 21 [%]**	**Mean time to first brood [days]**	**Offspring per surviving parental**	**Mean dry weight of parent after 21 d [mg]**	**Mean length of parent after 21d [mm]**
Blank control	Blank control	0	8.7 ± 1.85	117.3 ± 27.6	1.07 ± 0.14	4.50 ± 0.13
Solvent control	Solvent control	0	8.7 ± 1.19	115.2 ± 16.4	0.98 ± 0.15	4.42 ± 0.17
Pooled control	Pooled control	0	8.7 ± 1.52	116.3 ± 22.1	1.02 ± 0.15	4.46 ± 0.15
0.05	0.055	0	9.9 ± 0.92	127.3 ± 16.6	1.09 ± 0.17	4.55 ± 0.07
0.13	0.012	0	9.1 ± 1.10	127.4 ± 17.6	0.89 ± 0.27	4.56 ± 0.15
0.32	0.322	0	8.7 ± 1.78	113.0 ± 25.5	0.90 ± 0.34	4.55 ± 0.06
0.8	0.796	0	9.1 ± 1.11	118.7 ± 14.0	1.00 ± 0.12	4.58 ± 0.09
2.0	1.84	0	9.4 ± 1.09	122.4 ± 16.8	1.05 ± 0.12	4.61 ± 0.11
5.0	4.41	10	7.9 ± 3.40	115. ± 25.0	1.14 ± 0.32	4.61 ± 0.11
12.5	10.9	20	7.2 ± 2.00	93.5 ± 30.5*	0.81 ± 0.12*	4.21 ± 0.13*
NOEC (based on nominal)		2.0 mg/L	12.5 mg/L	5.0 mg/L	5.0 mg/L	5.0 mg/L

Spirotetramat [mg/L] (nominal)	Spirotetramat [mg/L] (mean measured)	Parent mortality at day 21 [%]	Mean time to first brood [days]	Offspring per surviving parental	Mean dry weight of parent after 21 d [mg]	Mean length of parent after 21d [mm]
LOEC (based on nominal)		5.0 mg/L	> 12.5 mg/L	12.5 mg/L	12.5 mg/L	12.5 mg/L
NOEC (based on mean measured) [a)]		10.9 mg/L	10.9 mg/L	4.41 mg/L	4.41 mg/L	4.41 mg/L
LOEC (based on mean measured) [a)]		> 10.9 mg/L	> 10.9 mg/L	10.9 mg/L	10.9 mg/L	10.9 mg/L

* Statistically significantly different from pooled control (alpha = 0.05)
[a)] Statisics were performed using only negative control

Conclusion: Mortality adult: NOEC 2.0 mg/L, LOEC 5.0 mg/L
Reproduction (offspring per surviving parental): NOEC: 5.0 mg/L, LOEC: 12.5 mg/L
Growth (weight and length): NOEC: 5.0 mg/L, LOEC: 12.5 mg/L
All endpoints are based on nominal concentrations.

Reference:	***Chironomus riparius* 28-day chronic toxicity test with BYI 08330 (tech.) in a water-sediment system using spiked water**
Author(s), year:	Dorgerloh, M., 2005 i
Report/Doc. number:	M-248099-02-2, 16.03.2005, Amended: 29.06.2006
Guideline(s):	OECD 219
GLP:	Yes
Deviations:	The concentration of the used solvent control was higher than recommended (0.2 mL/L instead of 0.1 mL/L), however no significant differences between solvent control and the water only control were noted.
Validity:	Acceptable

Test substance: Spirotetramat (BYI 08330), purity: 97.2 %, batch: 08045/0014

Material and methods:

Test species: Midge (*Chironomus riparius*)
- Number organisms: 4 replicates each with 20 larvae per treatment and control
- Age: First instar (L1) larvae

Type of test, duration: Static test (spiked water exposure), 28 d

Applied conc.:
- Nominal: 0 (control and solvent control), 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, 3.20 mg/L
- Initial measured: -- (control and solvent control), 0.058, n.a., n.a., 0.401, n.a., n.a., 2.95 mg/L
- Solvent: 0.2 mL/L Aceton

Test system:
- Water quality: M7 medium (according to OECD), the height of water column was 6.0 cm
- Sediment: 74 % quartz sand, 4 – 5 % sphagnum peat, 20 % Kaolin, 1 % $CaCO_3$ to adjust the pH to 7 ± 0.5; the wet sediment layer was 1.5 cm
- Size of test vessels: 0.6 L glass beakers (9.5 cm diameter)
- No. of replicates: 4 for biological evaluation and 1 for chemical analysis per each concentration and control

Test condition:

Temperature:	19.8 – 20.1 °C
pH:	8.3 – 8.7
Hardness:	320 – 338 mg $CaCO_3$/L
O_2 content:	6.9 – 8.5 mg O_2/L (> 60 % saturation)
Light regime:	16 hours light / 8 hours darkness, intensity: 500 – 1000 lux
Feeding:	3 x weekly with a commercial ornamental fish food extract (0.5 – 1 mg/larvae/day)
Test parameters:	The sex, the time point of emergence and the number of emerged midges were recorded daily. Behavioural differences compared to control were observed 3 times per week. For chemical analysis (HPLC/UV) of spirotetramat in the overlaying water column and the pore water of sediment samples were taken from fresh after 1 hours, 7 and 28 days from 0.05, 0.4 and 3.2 mg/L treatment.
Statistics:	Sex ratio: Chi^2-test EC_{50}: Probit analysis LOEC: ANOVA and multiple t-tests Comparison of control groups: Student-t test for homogeneous variances All statistical analyses are based on data of pooled controls.

Findings:

Analytical data:

Table 45: Measured concentration of spirotetramat in the overlaying water and the pore water

Spirotetramat [mg a.s./L]	1 hour		7 days		28 days	
	Analysed conc. [mg/L][a)]	% of nominal	Analysed conc. [mg/L][a)]	% of nominal	Analysed conc. [mg/L][a)]	% of nominal
	Overlaying water					
Control	< LOQ	--	n.a.	--	n.a.	--
Solvent control	< LOQ	--	n.a.	--	n.a.	--
0.05	0.0579	115.8	< LOQ	0	< LOQ	0
0.40	0.401	100.3	< LOQ	0	< LOQ	0
3.20	2.95	92.2	0.0529	1.7	< LOQ	0
	Pore water [b)]					
Control	< LOQ	--	n.a.	--	n.a.	--
Solvent control	< LOQ	--	n.a.	--	n.a.	--
0.05	< LOQ	0	< LOQ	0	< LOQ	0
0.40	< LOQ	0	< LOQ	0	< LOQ	0
3.20	0.155	0.3	0.0639	0.1	< LOQ	0

LOQ <0.00636

[a)] means of two samples

[b)] calculated to the real volume of pore water and the applied amount of a.s.

Effects:	Sex ratio: No relationship between treatment and sex ratio was found, therefore number of males and females midges was pooled for further endpoint calculations. Start of emergence: In controls the emergence started on day 14; at concentration levels up to 0.2 mg/L first midges emerged on day 15; at 0.4 and 0.8 mg/l emergence started on day 17 and 18, respectively For further details see.

Table 46: Effects of spirotetramat on midge (C. riparius) in a water-spiked test

Spiroteramat [mg a.s./L] initial nominal concentration (overlying water)	Number of emerged midges (out of 80)	Emergence of inserted larvae			Emergence rate (ER)	Development rate (1/d) pooled sex
		total [%]	male [%]	female [%]		
controls (pooled) [a)]	144	90.0	43.1	46.9	0.90	0.061
0.05	65	72.3	42.50	38.75	0.81	0.063
0.10	72	90.0	40.00	50.00	0.90	0.062
0.20	59	74.8	42.50	31.25	0.74*	0.062
0.40	54	67.5	36.25	31.25	0.68*	0.062
0.80	6	7.5	3.75	3.75	0.08*	0.061
1.60	0	0	-	-	-	-
3.20	0	0	-	-	-	-
NOEC					0.1 mg/L	0.8 mg/L
LOEC					0.2 mg/L	1.6 mg/L
EC_{50} (95% C.I.)					0.46 mg/L (n.d)**	> 0.8 mg/L

ER = midge emerged per vessel/number of introduced larvae

n.d. = not determined due to mathematical reasons

[a)] Result of t-test indicated no significant differences between control and solvent control, thus pooled control are used for statistical analysis.

* significant different from pooled control (alpha = 0.05)

** slope b (probit analysis): 4.429

Conclusions: Emergence of pooled sexes:
NOEC: 0.1 mg/L, LOEC: 0.2 mg/L, EC_{50}: 0.46 mg/L
Development rate of pooled sexes, males and females:
NOEC: 0.8 mg/L, LOEC: 1.6 mg/L, EC_{50}: > 0.8 mg/L
Endpoints based on initial nominal concentrations.

Comments: While no major deviations are noted from guidline OECD 219 significant deviation from USEPA recommendations are notable:
The EPA requires that the sediment be spiked with the test compound and allowed to equilibrate with overlying water prior to test initiation; the overlying water was spiked in this test and equilibrium was not achieved.
EPA also requires that mean-measured, rather than initial measured values, be used to perform statistical analyses and report endpoints.
EPA also recommends having endpoints calculated based on measured concentrations in overlying water, bulk sediment, and interstitial water.
EPA recommends a pH in the range of 6 to 7, the pH values measured in the test were outside this range.

5.4.3 Algae and aquatic plants

Effects on algal growth and growth rate (IIA 8.2.6)

Reference:	***Pseudokirchneriella subcapitata* - Growth inhibition test with BYI 08330 (tech.)**
Author(s), year:	Dorgerloh, M., 2004 c
Report/Doc. number:	DOM 23092, Edition Number: M-128874-01-2, 29.11.2004
Guideline(s):	Draft proposal for updating OECD 201, OPPTS 850.5400, JMAFF guideline (12 Nousan No 8147)
GLP:	Yes
Deviations:	EPA guideline: • The temperature is less than the EPA-specified temperatures (24-25ºC) and the lighting intensity is higher than EPA specified lighting. • The test duration was shorter than is accepted by EPA protocol; the EPA requires 96-120 hour tests. • Test concentrations dropped as low as 22% of the nominal, and test solutions were not centrifuged prior to analysis
Validity:	Acceptable

Test substance:	Spirotetramat (BYI 08330), purity: 97.2 %, batch: 08045/0014
Material and methods:	
Test species:	Green alga (*Pseudokirchneriella subcapitata)*
Number organisms:	1 x 10^4 cells/mL; 3 replicates for each concentration, 6 replicates for the medium and solvent control
Type of test, duration:	Static test, 72 h
Applied conc.:	
Nominal:	0 (medium control and solvent control), 0.31, 1.0, 3.1, 10, 31, 100 mg/L
Measured (mean):	-- (medium control and solvent control), 0.141, 0.471, 1.46, 5.38, 18.5, 63.2 mg/L
Solvent:	0.5 mL/L Aceton
Test conditions:	
Water quality:	Nutrient medium (according to OECD guideline)
Temperature	22.5 – 23.1 °C
pH	7.8 – 8.0 (0 h), 7.5 – 8.3 (96 h)
Incubation:	Continuous “cool white” illumination with fluorescent light (8000 lux ± 15 %)
Test parameters:	Cell numbers per volume were estimated photometrically (measuring the extinction in a single-beam-photometer at 578 nm) at 24, 48 and 72 hours. Morphological examinations were made under a microscope on each study day. For chemical analysis (HPLC method) of test the substance, samples of test solution were taken on day 0 and day 3. Test samples were analysed by direct injection into a HPLC-UV instrument without a centrifugation.
Statistics:	LOEC: ANOVA Procedure, t-test; EC_x: Probit analysis, comparison of control groups: Kolmogorov-Smirnov-test (normal distribution) and Cochran’s test procedure on variance homogeneity All statistical analyses are based on data of pooled control.
Findings:	
Analytical data:	Mean measured concentrations were in the range of 84 – 94 % and 22 – 48 % of nominal concentrations on day 0 and day 3, respectively.
Effects:	
Morphological effects:	None

Biomass & growth:

Table 47: Effects of spirotetramat (BYI 08330) on the green alga P. subcapitata

BYI 08330 [mg/L] (mean measured)	Inhibition after 72 h		
	Biomass (AUC)	Yield[1)]	Growth rate
0 (pooled control)[2)]	--	--	--
0.141	-51.1	-54.7	-11.3
0.471	-26.0	-22.2	-5.3
1.46	-15.1	-8.6	-2.2
5.38	40.9*	46.7*	18.1*
18.5	93.9*	101.2*	122.7*
63.2	98.5*	101.5*	130.7*
NOEC	1.46 mg/L	1.46 mg/L	1.46 mg/L
LOEC	5.38 mg/L	5.38 mg/L	5.38 mg/L
EC_{50} (95% C.I.)	6.58 mg/L (6.29 – 6.91 mg/L)	5.6 mg/L (5.46 – 5.83 mg/L)	8.15 mg/L (7.56 – 8.81 mg/L)

[1)] Yield = biomass at the end of exposure minus the biomass at the start of exposure

[2)] Results statistical analyses of control groups indicated no significant differences, thus pooled control are used for statistical analysis.

* Significant difference (alpha = 0.05) from the control

.

Conclusion: E_bC_{50} (0-72 h): 6.58 mg/L
E_rC_{50} (0-72 h): 8.15 mg/L,
NOEC (0-72 h): 1.46 mg/L (biomass and growth rate)
based on mean measured concentrations

Reference:	**Toxicity of BYI 08330 to the freshwater diatom *Navicula pelliculosa***
Author(s), year:	Kern, M.E.; Lam, C.V., 2005
Report/Doc. number:	EBFNX008, Edition Number: M-252794-01-1, 15.06.2005
Guideline(s):	Draft proposal for updating OECD 201 (2004), OPPTS 850.5400, FIFRA 123-2
GLP:	Yes
Deviations:	OECD: None of relevance US EPA: The number of replicates (3) were fewer than are accepted by EPA protocol (4 for Navicula sp.).
Validity:	Acceptable

Test substance: Spirotetramat (BYI 08330), purity: 97.1 %, batch: 08045/0014

Material and methods:

Test species: Freshwater diatom (*Navicular pelliculosa)*

Number organisms: 1×10^4 cells/mL; 3 replicates for each concentration, medium control and solvent control

Type of test, duration: Static test, 96 h

Applied conc.:

Nominal:	0 (medium control and solvent control), 0.20, 0.51, 1.3, 3.2, 8.0, 20 mg/L
Initial measured:	-- (medium control and solvent control), 0.22, 0.47, 1.25, 3.2, 8.23, 20.4 mg/L
Solvent:	0.5 mL/L Aceton
Test conditions:	
Water quality:	AAP Nutrient medium (according to OECD guideline)
Temperature	23.5 – 24.1 °C
pH	7.7 – 7.9 (0 h), 8.0 – 9.5 (96 h)
Incubation:	Continuous illumination (4026 – 4693 lux), orbital shaking at 100 rpm
Test parameters:	Cell densities were determined using a electronic particle counter after 24, 48, 72 and 96 hours. In addition the cell counts using a haemocytometer and a microscope were performed in order to confirmed measured cell densities. Morphological examinations were made under a microscope on each study day. For chemical analysis (HPLC method) of test the substance, samples of test solution were taken on day 0 and day 4. The test samples were directly analysed without a centrifugation.
Statistics:	NOEC: ANOVA followed by Dunnett's test; EC_{50}: Regression analysis, comparison of control groups: T-test All statistical analyses are based on data of pooled control.

Findings:

Analytical data:	Mean measured concentrations were in the range of 92 – 110 % and 0 (< LOQ) – 59 % of nominal concentrations on day 0 and day 4, respectively.
Effects:	
Morphological effects:	None
Biomass & growth:	

Table 48: Effects of spirotetramat (BYI 08330) on the freshwater diatom N. *pelliculosa*

BYI 08330 [mg/L] (initial measured)	BYI 08330 [mg/L] (geomean day 0/day 4)	Cumulative biomass Inhibition after 96 h [%]	Growth rate Inhibition after 96 h [%]
0 (pooled control) [a)]	0 (pooled control) [a)]	--	--
0.22	0.03	11	3.2
0.47	0.193	10	2.4
1.25	0.55	15*	4.4**
3.2	1.00	16*	3.5
8.23	4.73	62*	13*
20.4	15.4	99*	74*
NOEC		0.19 mg/L	1.0 mg/L
LOEC		0.55 mg/L	4.73 mg/L
EC_{50} (95% C.I.)		4.05 mg/L (3.73 – 4.38 mg/L)	15.0 mg/L (0 – 903 mg/L)

[a)] Result of t-test is indicated no significant differences between control and solvent control, thus pooled control are used for statistical analysis.

* Significant difference (alpha = 0.05) from the control

** Statistically significant from control but not considered biologically significant

Conclusion: E_bC_{50} (0-96 h): 4.05 mg/L

	E_rC_{50} (0-96 h): 15.0 mg/L, NOEC (0-96 h): 0.19 mg/L (biomass); 1.0 mg/L (growth rate) based on geometric mean measured concentrations (day 0 and day 4)

Reference:	**Toxicity of BYI 08330 to the blue-green alga *Anabaena flos-aqua***
Author(s), year:	Kern, M.E.; Lam, C.V., 2006
Report/Doc. number:	EBFNX007, Edition Number: M-264055-01-1, 12.01.2006
Guideline(s):	Draft proposal for updating OECD 201 (2004), OPPTS 850.5400, FIFRA 123-2
GLP:	Yes
Deviations:	All guidelines: The study results did not exhibit 50% inhibition at the highest test concentration for growth rate. US EPA: The temperature is less than the EPA-specified temperatures (24-25ºC).
Validity:	Acceptable, even if the inhibiton for growth rate is lower than recommended the enpoints are acceptable for EU risk assessment.

Test substance:	Spirotetramat (BYI 08330), purity: 97.99 %, batch: 08045/0014
Material and methods:	
Test species:	Blue-green alga (*Anabaena flos-aqua)*
Number organisms:	1 x 10^4 cells/mL; 3 replicates for each concentration, medium control and solvent control
Type of test, duration:	Static test, 96 h
Applied conc.:	
Nominal:	0 (medium control and solvent control), 0.51, 1.3, 3.2, 8.0, 20 mg/L
Initial measured:	-- (medium control and solvent control), 0.45, 1.29, 3.32, 9.05, 21.7 mg/L
Solvent:	0.5 mL/L Aceton
Test conditions:	
Water quality:	AAP Nutrient medium (according to OECD guideline)
Temperature	23.3 – 23.8 °C
pH	7.7 – 7.9 (0 h), 8.1 – 8.3 (96 h)
Incubation:	Continuous illumination with cool white fluorescents (2200 lux ± 10 %), orbital shaking at 100 rpm
Test parameters:	Cell densities were determined using a electronic particle counter after 24, 48, 72 and 96 hours. In addition the cell counts using a hemocytometer and a microscope were performed in order to confirmed measured cell densities. Morphological examinations were made under a microscope on each study day. For chemical analysis (HPLC method) of test the substance, samples of test solution were taken on day 0 and day 4. The test samples were directly analysed without a centrifugation.
Statistics:	NOEC: ANOVA followed by Dunnett's test; EC_{50}: Regression analysis, comparison of control groups: T-test All statistical analyses are based on data of pooled control.
Findings:	
Analytical data:	Mean measured concentrations were in the range of 87 – 113 % and 31 – 52 % of

nominal concentrations on day 0 and day 4, respectively.

Effects:

Morphological effects: None

Biomass & growth:

Table 49: Effects of spirotetramat (BYI 08330) on the blue-green algae Anabaena flos-aquae

BYI 08330 [mg/L] (initial measured)	BYI 08330 [mg/L] (geomean day 0/day 4)	Cumulative biomass Inhibition after 96 h [%]	Growth rate Inhibition after 96 h [%]
0 (pooled control) [a)]	0 (pooled control) [a)]	--	--
0.45	0.3	-43	3
1.29	0.718	-15	-13
3.32	2.08	-51	-13
9.05	5.68	-6	-3
21.7	15.1	48**	14
NOEC		5.68 mg/L	15.1 mg/L
LOEC		15.1 mg/L	> 15.1 mg/L
EC_{50} (95% C.I.)		15.2 mg/L (14.9 – 15.5 mg/L)	> 15.1 mg/L

[a)] Result of t-test is indicated no significant differences between control and solvent control, thus pooled control are used for statistical analysis.

** Biological significant based on % inhibition compared to the pooled controls

Conclusion: E_bC_{50} (0-96 h): 15.2 mg/L
E_rC_{50} (0-96 h): > 15.1 mg/L,
NOEC (0-96 h): 5.68 mg/L (biomass); 15.1 mg/L (growth rate)
based on geometric mean measured concentrations (day 0 and day 4)

Reference:	**Toxicity of BYI 08330 technical to the saltwater diatom *Skeletonema costatum***
Author(s), year:	Banman, C. S. & Lam, C. V., 2006
Report/Doc. number:	EBFNX009-1, Edition Number: M-271037-02-1, 16.06.2006
Guideline(s):	Draft proposal for updating OECD 201 (2004), OPPTS 850.5400, FIFRA 123-2
GLP:	Yes
Deviations:	OECD: None of relevance USEPA: -The photoperiod for studies involving *Skeletonema costatum* is 14 hour light/10 hour dark cycle; however, continuous illumination was used in this study. -Test samples were not centrifugated prior to chemical analyses.
Validity:	Acceptable

Test substance: Spirotetramat (BYI 08330), purity: 97.99 %, batch: 08045/0014

Material and methods:

Test species: Marine diatom (*Skeletonema costatum)*

Number organisms: 1 x 10^4 cells/mL; 3 replicates for each concentration, medium control and solvent control

Type of test, duration: Static test, 96 h

Applied conc.:

Nominal:	0 (medium control and solvent control), 0.256, 0.640, 1.60, 4.0, 10 mg/L
Initial measured:	-- (medium control and solvent control), 0.240, 0.622, 1.63, 4.15, 10.6 mg/L
Solvent:	0.5 mL/L Aceton
Test conditions:	
Water quality:	ASTM saltwater media (slightly modified: 40 mg/L silicates instead of 20 mg/L silicates, salinity 27 ‰
Temperature	19.4 – 21.0 °C
pH	8.0 – 8.4
Incubation:	Photoperiod: 16:8 (light/dark), intensity (3897 - 4682 lux), orbital shaking at 100 rpm
Test parameters:	Cell densities were determined using an electronic particle counter after 24, 48, 72 and 96 hours. In addition the cell counts using a haemocytometer and a microscope were performed in order to confirmed measured cell densities. Morphological examinations were made under a microscope on each study day. For chemical analysis (HPLC method) of test the substance, samples of test solution were taken on day 0 and day 4. . Test samples were analysed by direct injection into an HPLC instrument without centrifugation.
Statistics:	NOEC: ANOVA followed by Dunnett's test; EC_{50}: Linear regression analysis, comparison of control groups: T-test All statistical analyses are based on data of pooled controls.

Findings:

Analytical data:	Mean measured concentrations were in the range of 94 – 106 % and 25 – 46 % of nominal concentrations on day 0 and day 4, respectively.
Effects:	
Morphological effects:	None
Biomass & growth:	

Table 50: Effects of spirotetramat (BYI 08330) on the marine diatom S. costatum.

BYI 08330 [mg/L] (initial measured)	BYI 08330 [mg/L] (geomean)	Cumulative biomass Inhibition after 96 h [%]	Growth rate Inhibition after 96 h [%]
0 (pooled control) [a)]	0 (pooled control) [a)]	--	--
0.24	0.124	-22	-10
0.622	0.394	57*	27*
1.63	1.03	87*	61*
4.15	2.7	105*	115*
10.6	6.95	104*	111*
NOEC		0.124 mg/L	
LOEC		0.394 mg/L	
EC_{50} (95% C.I.)		0.36 mg/L (0.27 – 0.46 mg/L)	0.98 mg/L (0.92 – 1.05 mg/L)

[a)] no statistically significant difference between dilution control and solvent control

* Significant difference (alpha = 0.05) from the control

Conclusion:	E_bC_{50} (0-96 h): 0.36 mg/L E_rC_{50} (0-96 h): 0.98 mg/L NOEC (0-72 h): 0.12 mg/L (biomass and growth rate) based on geometric mean measured concentrations

Effects on aquatic plants

Reference:	***Lemna gibba* G3 - Growth inhibition test with BYI 08330 (tech.) under static-renewal test conditions**
Author(s), year:	Dorgerloh, M., 2005 j
Report/Doc. number:	DOM 24019, Edition Number: M-255296-01-2, 29.07.2005
Guideline(s):	OECD 221, EPA OPPTS 850.4400
GLP:	Yes
Deviations:	EPA guideline: -The light intensity was higher than the EPA recommended light intensity. -All test concentrations have not been centrifuged prior to analysis. -Precipitate was observed in the highest test level.
Validity:	Acceptable

Test substance:	Spirotetramat (BYI 08330), purity: 97.2 %, batch: 08045/0014
Material and methods:	
Test species:	Duckweed (*Lemna gibba*)
Number organisms:	12 fronds per vessel; 3 replicates for each concentration, control and solvent control
Type of test, duration:	Static renewal test (renewal of test medium on day 0, 3 and 5), 7 d
Applied conc.:	
Nominal:	0 (medium and solvent control), 0.30, 0.95, 3.05, 9.77, 31.3, 100 mg/L
Measured (mean):	-- (medium and solvent control), 0.14, 0.47, 1.54, 6.06, 20.5, 71.4 mg/L
Solvent	0.5 mL/L Aceton
Test conditions:	
Water quality:	20X-AAP growth medium (according to OECD guideline), the pH was adjusted to 7.5 ± 0.1
Temperature	23.5 – 24.8 °C
pH	7.2 – 7.6 (new test media), 8.0 – 8.7 (aged test media); control: 7.4 (0 d), 8.7 (7 d)
Incubation:	Continuous illumination with white fluorescent light (mean intensity: 8.04 kLux) in a growth incubator.
Test parameters:	Biomass: Frond numbers and total frond area were determined using LemnaTec Scanalyzer machine on day 0, 3, 5 and 7, respectively. Dry weights of plants (needed for yield calculations) were measured at the end of the study (final biomass). For chemical analysis (HPLC-MS/MS, HPLC-UV) of test the substance (BYI 08330) samples of test solution were taken from freshly prepared test media (0 d, 3 d, 5 d) and from aged test media (3 d, 5 d, 7 d). Test samples were analysed by direct injection into an HPLC instrument without centrifugation.

Statistics: LOEC: ANOVA (α = 0.05, one sided) and Dunnett's multiple t-tests

Findings:

Analytical data: Overall mean measured concentrations ranged from 47 % to 71 % of nominal. An overview of all measured concentrations is given in the table:

Table 51: Analytical results of spirotetramat

Day	Nominal concentration of BYI 08330 [mg/L]	Actual concentration of BYI 08330				
		Detection 1 [mg/L]	Detection 2 [mg/L]	Mean [mg/L]	% of nominal	Time weighted mean [mg/L]*
0	Control	< 0.0245	< 0.0245	< 0.0245	-	
3 aged		< 0.0245	< 0.0245	< 0.0245	-	
3 new		< 0.0245	< 0.0245	< 0.0245	-	-
5 aged		< 0.0245	< 0.0245	< 0.0245	-	
5 new		< 0.0245	< 0.0245	< 0.0245	-	
7		< 0.0245	< 0.0245	< 0.0245	-	
0	Solvent control	< 0.0245	< 0.0245	< 0.0245	-	
3 aged		< 0.0245	< 0.0245	< 0.0245	-	
3 new		< 0.0245	< 0.0245	< 0.0245	-	-
5 aged		< 0.0245	< 0.0245	< 0.0245	-	
5 new		< 0.0245	< 0.0245	< 0.0245	-	
7		< 0.0245	< 0.0245	< 0.0245	-	
0	0.30	0.246	0.246	0.246	82	
3 aged		0.122	0.120	0.121	40	
3 new		0.258	0.256	0.257	86	0.14
5 aged		0.141	0.410	0.141	47	
5 new		0.112	0.107	0.109	36	
7		0.0604	0.0562	0.0583	19	
0	0.95	0.740	0.742	0.741	78	
3 aged		0.362	0.361	0.362	38	
3 new		0.779	0.779	0.779	82	0.47
5 aged		0.462	0.457	0.459	48	
5 new		0.426	0.423	0.424	45	
7		0.227	0.227	0.227	24	
0	3.05	2.34	2.34	2.34	77	
3 aged		1.08	1.08	1.08	35	
3 new		2.41	2.40	2.40	79	1.54
5 aged		1.50	1.51	1.50	49	
5 new		1.63	1.61	1.62	53	
7		0.869	0.865	0.867	28	
0	9.77	9.12	9.11	9.11	93	
3 aged		4.76	4.70	4.73	48	
3 new		7.66	7.62	7.64	78	6.06
5 aged		4.91	4.93	4.92	50	
5 new		6.31	6.28	6.29	64	
7		4.49	4.48	4.48	46	
0	31.3	27.5	27.4	27.5	88	
2 aged		15.3	15.3	15.3	49	
2 new		26.5	26.5	26.5	85	20.5
5 aged		17.7	17.7	17.7	57	
5 new		22.9	23.0	22.9	73	
7		16.4	16.3	16.4	52	
0	100**	79,4	79,6	79,5	80	
2 aged		48,3	48,5	48,4	48	
2 new		87,0	85,8	86,4	86	71.4
5 aged		62,9	62,7	62,8	63	
5 new		114	114	114	114	
7		63,8	63,7	63,8	64	

Effects:

Morphological effects: None visual effects were observed in concentration levels up to 0.47 mg/L. At 1.54 mg/l slight chlorosis was observed at day 7. At 6.06 mg/L and higher concentration levels strong chlorosis and necrosis were noted.

Biomass & growth: Doubling time T_d of the control = 2.2 d (fulfilled the validity criterion), further details see also tables below:

Table 52: Effects of spirotetramat on duckweed Lemna gibba

Spirotetramat [mg/L] (nominal)	Spirotetramat [mg/L] time weighted mean measured	Yield % inhibition in 7 d	Growth rate% inhibition in 7 d	
			based on frond number	based on total frond area
0.30	0.14	-3.5	2.4	-3.6
0.95	0.47	-5.5	5.8	-4.0
3.05	1.54	-2.3	5.7	-2.7
9.77	6.06	45.7*	34*	50.1*
31.3	20.5	87.4*	70.2*	91.8*
100[a)]	71.4	97.6*	113.5*	116.1*

* Significant difference (alpha = 0.05) from the control

Table 53: Toxicity of spirotetramat on duckweed Lemna gibba based on time weighted mean measured concentrations

Endpoint	Time scale	NOEC [mg/L]	LOEC [mg/L]	EC_{50} (95% C.I.) [mg/L][a)]	EC_{50} (95% C.I.) [mg/L][b)]
Growth rate (based on frond number)	7 d	1.54	6.06	10.4 (7.96 – 13.8)	9.96 (7.85 – 12.49)
Growth rate (based on total area of plants	7 d	1.54	6.06	6.21 (5.33 – 7.18)	6.21 (5.55 – 6.91)
Yield (based on frond number)	7 d	1.54	6.06	4.62 (2.04 – 10.3)	4.49 (2.70 – 7.20)
Yield (based on biomass – dry weight)	7 d	1.54	6.06	6.96 (5.76 – 8.38)	7.04 (6.07 – 8.12)
Visual effects on plants	7 d	0.47	1.54	--	--

[a)] The 100 mg/L concentration level was excluded from EC50 calculations because of exceeded water solubility

[b)] The 100 mg/L concentration level was included into the EC50 calculations.

Conclusion: E_yC_{50} (7 d): 4.62 mg/L
E_rC_{50} (7 d): 6.21 mg/L
NOEC (7d): 1.54 mg/L (for biomass/yield and growth rate)
based on time weighted mean measured concentrations

5.4.4 Other aquatic organisms (including sediment)

None.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Conclusion of environmental classification and labelling according to Directive 67/548/EEC

Hazard symbol(s)	
Indications of danger	N Dangerous for the environment
Risk phrases	R51/53 Toxic to aquatic organisms, may cause long-term adverse effect in the aquatic environment.
Safety phrases	S56 Dispose of this material and its container to hazardous or special waste collection point. S57 Use appropriate container to avoid environmental contamination. S60 This material and its container must be disposed of as hazardous waste. S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

Justification for the proposal

N Applicable as R51/53 has been assigned.

R51/53 In ecotoxicity tests with standard species (fish, daphnia and algae), *Lepomis macrochirus* is the most sensitive species with a EC50 value of 2.2 mg/l (Dorgerloh 2005a). Spirotetramat is not readily biodegradable (Weyers, 2005).

S56 and S57 Because of the toxicity of spirotetramat to aquatic organisms and potential long term adverse effects to the aquatic environment

S60 Applicable to all dangerous substances.

S61 Applicable as R51/53 has been assigned.

Conclusion of environmental classification according to Regulation EC 1272/2008

Aquatic Acute 1

H400 'Very toxic to aquatic life'

'Warning' and environmental warning label.

M factor: 1

Justification for the proposal

Spirotetramt is acute very toxic to eastern oyster (*Crassostrea virginica*) and diatomen *Skeletonema costatum* with $EC_{(r)}50$ values of 0.85 mg/L and 0.96 mg/L. Spirotetramat is not readily biodegradable because in a Manometric Respirometry Test no degradation (maximum 1 %) was determined within 28 days. However, rapid degradation was demonstrated in a water/sediment study with a DT50 of 0.78 for the whole system. Thus spirotetramat meets the criterion of the degradation > 70 % within a 28-day period the aquatic environment.

An M factor of 1 is applicable based on $0.1 < L(E)C_{50} \leq 1$ mg/l

Spirotetramat fulfils the criteria for classification as aquatic environmental hazard based on the CLP Regulation and should be classified.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Spirotetramat was shown to hydrolyse in strong dependence of the pH value and temperature, $DegT_{50}$ values at 25 °C were 32.5, 8.6 and 0.32 days at pH 4, 7 and 9, respectively. Hydrolysis rates decreased with decreasing temperature. Photodegradation of spirotetramat can be considered a significant route for the elimination of the compound from the environment.

Spirotetamat is not readily biodegradable, but meets the criterion for rapid degradation (> 70 % within 28 days in the aquatic environment) with a $DT_{50whole\ system}$ of 0.78 days in a water/sediment study.

Spirotetramat has a low potential of bioaccumulation in aquatic system because of a measured $logP_{ow}$ value of 2.51 at pH 7.

Spirotetramat is acute very toxic to eastern oyster (*Crassostrea virginica*) and diatomen *Skeletonema costatum* with $EC_{(r)}50$ values of 0.85 mg/L and 0.96 mg/L.

Classification categories	aquatic environmental hazard **acute category 1**	
GHS Pictogram		
Signal Word	Warning	
Hazard Statement	H400	'Very toxic to aquatic life'
	EUH 401	'To avoid risk to human health and the environment, comply with instruction for use.'
M-factor	1	
Precautionary statements — Prevention	P273	Avoid release to the environment
	P391	Collect spillage
	P501	Dispose of contents/container to ….

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

The DS proposed to classify the substance as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) in accordance with the CLP Regulation, with an M-factor of 1 for both. The corresponding classification according to the DSD is N; R50/53. The proposal is based on short-term marine invertebrate and marine diatom toxicity results (96-h EC_{50} of 0.85 mg/L and 96-h E_rC_{50} of 0.96 mg/L, respectively) for the acute CLP and the DSD classification, while a long-term invertebrate toxicity result (28-d NOEC of 0.1 mg/L in a spiked water-sediment test with *Chironomus riparius*) together with the fact that the substance is not rapidly (or readily) biodegradable supports chronic classification under the CLP Regulation.

Comments received during public consultation

Three MSCAs commented during the public consultation, two indicating their support for the proposed environmental classification. One MSCA queried the use of oyster growth data for acute classification purposes, and also suggested that a species sensitivity distribution might be appropriate to take account of the range of algal data available, particularly as the 95% confidence intervals for the most sensitive result straddle the 1 mg/L threshold. A second Member State, while noting that toxicity data from a sediment-dwelling species had been used for chronic classification, did not object to this.

Additional key elements

None.

Assessment and comparison with the classification criteria

Degradability: Spirotetramat hydrolyses under standard conditions, with calculated half-lives at 25 °C of 32.5, 8.6 and 0.32 days at pH 4, 7 and 9, respectively (longer half-lives may be expected at lower temperatures). One major product was formed in all tests (Spirotetramat-enol), which can be considered to be stable to hydrolysis. Aqueous photolysis is rapid, with extensive photo-rearrangement after 7 days' irradiation and an estimated half-life of about 9 – 12 days under natural summer sunlight conditions at pH 5. Maximum amounts of four products formed were 42.9, 22.9, 11.5 and 19.3% of applied radioactivity (AR), respectively. Whilst photolysis is not relevant for classification purposes, it might be a factor in the interpretation of aquatic toxicity tests.

Spirotetramat failed a test for ready biodegradation, achieving at most 1% mineralisation in 28 days. Simulation tests in two aerobic water-sediment systems using radio-labelled substance indicated fast primary degradation, with no Spirotetramat detectable in the water or sediment phases in either system after 7 days. The first order degradation DT_{50} value for the whole system was 1 day in both cases, but a maximum of only 24% mineralisation occurred over 120 days in one system. Major metabolites were Spirotetramat-enol (reaching a maximum in the total system of 92 – 99.0% of AR after 7 – 14 days, decreasing thereafter) and Spirotetramat-ketohydroxy (with an increasing trend towards the end of the studies, reaching a maximum in the total system 50.8% of AR at study termination). Other identified metabolites (Spirotetramat-MA-amide, Spirotetramat-oxo-enol isomer and Spirotetramat-di-hydroxy) did not exceed 9.8% of AR in the total system individually. Non-extractable residues accounted for a maximum of 40.7% of AR by day 91. Aerobic degradation in soils followed a similar pattern, with rapid primary degradation, formation of non-extractable residues and limited mineralisation (up to 19.4% of AR after 50 days in EU soils, but only 15.3% of AR after 360 days in a US soil).

Despite hydrolysis half-lives above 16 days at some relevant pH values, lack of ready biodegradation and limited mineralization in water-sediment and soil simulation tests, the substance does undergo rapid primary degradation in aquatic systems. The conclusion about rapid degradability therefore depends on whether any of the metabolites are classifiable.

The most sensitive ecotoxicity values for one of the major metabolites, Spirotetramat-enol, are a 48-h LC_{50} of 74.9 mg/L for *Chironomus riparius* (static, water only exposure, nominal concentrations) and a 7-d E_rC_{50} of 19.3 mg/L and a 7-d $NOEC_{yield}$ of 0.95 mg/L for *Lemna gibba* (both based on nominal concentrations, since test concentrations were well maintained). Whilst the acute *Lemna* value would trigger chronic classification using the surrogate approach (this metabolite has a degradation DT_{50} of 37.9 – 59 days in aerobic water/sediment systems), the 7-d NOE_rC is 3.05 mg/L, which is above the classification threshold. Since growth rate is the preferred measure for classification purposes, this means that the *Lemna* data do not lead to classification for this metabolite. Nevertheless, the absence of a chronic toxicity result for *Chironomus riparius* means that the surrogate approach is also appropriate for invertebrates, and Spirotetramat-enol is therefore classifiable as H412 (harmful to aquatic life with long lasting effects). No information was presented in the CLH report for other metabolites.

On this basis, Spirotetramat does not meet the criteria for being rapidly degradable (nor readily biodegradable) in the environment.

Bioaccumulation: The log n-octanol-water partition coefficient (K_{ow}) of Spirotetramat is 2.5 at pH 4 – 9. It is therefore not considered to be a bioaccumulative substance for classification purposes.

Ecotoxicity: The lowest reliable ecotoxicity results were as follows (the key studies are highlighted in bold):

Trophic level	**Species**	**Short-term result**	**Long-term result**
Fish	*Cyprinodon variegatus*	96-h LC_{50} = 1.96 mg/L*	-
	Pimephales promelas	-	33-d NOEC = 0.534 mg/L
Aquatic invertebrates	*Daphnia magna*	48-h EC_{50} > 42.7 mg/L	21-d NOEC = 2.0 mg/L
	Americamysis bahia	96-h EC_{50} = 5.5 mg/L	**-**
	Chironomus riparius	48-h EC_{50} = 1.30 mg/L	**-**
	Crassostrea virginica	**96-h EC_{50} (shell deposition) = 0.85 mg/L**	**-**
Aquatic algae and plants	*Pseudokirchneriella subcapitata*	72-h E_rC_{50} = 8.15 mg/L	72-h NOE_rC = 1.46 mg/L
	Navicula pelliculosa	96-h E_rC_{50} = 15.0 mg/L	96-h NOE_rC = 1.00 mg/L
	Anabaena flos-aquae	96-h E_rC_{50} > 15. 1 mg/L	96-h NOE_rC = 5.1 mg/L
	Skeletonema costatum	96-h E_rC_{50} = 0.96 mg/L	**96-h NOE_rC = 0.12 mg/L**
	Lemna gibba	7-d E_rC_{50} = 6.21 mg/L	7-d NOE_rC = 1.54 mg/L

Note: *This is a marine species, but three freshwater fish species had acute LC_{50} values within a factor of two of this result, so there appears to be good consistency in acute sensitivity amongst fish species.

All values except the long-term *Daphnia* result were based on mean measured concentrations. The purity profile of the key studies complies with the specified

composition in Section 1 of the CLH report. Although a long-term result is not available for the most sensitive fish species, and there are no acute data for the only species for which long-term data are available, the very similar acute sensitivity for four species suggests that this is not a significant issue in terms of using the chronic data directly rather than the surrogate approach.

Daphnia are less acutely sensitive to Spirotetramat than other aquatic invertebrates (including another crustacean, an insect and a mollusc) by an order of magnitude. As the substance is an insecticide, it is important that an insect is present in the data set. An acute result is available for *Chironomus riparius* from a test using water-only exposure (this test is considered valid, although there were some deviations from standard test guidelines). The DS chose to include a 28-day study on *Ch. riparius* using spiked water but with sediment present, and used this value to derive the chronic classification under the CLP Regulation. The results were based on nominal concentrations, but this was a static test, and test substance concentrations in the overlying water were below the limit of quantitation (0.00636 mg/L) in two of three treatment groups after 7 days, and in all exposures after 28 days (most likely due to rapid hydrolysis, as the pH was in the range 8.3 – 8.7). It is therefore unclear what caused the observed toxicity, and the test system might not have achieved equilibrium. In addition, it cannot be ruled out that the test organisms were exposed to the substance or metabolites adsorbed to the sediment surface. Therefore although the DS considers the study to be valid, the RAC did not consider it to be relevant for classification purposes. This means that no suitable chronic toxicity result was available for the three most sensitive invertebrate species, so the surrogate approach needed to be considered for the invertebrate trophic group.

Another consideration is that the most sensitive invertebrate acute toxicity result was for the marine mollusc *Crassostrea virginica*, with a 96-h EC_{50} for shell deposition of 0.85 mg/L (95% confidence intervals: 0.59 – 1.3 mg/L). The results were based on mean measured concentrations (which were in the range of 59–100% of the nominal). As noted during the public consultation, this end point is related to growth rather than mortality/immobilization (which is the measure used for other invertebrate species). However, the RAC recognised that this test is intended to give information on acute toxicity for this species, and the result is within a factor of 2 – 3 of acute toxicity values for both fish and another invertebrate. It is also supported by data on marine diatoms (but see caveats below), so is considered relevant for the classification of this substance.

As might be expected from the potential for rapid hydrolysis and photolysis, the concentrations in the algal studies were not well maintained. For example, mean measured concentrations were in the range 22–48% of nominal by day 3 for *Pseudokirchneriella subcapitata*, and 0–59% of nominal on day 4 for *Navicula pelliculosa*. Results were therefore expressed as geometric mean concentrations (or time weighted mean measured concentrations in the case of *Lemna*, as concentrations were better maintained by the static renewal regime). The most sensitive aquatic algal/plant species was the marine diatom *Skeletonema costatum*, with a 96-h E_rC_{50} of 0.98 mg/L (95% confidence interval: 0.92 – 1.05 mg/L).

<u>Classification according to CLP</u>

Acute aquatic hazard: The lowest reliable short-term aquatic toxicity result was a 96-h EC_{50} of 0.85 mg/L for the mollusc *Crassostrea virginica*. This result was within a factor of 2 – 3 of acute toxicity values for both fish and other invertebrates. It was also supported by acute toxicity data on a marine diatom species which was also slightly below 1 mg/L. Spirotetramat is therefore classifiable as Aquatic Acute 1 (H400), with an M-factor of 1 ($0.1 < L(E)C_{50} < 1$ mg/L).

Chronic aquatic hazard: Spirotetramat is not considered to be rapidly degradable. Reliable and relevant long-term aquatic toxicity data was only available for the fish and aquatic algae/plant trophic levels, with lowest NOEC values of 0.534 and 0.12 mg/L, respectively.

These concentrations were below the threshold value of 1 mg/L for non-rapidly degradable substances, leading to classification as Aquatic Chronic 2 (H411). for invertebrates Due to the lack of reliable chronic toxicity data for the most acutely sensitive species, the surrogate approach was used. Based on the lowest acute $L(E)C_{50}$ of 0.85 mg/L combined with the substance's lack of rapid degradability, classification as Aquatic Chronic 1 (H400) was considered appropriate.

In summary, Spirotetramat is therefore classifiable as Aquatic Chronic 1 (H400), with an M-factor of 1.

Classification according to DSD

The lack of ready biodegradation and a 96-h EC_{50} of 0.85 mg/L for invertebrates (with a similar value for a marine diatom) meant that Spirotetramat fulfilled the criteria for classification with N; R50-53. The following specific concentration limits are therefore applicable:

Concentration of Spirotetramat in the mixture, C (w/w)	**Classification of the mixture**
$C \geq 25\%$	N; R50-53
$2.5\% \leq C < 25\%$	N; R51-53
$0.25\% \leq C < 2.5\%$	R52-53

In summary, the RAC agrees with the original proposal of the DS, although the basis for the lack of rapid degradability and chronic classification are different.

In depth analyses by the RAC

None.

6 OTHER INFORMATION

7 REFERENCES

7.1 Physchem. properties

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Annex II Data and Information					
Bogdoll, B.	KIIA 2.3.2 /01	2005	Henry's law constant of BYI 08330(AE 1302943) at pH 7 and pH 9 Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: AF05/085, Edition Number: M-262215-01-1 Date: 08.12.2005 Non GLP, unpublished	Yes	BCS
Bogdoll, B.; Lemke, G.	KIIA 2.2 /02	2006	Relative density of BYI 08330 - Technical substance Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA06/025, Edition Number: M-270041-01-1 Date: 13.04.2006 GLP, unpublished	Yes	BCS
Bogdoll, B.; Lemke, G.	KIIA 2.4.1 /02	2006	Physical characteristics - Color, physical state and odor of BYI 08330 - Technical substance Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA06/026, Edition Number: M-270051-01-1 Date: 20.04.2006 GLP, unpublished	Yes	BCS
Bogdoll, B.; Lemke, G.	KIIA 2.4.2 /02	2006	Physical characteristics - Color, physical state and odor of BYI 08330 - Technical substance Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA06/026, Edition Number: M-270051-01-1 Date: 20.04.2006 GLP, unpublished	Yes	BCS
Bogdoll, B.; Lemke, G.	KIIA 2.6 /02	2006	Water solubility of BYI 08330 (AE 1302943) in distilled water (Flask Method) Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA06/033, Edition Number: M-270060-01-1 Date: 20.04.2006 GLP, unpublished	Yes	BCS

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Bogdoll, B.; Lemke, G.	KIIA 2.14 /02	2006	Surface tension of BYI 08330 - Technical substance Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA06/024, Edition Number: M-270037-01-1 Date: 13.04.2006 GLP, unpublished	Yes	BCS
Bogdoll, B.; Lemke, G.	KIIA 2.16 /01	2006	Determination of the pH-Value of BYI 08330 (AE 1302943) Pure and technical substance Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA06/023, Edition Number: M-269907-01-1 Date: 13.04.2006 GLP, unpublished	Yes	BCS
Bogdoll, B.; Lemke, G.	KIIA 2.18 /01	2006	The oxidation or reduction properties of BYI 08330 - Technical substance Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA06/027, Edition Number: M-270048-01-1 Date: 20.04.2006 GLP, unpublished	Yes	BCS
Franke, J.	KIIA 2.1.1 /01	2004	BYI 08330, Mix-Batch 08045/0003 - Melting point, boiling point, thermal stability Siemens Axiva GmbH & Co. KG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20040156.01, Edition Number: M-063268-01-1 Date: 26.03.2004 GLP, unpublished	Yes	BCS
Franke, J.	KIIA 2.1.2 /01	2004	BYI 08330, Mix-Batch 08045/0003 - Melting point, boiling point, thermal stability Siemens Axiva GmbH & Co. KG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20040156.01, Edition Number: M-063268-01-1 Date: 26.03.2004 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Franke, J.	KIIA 2.1.3 /01	2004	BYI 08330, Mix-Batch 08045/0003 - Melting point, boiling point, thermal stability Siemens Axiva GmbH & Co. KG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20040156.01, Edition Number: M-063268-01-1 Date: 26.03.2004 GLP, unpublished	Yes	BCS
Franke, J.	KIIA 2.3.1 /01	2004	BYI 08330, Mix-Batch 08045/0003 - Vapour pressure Siemens Axiva GmbH & Co. KG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20040156.02, Edition Number: M-066171-01-1 Date: 26.04.2004 GLP, unpublished	Yes	BCS
Heinemann, O.	KIIA 2.9.1 /01	2004	[Azaspirodecenyl-3-14C]- and [azaspirodecenyl-5-14C]BYI08330: Hydrolytic degradation Bayer CropScience AG, Report No.: MEF-04/176, Edition Number: M-093124-01-2 Date: 08.09.2004 GLP, unpublished	Yes	BCS
Heinemann, O.	KIIA 2.9.3 /01	2004	BYI08330: Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation in water Bayer CropScience AG, Report No.: MEF-04/080, Edition Number: M-092941-01-2 Date: 28.09.2004 GLP, unpublished	Yes	BCS
Heinemann, O.	KIIA 2.9.4 /01	2004	BYI08330: Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation in water Bayer CropScience AG, Report No.: MEF-04/080, Edition Number: M-092941-01-2 Date: 28.09.2004 GLP, unpublished	Yes	BCS
Hellpointner, E.	KIIA 2.10 /01	2004	Calculation of the chemical lifetime of BYI 08330 in the troposphere Bayer CropScience AG, Report No.: MEF-04/400, Edition Number: M-092840-01-1 Date: 14.09.2004 Non GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Kaussmann, M.	KIIA 2.5.1.1 /01	2004	Spectral data set of BYI 08330 (Spirotetramat) - Amendment no. 1 Bayer CropScience AG, Report No.: 15-600-2244, Edition Number: M-182543-02-1 Date: 02.12.2004, Amended: 28.08.2006 GLP, unpublished	Yes	BCS
Kaussmann, M.	KIIA 2.5.1.2 /01	2004	Spectral data set of BYI 08330 (Spirotetramat) - Amendment no. 1 Bayer CropScience AG, Report No.: 15-600-2244, Edition Number: M-182543-02-1 Date: 02.12.2004, Amended: 28.08.2006 GLP, unpublished	Yes	BCS
Kaussmann, M.	KIIA 2.5.1.3 /01	2004	Spectral data set of BYI 08330 (Spirotetramat) - Amendment no. 1 Bayer CropScience AG, Report No.: 15-600-2244, Edition Number: M-182543-02-1 Date: 02.12.2004, Amended: 28.08.2006 GLP, unpublished	Yes	BCS
Kaussmann, M.	KIIA 2.5.1.4 /01	2004	Spectral data set of BYI 08330 (Spirotetramat) - Amendment no. 1 Bayer CropScience AG, Report No.: 15-600-2244, Edition Number: M-182543-02-1 Date: 02.12.2004, Amended: 28.08.2006 GLP, unpublished	Yes	BCS
Kaussmann, M.	KIIA 2.5.1.5 /01	2004	Spectral data set of BYI 08330 (Spirotetramat) - Amendment no. 1 Bayer CropScience AG, Report No.: 15-600-2244, Edition Number: M-182543-02-1 Date: 02.12.2004, Amended: 28.08.2006 GLP, unpublished	Yes	BCS
Lemke, G.; Muehlberger, B.	KIIA 2.8.1 /01	2003	BYI 08330 (AE 1302943) - Partition coefficient 1-octanol/water (HPLC-method) Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA03/036, Edition Number: M-103244-01-1 Date: 13.11.2003 GLP, unpublished	Yes	BCS
Lemke, G.; Muehlberger, B.	KIIA 2.8.2 /01	2003	BYI 08330 (AE 1302943) - Partition coefficient 1-octanol/water (HPLC-method) Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA03/036, Edition Number: M-103244-01-1 Date: 13.11.2003 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Muehlberger, B.	KIIA 2.4.1 /01	2003	BYI 08330 (AE 1302943) - Physical characteristics color, appearance and odor Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA03/035, Edition Number: M-103239-01-1 Date: 03.11.2003 GLP, unpublished	Yes	BCS
Muehlberger, B.	KIIA 2.4.2 /01	2003	BYI 08330 (AE 1302943) - Physical characteristics color, appearance and odor Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA03/035, Edition Number: M-103239-01-1 Date: 03.11.2003 GLP, unpublished	Yes	BCS
Muehlberger, B.; Eyrich, U.	KIIA 2.7 /01	2004	BYI 08330 (AE 1302943) - Solubility in organic solvents Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA03/033, Edition Number: M-122802-01-1 Date: 17.02.2004 GLP, unpublished	Yes	BCS
Muehlberger, B.; Eyrich, U.	KIIA 2.9.5 /01	2005	BYI 08330 (AE 1302943) - Determination of the dissociation constant Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: M-261598-01-1, Edition Number: M-261598-01-1 Date: 24.11.2005 GLP, unpublished	Yes	BCS
Muehlberger, B.; Lemke, G.	KIIA 2.2 /01	2004	BYI 08330 (AE 1302943) - Relative density Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA03/039, Edition Number: M-063293-01-1 Date: 02.04.2004 GLP, unpublished	Yes	BCS
Muehlberger, B.; Lemke, G.	KIIA 2.14 /01	2004	BYI 08330 (AE 1302943) - Surface tension Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA03/065, Edition Number: M-063303-01-1 Date: 02.04.2004 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Muehlberger, B.; Strunk, B.	KIIA 2.6 /01	2003	BYI 08330 (AE 1302943) - Water solubility at pH4, pH7 and pH9 (Flask method) Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA03/034, Edition Number: M-103256-01-1 Date: 21.11.2003 GLP, unpublished	Yes	BCS
Olenik, B.	KIIA 2.17.1 /01	2006	Storage stability of BYI 08330 (Spirotetramat) - Amendment 2 Bayer CropScience AG, Report No.: 151552237, Edition Number: M-272433-03-1 Date: 07.06.2006, Amended: 24.08.2006 GLP, unpublished	Yes	BCS
Olenik, B.	KIIA 2.17.2 /01	2006	Stability to elevated temperature, metals and metal ions of Spirotetramat (BYI 08330) Amendment No.1 Bayer CropScience AG, Report No.: 15-160-2287, Edition Number: M-271221-02-1 Date: 13.04.2006, Amended: 24.07.2006 GLP, unpublished	Yes	BCS
Smeykal, H.	KIIA 2.11.1 /01	2006	BYI 08330 (Spirotetramat); Substance, technical Flammability (Solids) A.10. Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20060305.01, Edition Number: M-269989-01-1 Date: 26.04.2006 GLP, unpublished	Yes	BCS
Smeykal, H.	KIIA 2.11.2 /01	2006	BYI 08330 (Spirotetramat); Substance, technical - auto – flamma-bility (solids - determination of relative self-ignition temperature) A.16. Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20060305.03, Edition Number: M-270010-01-1 Date: 26.04.2006 GLP, unpublished	Yes	BCS
Smeykal, H.	KIIA 2.11.2 /02	2006	BYI 08330 (Spirotetramat); Substance, technical - auto – flamma-bility (Bowews-Cameron-Cage test) Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20060305.05, Edition Number: M-270036-01-1 Date: 26.04.2006 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Smeykal, H.	KIIA 2.13 /01	2006	BYI 08330 (Spirotetramat); Substance, technical - Explosive properties A.14. Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20060305.02, Edition Number: M-269992-01-1 Date: 26.04.2006 GLP, unpublished	Yes	BCS
Smeykal, H.	KIIA 2.15 /01	2006	BYI 08330 (Spirotetramat); Substance, technical - Oxidizing properties A.17 Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20060305.04, Edition Number: M-270029-01-1 Date: 26.04.2006 GLP, unpublished	Yes	BCS
Stupp, H. - P.	KIIA 2.9.2 /01	2005	BYI08330: Phototransformation of BYI08330 in sterile water Bayer CropScience AG, Report No.: MEF-05/206, Edition Number: M-266695-01-2 Date: 15.11.2005 GLP, unpublished	Yes	BCS
Stupp, H. P.	KIIA 2.9.4 /02	2005	[Azaspirodecenyl-3-14C]BYI08330 and [Azaspirodecenyl-5-14C]BYI08330: Phototransformation in natural water Bayer CropScience AG, Report No.: M-266753-01-2, Edition Number: M-266753-01-2 Date: 15.11.2005 GLP, unpublished	Yes	BCS

7.2 Human health hazard assessment

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Annex II Data and Information					
Brendler-Schwaab, S.	KIIA 5.4.5 /01	2003	BYI 08330 - Unscheduled DNA synthesis test with rat liver cells in vivo Bayer HealthCare, Wuppertal, Germany Bayer CropScience AG, Report No.: AT00526, Edition Number: M-116087-01-2 Date: 10.07.2003 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Cappon, G. D.; Fleeman, T. L.; Chapin, R. E.; Hurtt, M. E.	KIIA 5.10 /03	2005	Effects of feed restriction during organogenesis on embryo-fetal development in rabbit Publisher:2005 Wiley-Liss, Inc., Journal:Birth Defects Research (Part B), Volume:74, Pages:424-430, Year:2005, Report No.: Lit. 8639, Edition Number: M-274544-01-1 Non GLP, published also filed: KIIIA1 10.3.1.3 /01	No	
Christenson, W. R.	KIIA 5.10 /08	2008	Technical grade spirotetramat (BYI 08330): Further examination of the finding of brain dilatation in the on year dog study Bayer CropScience LP, RTP, NC, USA Bayer CropScience AG, Report No.: G201862, Edition Number: M-298505-01-1 Date: 03.03.2008 Non GLP, unpublished	Yes	BCS
Christenson, W. R.	KIIA 5.10 /09	2008	Comparison of neurotoxic potential, declines in thyroid hormone and data on sensitivity of the young in studies conducted with Spiromesifen, Spirodiclofen, and Spirotetramat Bayer CropScience, Research Triangle Park, NC, USA Bayer CropScience AG, Report No.: M-298729-01-1, Edition Number: M-298729-01-1 Date: 10.03.2008 Non GLP, unpublished	Yes	BCS
Clark, R. L.; Robertson, R. T.; Peter, C. P.; Bland, J. A.; Nolan, T. E.; Oppenheimer, L.; Bokelman, D. L.	KIIA 5.10 /04	1986	Association between adverse maternal and embryo-fetal effects in Norfloxacin-treated and food-deprived rabbits Publisher:Society of Toxicology, Journal:Fundamental and Applied Toxicology, Volume:7, Pages:272-286, Year:1986, Report No.: M-296863-01-1, Edition Number: M-296863-01-1 Non GLP, published	No	
Eigenberg A. D.	KIIA 5.3.7 /01	2006	A subacute dermal toxicity study in rats with BYI 08330 Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201505, Edition Number: M-275227-01-1 Date: 20.06.2006 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Eigenberg, D. A.	KIIA 5.2.1 /01	2004	An acute oral LD50 study in the rat with BYI 08330 Bayer CropScience LP, Research Triangle Park, NC, USA Bayer CropScience AG, Report No.: 200398, Edition Number: M-069299-01-1 Date: 06.05.2004 GLP, unpublished	Yes	BCS
Eigenberg, D. A.	KIIA 5.2.2 /01	2004	An acute dermal LD50 study in the rat with BYI 08330 Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience AG, Report No.: 200399, Edition Number: M-066937-01-2 Date: 28.04.2004 GLP, unpublished	Yes	BCS
Eigenberg, D. A.	KIIA 5.3.1 /03	2004	Technical grade BYI 08330: A subacute toxicity feeding study in the Beagle dog Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201012, Edition Number: M-182239-01-1 Date: 13.12.2004 GLP, unpublished	Yes	BCS
Eigenberg, D. A.	KIIA 5.3.3 /01	2005	Technical grade BYI 08330 - A 90-day subchronic toxicity feeding study in the Beagle dog Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201223, Edition Number: M-254183-01-1 Date: 09.05.2005 GLP, unpublished	Yes	BCS
Eigenberg, D. A.	KIIA 5.3.4 /01	2006	A chronic toxicity feeding study in the beagle dog with technical grade BYI 08330 Bayer CropScience, Kansas City, MO, USA Bayer CropScience AG, Report No.: 201486, Edition Number: M-274969-01-1 Date: 06.07.2006 GLP, unpublished	Yes	BCS
Esdaile, D.	KIIA 5.2.6 /03	2004	BYI 08330 - Evaluation of potential dermal sensitization in the local lymph node assay Bayer CropScience SA, Sophia Antipolis, France Bayer CropScience AG, Report No.: SA 04120, Edition Number: M-090707-01-2 Date: 09.09.2004 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Gilmore, R. G.; Fickbohm, B. L.	KIIA 5.7.1 /01	2005	An acute oral neurotoxicity screening study with technical grade BYI 08330 in Wistar Rats Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201283, Edition Number: M-254187-01-1 Date: 13.04.2005 GLP, unpublished	Yes	BCS
Herbold, B.	KIIA 5.4.1 /01	2002	BYI 08330 - Salmonella/microsome test plate incorporation and preincubation method Bayer HealthCare, Wuppertal, Germany Bayer CropScience AG, Report No.: AT00056, Edition Number: M-065358-01-2 Date: 24.10.2002 GLP, unpublished	Yes	BCS
Herbold, B.	KIIA 5.4.1 /02	2006	BYI 08330 - Salmonella/microsome test - Plate incorporation and preincubation method Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT03070, Edition Number: M-272000-01-2 Date: 24.05.2006 GLP, unpublished	Yes	BCS
Herbold, B.	KIIA 5.4.2 /01	2002	BYI 08330 - In vitro chromosome aberration test with chinese hamster V79 cells Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT00055, Edition Number: M-065342-01-2 Date: 24.10.2002 GLP, unpublished	Yes	BCS
Herbold, B.	KIIA 5.4.2 /02	2003	BYI 08330 - Cytogenetic screening with chinese hamster V79 cells Bayer HealthCare, Wuppertal, Germany Bayer CropScience AG, Report No.: AT00194, Edition Number: M-075136-01-2 Date: 13.01.2003 Non GLP, unpublished	Yes	BCS
Herbold, B.	KIIA 5.4.3 /01	2002	BYI 08330 - V79/HPRT-test in vitro for the detection of induced forward mutations Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT00137, Edition Number: M-072857-02-2 Date: 03.12.2002, Amended: 04.05.2006 GLP, unpublished	Yes	BCS

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Herbold, B.	KIIA 5.4.4 /01	2002	BYI 08330 - Micronucleus-test on the male mouse Bayer HealthCare, Wuppertal, Germany Bayer CropScience AG, Report No.: AT00048, Edition Number: M-065314-01-2 Date: 24.10.2002 GLP, unpublished	Yes	BCS
Herbold, B.	KIIA 5.8 /02	2005	BYI 08330-CIS-Ketohydroxy (Project: BYI 08330) - Salmonella/microsome test - Plate incorporation and preincubation method Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT02735, Edition Number: M-262850-01-3 Date: 15.12.2005 GLP, unpublished	Yes	BCS
Herbold, B.	KIIA 5.8 /06	2005	BYI 08330-mono-hydroxy (Project: BYI 08330) - Salmonella/microsome test - Plate incorporation and preincubation method Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT02716, Edition Number: M-262976-01-2 Date: 13.12.2005 GLP, unpublished	Yes	BCS
Herbold, B.	KIIA 5.8 /08	2006	BYI 08330-di-hydroxy (Project: BYI 08330) - Salmonella/microsome test - Plate incorporation and preincubation method Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT03069, Edition Number: M-271980-01-2 Date: 24.05.2006 GLP, unpublished	Yes	BCS
Holzum, B.	KIIA 5.6.11 /01	2001	BYI 08330 - Pilot developmental toxicity study in rabbits after oral administration Bayer AG, Wuppertal, Germany Bayer CropScience AG, Report No.: T3062735, Edition Number: M-084392-01-2 Date: 14.11.2001 Non GLP, unpublished	Yes	BCS
Honarvar, N.	KIIA 5.4.4 /02	2003	Chromosome aberration assay in bone marrow cells of the mouse with BYI 08330 RCC Cytotest Cell Research, Rossdorf, Germany Bayer CropScience AG, Report No.: AR00070, Edition Number: M-084116-01-2 Date: 24.03.2003 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Jensen, T. L.	KIIA 5.5.3 /02	2005	A revised homogeneity and stability study of BYI 08330 technical in rodent ration Bayer CropScience, Kansas City, MO, USA Bayer CropScience AG, Report No.: 201363, Edition Number: M-258710-01-1 Date: 04.10.2005 GLP, unpublished	Yes	BCS
Kehrig, B.; Steffens, W.	KIIA 5.9.1 /02	2006	Occupational medical experiences with Spirotetramat/BYI 08330 Bayer Industry Services, Dormagen, Germany Bayer CropScience AG, Report No.: M-277039-01-1, Edition Number: M-277039-01-1 Date: 30.08.2006 Non GLP, unpublished	Yes	BCS
Kennel, P.	KIIA 5.5.4 /01	2005	BYI 08330 - Evaluation of the potential reproductive toxicity in the male rat following daily oral administration by gavage Bayer CropScience SA, Sophia Antipolis, France Bayer CropScience AG, Report No.: SA 04181, Edition Number: M-252001-01-2 Date: 23.05.2005 GLP, unpublished	Yes	BCS
Klaus, A. M.	KIIA 5.6.10 /01	2001	BYI 08330 - Pilot study on developmental toxicity in rats after oral administration Bayer AG, Wuppertal, Germany Bayer CropScience AG, Report No.: T3068559, Edition Number: M-021476-01-2 Date: 08.05.2001 Non GLP, unpublished	Yes	BCS
Klaus, A. M.	KIIA 5.6.10 /03	2004	BYI 08330 - Synonym: FHN 08330 - Supplementary developmental toxicity study in rats after oral administration Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT01512, Edition Number: M-091750-01-2 Date: 07.10.2004 GLP, unpublished	Yes	BCS
Klaus, A.M.	KIIA 5.6.10 /02	2004	BYI 08330, Synonym: FHN 08330 - Developmental toxicity study in rats after oral administration Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT01413, Edition Number: M-086404-01-2 Date: 23.08.2004 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Klaus, A.-M.	KIIA 5.6.11 /02	2004	BYI 08330 - Developmental toxicity study in rabbits after oral administration Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT01003, Edition Number: M-122324-01-2 Date: 17.02.2004 GLP, unpublished	Yes	BCS
Klempner, A.	KIIA 5.1.1 /01	2006	[Azaspirodecenyl-3-14C]BYI 08330: Distribution of the total radioactivity in male and female rats determined by quantitative whole body autoradiography (QWBA) including determination of the total radioactivity in excreta and exhaled 14CO2 Bayer CropScience AG, Report No.: MEF-06/15, Edition Number: M-269337-01-2 Date: 21.02.2006 GLP, unpublished	Yes	BCS
Klempner, A.	KIIA 5.1.1 /02	2006	[Azaspirodecenyl-3-14C]BYI 08330: Adsorption, distribution, excretion and metabolism in the rat Bayer CropScience AG, Report No.: MEF-048/04, Edition Number: M-268709-02-2 Date: 15.02.2006, Amended: 15.09.2006 GLP, unpublished	Yes	BCS
Klempner, A.	KIIA 5.1.1 /04	2006	[Azaspirodecenyl-3-14C]BYI 08330: Depletion of residues and metabolites in plasma, urine, liver, kidney and testis of the male rat Bayer CropScience AG, Report No.: MEF-06/328, Edition Number: M-275731-01-2 Date: 11.08.2006 GLP, unpublished	Yes	BCS
Klempner, A.	KIIA 5.1.1 /07	2006	[Azaspirodecenyl-3-14C]BYI 08330-enol-glucoside supplemental study: adsorption, distribution, excretion and metabolism in the rat Bayer CropScience AG, Report No.: MEF-06/006, Edition Number: M-268645-01-2 Date: 09.02.2006 GLP, unpublished	Yes	BCS
Klempner, A.	KIIA 5.1.1 /08	2006	[Azaspirodecane-3-14C]BYI 08330-ketohydroxy: Adsorption, distribution, excretion and metabolism in the rat Bayer CropScience AG, Report No.: MEF-06/007, Edition Number: M-268931-01-2 Date: 20.02.2006 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Leuschner, J.	KIIA 5.2.4 /01	2002	Acute skin irritation test (patch test) of BYI 8330 in rabbits LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany Bayer CropScience AG, Report No.: R8147, Edition Number: M-062870-01-2 Date: 25.04.2002 GLP, unpublished	Yes	BCS
Leuschner, J.	KIIA 5.2.5 /01	2002	Acute eye irritation study of BYI 08330 by instillation into the conjunctival sac of rabbits LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany Bayer CropScience AG, Report No.: R8146, Edition Number: M-062864-01-3 Date: 25.04.2002 GLP, unpublished	Yes	BCS
Matsuoka, T.; Mizoguchi, Y.; Serizawa, K.; Ishikura, T.; Mizuguchi, H.; Asano, Y.	KIIA 5.10 /07	2006	Effects of stage and degree of restricted feeding on pregnancy outcome in rabbits Journal:The Journal of Toxicological Science, Volume:31, Issue:2, Pages:169-175, Year:2006, Report No.: M-298488-01-1, Edition Number: M-298488-01-1 Non GLP, published	No	
Matsuzawa, T.; Nakata, , M.; Goto, I.; Tsushima, M.	KIIA 5.10 /05	1981	Dietary deprivation induces fetal loss and abortion in rabbits Publisher:Elsevier/North-Holland Scientific Publishers Ltd., Location:Netherlands, Journal:Toxicology, Volume:22, Issue:--, Pages:255-259, Year:1981, Report No.: M-296871-01-1, Edition Number: M-296871-01-1 Non GLP, published	No	
Mihail, F.; Kroetlinger, F.	KIIA 5.3.1 /01	1998	Cyclic ketoenols BSN 3457, BSN 2342, FHN 7504, FHN 8330 - Subacute exploratory toxicity studies in rats (application by feed over 4 weeks) Bayer AG, Wuppertal, Germany Bayer CropScience AG, Report No.: T0061869, Edition Number: M-040236-01-2 Date: 13.02.1998 Non GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Pauluhn, J.	KIIA 5.2.3 /01	2002	BYI 08330 (c.n.: --) - Study on acute inhalation toxicity in rats according to OECD no. 403 Bayer AG, Wuppertal, Germany Bayer CropScience AG, Report No.: 32020, Edition Number: M-064654-01-2 Date: 15.05.2002 GLP, unpublished	Yes	BCS
Petrere, J. A.; Rohn, W. R.; Grantham, L. E.; Anderson, J. A.	KIIA 5.10 /06	1993	Food restriction during organogenesis in rabbits: Effects ob reproduction and the offspring Publisher:Society of Toxicology, Journal:Fundamental and Applied Toxicology, Volume:21, Issue:--, Pages:517-522, Year:1993, Report No.: M-296872-01-1, Edition Number: M-296872-01-1 Non GLP, published	No	
Schladt, L.	KIIA 5.3.1 /02	2001	BYI 08330 - Subacute study with mice (keto-enol design) Bayer AG, Wuppertal, Germany Bayer CropScience AG, Report No.: T2070951, Edition Number: M-035927-01-2 Date: 13.09.2001 Non GLP, unpublished	Yes	BCS
Schmitt, W.	KIIA 5.1.1 /03	2006	Physiology based pharmacokinetic simulation of BYI 08330 in male rats Bayer Technology Services GmbH, Leverkusen, Germany Bayer CropScience AG, Report No.: BTS-WSM0602, Edition Number: M-274844-01-2 Date: 21.07.2006 Non GLP, unpublished	Yes	BCS
Schmitt, W.	KIIA 5.1.1 /05	2006	PBPK-Simulation of BYI 08330 in male rats at high doses Bayer Technology Services GmbH, Leverkusen, Germany Bayer CropScience AG, Report No.: BTS-WSM0603-1, Edition Number: M-274847-02-2 Date: 21.07.2006, Amended: 01.09.2006 Non GLP, unpublished	Yes	BCS
Schuengel, M.	KIIA 5.8 /01	2005	BYI 08330-CIS-Ketohydroxy - Acute toxicity in the rat after oral administration Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT02506, Edition Number: M-258306-01-2 Date: 14.10.2005 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Schuengel, M.	KIIA 5.8 /03	2006	BYI 08330-desmethyl-ketohydroxy - Acute toxicity in the rat after oral administration Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT02927, Edition Number: M-269279-01-2 Date: 06.04.2006 GLP, unpublished	Yes	BCS
Schuengel, M.	KIIA 5.8 /05	2005	BYI 08330-mono-hydroxy - Acute toxicity in the rat after oral administration Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT02687, Edition Number: M-262070-01-2 Date: 08.12.2005 GLP, unpublished	Yes	BCS
Schuengel, M.	KIIA 5.8 /07	2006	BYI 08330-di-hydroxy - Acute toxicity in the rat after oral administration Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT02995, Edition Number: M-270700-01-2 Date: 05.05.2006 GLP, unpublished	Yes	BCS
Steffens, W.	KIIA 5.9.1 /01	2005	Assessment of potential skin sensitization incidence in workers in handling BYI 08330 Bayer CropScience AG, Report No.: M-257035-01-1, Edition Number: M-257035-01-1 Date: 05.09.2005 Non GLP, unpublished	Yes	BCS
Temerowski, M.	KIIA 5.6.10 /04	2008	Spirotetramat (syn. BYI 08330) - Request for historical control data Bayer CropScience AG, Report No.: M-296860-01-1, Edition Number: M-296860-01-1 Date: 30.01.2008 Non GLP, unpublished	Yes	BCS
Temerowski, M.	KIIA 5.9.2 /01	2006	BYI 08330 (Spirotetramat) - Assessment of literature research in various databases Bayer CropScience AG, Report No.: M-275046-01-1, Edition Number: M-275046-01-1 Date: 27.07.2006 Non GLP, unpublished also filed: KIIA 5.9.3 /01 also filed: KIIA 5.9.4 /01	Yes	BCS

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Temerowski, M.	KIIA 5.9.3 /01	2006	BYI 08330 (Spirotetramat) - Assessment of literature research in various databases Bayer CropScience AG, Report No.: M-275046-01-1, Edition Number: M-275046-01-1 Date: 27.07.2006 Non GLP, unpublished also filed: KIIA 5.9.2 /01 also filed: KIIA 5.9.4 /01	Yes	BCS
Temerowski, M.	KIIA 5.9.4 /01	2006	BYI 08330 (Spirotetramat) - Assessment of literature research in various databases Bayer CropScience AG, Report No.: M-275046-01-1, Edition Number: M-275046-01-1 Date: 27.07.2006 Non GLP, unpublished also filed: KIIA 5.9.2 /01 also filed: KIIA 5.9.3 /01	Yes	BCS
Temerowski, M.	KIIA 5.10 /01	2008	Spirotetramat (syn. BYI 08330) - High dose reproductive effects in male rats and their relevance to humans Bayer CropScience AG, Report No.: M-297775-01-1, Edition Number: M-297775-01-1 Date: 20.02.2008 Non GLP, unpublished	Yes	BCS
Temerowski, M.	KIIA 5.10 /02	2008	Spirotetramat (syn. BYI 08330) - Setting of an ARfD for Spirotetramat Bayer CropScience AG, Report No.: M-296931-01-1, Edition Number: M-296931-01-1 Date: 31.01.2008 Non GLP, unpublished	Yes	BCS
Tinwell, H.	KIIA 5.5.4 /02	2006	BYI 08330-Enol - Investigation of the testicular/sperm toxicity in the rat following 21 days of exposure by gavage Bayer CropScience SA, Sophia Antipolis, France Bayer CropScience AG, Report No.: SA06011, Edition Number: M-273959-01-1 Date: 30.06.2006 Non GLP, unpublished	Yes	BCS
Totis, M.	KIIA 5.1.1 /06	2006	[Azaspirodecenyl-3-14C]-BYI 08330: Comparison of the in vitro metabolism in liverbeads from male rat, mouse and human Bayer CropScience SA, Sophia Antipolis, France Bayer CropScience AG, Report No.: SA05319, Edition Number: M-274118-02-2 Date: 06.07.2006, Amended: 09.08.2006 GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Vohr, H. W.	KIIA 5.2.6 /02	2004	BYI 08330 - Study for the skin sensitization effect in guinea pigs (Buehler Patch test) Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT01317, Edition Number: M-078494-01-2 Date: 13.07.2004 GLP, unpublished	Yes	BCS
Vohr, H.-W.	KIIA 5.2.6 /01	2002	BYI 08330 - Study for the skin sensitization effect in guinea pigs (guinea pig maximization test according to Magnusson and Kligman) Bayer AG, Wuppertal, Germany Bayer CropScience AG, Report No.: 32273, Edition Number: M-076253-01-2 Date: 29.07.2002 GLP, unpublished	Yes	BCS
Wahle, B. S.	KIIA 5.3.2 /01	2005	Technical grade BYI 08330: A subchronic toxicity testing study in the rat Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201136, Edition Number: M-252787-01-1 Date: 01.06.2005 GLP, unpublished	Yes	BCS
Wahle, B. S.	KIIA 5.5.1 /01	2005	Technical grade BYI 08330 (common name Spirotetramat): a chronic toxicity testing study in the rat Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201285, Edition Number: M-260765-01-1 Date: 15.11.2005 GLP, unpublished	Yes	BCS
Wahle, B. S.	KIIA 5.5.2 /01	2006	Technical grade BYI 08330 (common name Spirotetramat): An oncogenicity testing study in the rat Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201358, Edition Number: M-273643-01-1 Date: 08.03.2006 Non GLP, unpublished	Yes	BCS
Wahle, B. S.	KIIA 5.5.3 /01	2006	Revised report : Technical grade BYI 08330 (common name Spirotetramat): An oncogenicity testing study in the mouse Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201359-1, Edition Number: M-275506-02-1 Date: 13.03.2006, Amended: 17.03.2006 Non GLP, unpublished	Yes	BCS

Author(s)	Annex point/ reference number	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Wahle, M. S.	KIIA 5.3.2 /02	2005	Technical grade BYI 08330: A subchronic toxicity testing study in the mouse Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201284, Edition Number: M-255359-01-1 Date: 14.07.2005 GLP, unpublished	Yes	BCS
Wirnitzer, U.	KIIA 5.8 /04	2006	BYI 08330-desmethyl-ketohydroxy (Project: BYI 08330) - Salmonella/microsome test - Plate incorporation and preincubation method Bayer HealthCare AG, Wuppertal, Germany Bayer CropScience AG, Report No.: AT03027, Edition Number: M-271090-01-2 Date: 09.05.2006 GLP, unpublished	Yes	BCS
Young, A. D.	KIIA 5.6.1 /01	2006	Technical grade BY1 08330 - A dose range-finding reproductive toxicity study in the Wistar rat (revised report) Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201300-1, Edition Number: M-273578-02-1 Date: 30.05.2006, Amended: 12.07.2006 GLP, unpublished	Yes	BCS
Young, A. D.	KIIA 5.6.1 /02	2006	Technical grade BYI 08330 (common name Spirotetramat): A two generation reproductive toxicity study in the Wistar rat Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201426-1, Edition Number: M-274619-02-1 Date: 30.05.2006, Amended: 13.07.2006 GLP, unpublished	Yes	BCS

- **Environmental hazard assessment**

1.1.1 Fate and Behaviour in the environment

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Heinemann, O.	2004a	[Azaspirodecenyl-3-^{14}C]- and [azaspirodecenyl-5-^{14}C]BYI08330: Hydrolytic degradation Bayer CropScience AG, Report No.: MEF-04/176, Edition Number: M-093124-01-2 Date: 08.09.2004 GLP, unpublished	Yes	BCS
Menke, U.	2006c	BYI08330: Aerobic aquatic metabolism Bayer CropScience AG, Report No.: MEF-04/511, Edition Number: M-269307-01-2 Date: 29.03.2006 GLP, unpublished	Yes	BCS
Roepke, B.	2006b	Kinetic evaluation of the aerobic aquatic metabolism of BYI08330, BYI08330-enol and BYI08330-ketohydroxy in water sediment systems Bayer CropScience AG, Report No.: MEF-06/279, Edition Number: M-277415-01-1 Date: 30.08.2006 Non GLP, unpublished	Yes	BCS
Stupp, H. -P.	2005a	BYI08330: Phototransformation of BYI08330 in sterile water Bayer CropScience AG, Report No.: MEF-05/206, Edition Number: M-266695-01-2 Date: 15.11.2005 GLP, unpublished	Yes	BCS
Weyers, A.	2005	BYI 08330 - Biodegradation Bayer Industry Services, Leverkusen, Germany Bayer CropScience AG, Report No.: 2005/0077/01, Edition Number: M-263287-01-1 Date: 16.11.2005 GLP, unpublished	Yes	BIS

1.1.2 Aquatic Toxicity

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Banman, C. S.; Lam, C. V.	2005	Acute toxicity of BYI 08330 technical to the sheepshead minnow (Cyprinodon variegatus) under flow-trough conditions Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: EBFNX006, Edition Number: M-255363-01-1 Date: 27.07.2005 GLP, unpublished	Yes	BCS
Banman, C. S.; Lam, C. V.	2006	Toxicity of BYI 08330 technical to the saltwater diatom Skeletonema costatum Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: EBFNX009-1, Edition Number: M-271037-02-1 Date: 23.02.2006, Amended: 16.06.2006 GLP, unpublished	Yes	BCS
Cafarelle, M. A.	2005a	BYI 08330 - Acute toxicity to mysids (Americamysis bahia) under flow-through conditions Springborn Smithers Laboratories, Wareham, MA, USA Bayer CropScience AG, Report No.: EBFNX010, Edition Number: M-270200-01-1 Date: 06.01.2005 GLP, unpublished	Yes	BCS
Cafarella, M. A.	2005b	BYI 08330 - Acute toxicity to eastern oysters (*Crassostrea virginica*) under flow-through conditions Springborn Smithers Laboratories, Inc., Wareham, MA, USA Bayer CropScience AG, Report No.: EBFNX011, Edition Number: M-257677-01-1 Date: 15.06.2005 GLP unpublished	Yes	Spring born

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Dorgerloh, M.	2004a	Acute toxicity of BYI 08330 (tech.) to fish *(Oncorhynchus mykiss*) Bayer CropScience AG, Report No.: DOM 24025, Edition Number: M-182649-01-2 Date: 16.12.2004 GLP unpublished	Yes	BCS
Dorgerloh, M.	2004b	Acute toxicity of BYI 08330 (tech.) to fish (*Cyprinus carpio*) Bayer CropScience AG, Report No.: DOM24022, Edition Number: M-128667-01-2 Date: 25.11.2004 GLP unpublished	Yes	BCS
Dorgerloh, M.	2004c	*Pseudokirchneriella subcapitata* - Growth inhibition test with BYI 08330 (tech.) Bayer CropScience AG, Report No.: DOM 23092, Edition Number: M-128874-01-2 Date: 29.11.2004 GLP unpublished	Yes	BCS
Dorgerloh, M.	2005a	Acute toxicity of BYI 08330 (tech.) to fish (*Lepomis macrochirus*) Bayer CropScience AG, Report No.: DOM 24056, Edition Number: M-242689-01-2 Date: 12.01.2005 GLP unpublished	Yes	BCS
Dorgerloh, M.	2005b	Early-life stage toxicity of BYI 08330 tech. to fish (*Pimephales promelas*) Bayer CropScience AG, Report No.: EBFN0305, Edition Number: M-260676-01-2 Date: 16.11.2005 GLP unpublished	Yes	BCS

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Dorgerloh, M.	2005c	Acute toxicity of BYI 08330 (tech.) to the waterflea *Daphnia magna* under static conditions Bayer CropScience AG, Report No.: DOM 24004, Edition Number: M-242683-01-2 Date: 14.01.2005 GLP unpublished	Yes	BCS
Dorgerloh, M.	2005e	Influence of BYI 08330 (tech.) on development and reproductive output of the waterflea *Daphnia magna* in a static renewal laboratory test system Bayer CropScience AG, Report No.: EBFN0245, Edition Number: M-251843-01-2 Date: 13.05.2005 GLP unpublished	Yes	BCS
Dorgerloh, M.	2005g	Acute toxicity of BYI 08330 (tech.) to larvae of *Chironomus riparius* in a 48 h water-only study Bayer CropScience AG, Report No.: EBFNX072, Edition Number: M-262632-01-2 Date: 08.12.2005 GLP unpublished	Yes	BCS
Dorgerloh, M.	2005i	*Chironomus riparius* 28-day chronic toxicity test with BYI 08330 (tech.) in a water-sediment system using spiked water Bayer CropScience AG, Report No.: EBFN0050, Edition Number: M-248099-02-2 Date: 16.03.2005, Amended: 29.06.2006 GLP unpublished	Yes	BCS

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Dorgerloh, M.	2005j	*Lemna gibba* G3 - Growth inhibition test with BYI 08330 (tech.) under static-renewal test conditions Bayer CropScience AG, Report No.: DOM 24019, Edition Number: M-255296-01-2 Date: 29.07.2005 GLP unpublished	Yes	BCS
Kern, M.E.; Lam, C.V.	2005	Toxicity of BYI 08330 to the freshwater diatom *Navicula pelliculosa* Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: EBFNX008, Edition Number: M-252794-01-1 Date: 15.06.2005 Non GLP unpublished	Yes	BCS
Kern, M. E.; Lam, C. V.	2006	Toxicity of BYI 08330 to the blue-green alga *Anabaena flos-aquae* Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: EBFNX007, Edition Number: M-264055-01-1 Date: 12.01.2006 GLP unpublished	Yes	BCS

1) ANNEXES