

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:

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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	Skin Sens. 1; H317 Eye Irrit. 2; H319 Repr. 2, H361fd Aquatic Acute 1; H400; M-Factor 1 Aquatic Chronic 1; H410; M-Factor 1	Xi, R36 R43 Repr. Cat. 3; R62 - 63 N; R50/53
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Sens. 1; H317 Eye Irrit. 2; H319 Repr. 2, H361fd Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Xi, R36 R43 Repr. Cat. 3; R62 - 63 N; R50/53

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; R50/53

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, H410,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.			Conclusive, but not sufficient for classification
2.2.	Flammable gases	No gas.			Conclusive, but not sufficient for classification
2.3.	Flammable aerosols	No aerosol.			Conclusive, but not sufficient for classification
2.4.	Oxidising gases	No gas.			Conclusive, but not sufficient for classification
2.5.	Gases under pressure	No gas.			Conclusive, but not sufficient for classification
2.6.	Flammable liquids	No liquid.			Conclusive, but not sufficient for classification
2.7.	Flammable solids	Not a highly flammable solid.			Conclusive, but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not applicable.			Conclusive, but not sufficient for classification
2.9.	Pyrophoric liquids	No liquid.			Conclusive, but not sufficient for classification
2.10.	Pyrophoric solids	Not applicable. Stable.			Conclusive, but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not applicable. Stable.			Conclusive, but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not applicable. Stable.			Conclusive, but not sufficient for classification
2.13.	Oxidising liquids	No liquid.			Conclusive, but not sufficient for classification
2.14.	Oxidising solids	No oxidizing properties.			Conclusive, but not sufficient for classification
2.15.	Organic peroxides				Data lacking
2.16.	Substance and mixtures corrosive to metals				Data lacking
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	No classification	-	-	Conclusive, but not sufficient for

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification ¹⁾	Reason for no classification ²⁾
	sensitisation				classification
3.4.	Skin sensitisation	Cat. 1, H317			
3.5.	Germ cell mutagenicity	No classification	-	-	Conclusive, but not sufficient for 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	Aquatic Acute 1 - H400 Aquatic Chronic 1 - H410.	M-Factor 1 M-Factor 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:

Hazard statements:

Precautionary statements:

Suppl. Hazard:

Warning

H317, H319, H361fd, H400, H410

P201, 202, 260, 263, 264, 270, 272, 280, P373, P391, P501

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.			Conclusive, but not sufficient for classification
Oxidising properties	No oxidizing properties.			Conclusive, but not sufficient for classification.
Flammability	No self-ignition temperature was observed up to the melting point (142°C) or up to the maximum test temperature of 401°C.			Conclusive, but not sufficient for classification.
Other physico-chemical properties <i>[Add rows when relevant]</i>				
Thermal stability	Endothermic effect in temp. range of 135 – 165°C (melting) and two exothermal effects in the range of 200 – 300°C and 380 – 450 °C with a maximum overall energy of 246 J/g.			Conclusive, but not sufficient for classification
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	No classification	-	-	Conclusive, but not sufficient for classification

lactation				
Environment	R50/53			

¹⁾ Including SCLs

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

Labelling:

<u>Indication of danger:</u>	Xn, Xi, N
<u>R-phrases:</u>	R36, R43, R62, R63, R50/53
<u>S-phrases:</u>	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 Authorities 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 1A, 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-arcuation 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 + 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 environment, classification as R50/53 (DSD) or H400 (CLP aquatic environment hazards acute category 1 very toxic to aquatic organisms) and H410 (CLP aquatic environment hazards chronic category 1 Very toxic to aquatic life with long lasting effects) are proposed.

The classification as R53 according to Directive 67/548/EEC is based on the fact that the active substance is not considered as ready biodegradable/rapidly degradable.

Spirotetramat is of high acute toxicity to marine algae *Skeletonema costatum* with an ErC50 = 0.96 mg/L and to marine invertebrate *Crassostrea virginica* with an EC50 = 0.85 mg/l. Spirotetramat therefore fulfills the criteria for the proposed classification as R50 according to Directive 67/548/EEC and the criteria for the proposed classification as H400 according to Regulation EC 1272/2008.

Spirotetramat is not rapidly degradable and of high chronic toxicity to sediment dwelling organism (*Chironomus riparius*) with a NOEC_{EMERGENCE} = 0.1 mg/L. Therefore Spirotetramat fulfills the criteria for the proposed classification as H410 according to Regulation EC 1272/2008.

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

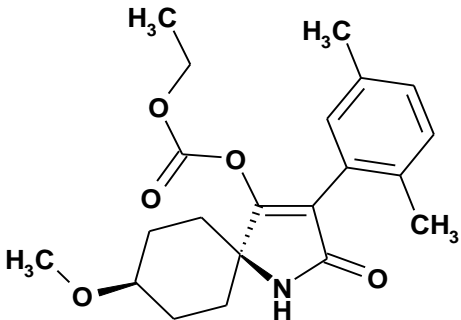
Part B.

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-xyllyl)-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 endothermic 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
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: 5.6×10^{-9} Pa for 20 °C 1.5×10^{-8} Pa for 25 °C 1.5×10^{-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

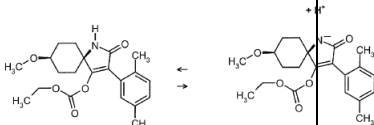
Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
B.2.1.6 Henry's law constant IIA 2.3.2	Calculation		Henry's law constants at 20 °C at different pH values: at pH 4: $6.24 \times 10^{-8} \text{ Pa} \times \text{m}^3 \times \text{mol}^{-1}$ at pH 7: $6.99 \times 10^{-8} \text{ Pa} \times \text{m}^3 \times \text{mol}^{-1}$ at pH 9: $1.09 \times 10^{-7} \text{ Pa} \times \text{m}^3 \times \text{mol}^{-1}$	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 %	^1H and ^{13}C spectra were obtained using a Bruker Avance 400 spectrometer. ^1H -NMR (400.13 MHz, CDCl_3) ^{13}C -NMR (100.61 MHz, CDCl_3) The structure and the assignment 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
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 $[\text{M}+\text{H}]^+$ ion m/z 374.3 and $[2\text{M}+\text{H}]^+$ 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

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference	
IIA 2.5.1.5 Wavelengths at which UV/VIS molar extinction occurs, max >290 nm		BYI 08330, M26802, purity 99.2 %	Peak maxima ity [nm] cm ² /mol 211 10 ³ 276	molar absorptivity [1000 22.0 x 0.8 x 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		
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 water (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 at 20°C pH 7 at 20°C pH 9 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 at 20°C	33.5 mg/L 29.9 mg/L 19.1 mg/L 33.4 mg/L	The results are acceptable	Muehlberger, B., Strunk, B.; 2003 M-103256-01-1 Bogdoll, B., Lemke, G.; 2006 M-270060-01-1

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference	
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 %	°C] ethanol n-hexane 5 toluene dichloromethane 600 acetone - 120 ethyl acetate dimethyl sulfoxide - 300	[g/L at 20 44 0.05 60 > 100 67 200	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 (pH 7) Pow:324 log Pow 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: pH 4: 324 (Pow); 2.51 (log Pow) pH 7: 324 (Pow); 2.51 (log Pow) pH 9: 316 (Pow); 2.50 (log Pow)	The results are acceptable	Lemke, G., Muehlberger, B.; 2003 M-103244-01-1	
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	

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
<p>B.2.1.16 Direct phototransformation in sterile water using artificial light IIA 2.9.2</p>	<p>EPA 162-1, DACO 8.2.3.3.2</p>	<p>[Azaspirodecenyl-3-¹⁴C]-BYI 08330, radiochemical purity > 98 %, specific radioactivity 3.67 MBq/mg</p> <p>[Azaspirodecenyl-5-¹⁴C]-BYI 08330, radiochemical purity > 98 %, specific radioactivity 4.03 MBq/mg</p>	<p>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).</p> <p>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.</p> <p>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.</p> <p>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.</p>	<p>The results are acceptable.</p>	<p>Stupp, H.-P.; 2005 M-266695-01-1</p>

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 irradiated under the same study conditions. Using the UV absorption data and the degradation kinetics of both the experiments a mean quantum yield of $\Phi = 0.00571$ was calculated. Estimates based on two different arithmetic models (GC-Solar and Frank & Klopffer) 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

Test or Study & Annex point	Guideline and method	Test material purity and specification	Findings	Comments	Reference
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
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.	Muehleberger, 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 molar absorptivity [1000 [nm] cm ² /mol] 201 26823.64 213 20704.35 at pH 10: λ max molar absorptivity [1000 [nm] cm ² /mol] 212 22417.81	Acceptable	Y	Olenik, B. (2008) M-306462-01-1

According to Dir. 91/414/EEC, granulometry is not required for active substances. Thus, no study considering this end-point has been provided.

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 5.6×10^{-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:

- Spirotetramat is not a highly flammable solid in the sense of EC guideline A.10
- 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 active substance is a solid; its melting point is > 40 °C; therefore, criteria regarding the flash point are not applicable
- Not explosive in the sense of EC guideline A.14.

- Spirotetramat has no oxidizing properties in the sense of EC guideline A.17.
- 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

3.1.3 Conclusions on classification and labelling

No classification and labelling for physico chemical properties is proposed.

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. A comparative in vitro metabolism study with liver cells from male rats, male mice and male humans revealed differences in the proportions of metabolites formed by the different species. In the liver cells from all species, BYI 08330 was completely metabolized and no parent compound was detected at the end of the incubation. BYI 08330-enol was the first and most prominent metabolite accounting for 66 – 92 % of total metabolites. In the rat, the BYI 08330-enol was further metabolised by oxidation reactions to BYI 08330-desmethyl-enol (oxidative demethylation), BYI 08330-enol-alcohol (oxidation of aromatic methyl group) and BYI 08330-ketohydroxy (oxidation of the azaspirodecenyl moiety). Oxidation products accounted for ca. 14 %. Conjugation was not detected as an in vitro metabolic transformation. In the mouse, oxidative degradation of BYI 08330-enol was detected as a minor “in vitro” metabolic reaction, only (4 % of oxidation products). Conjugation with glucuronic acid to BYI 08330-enol-GA was very prominent with the conjugate accounting for ca. 30 %. In human liver cells, conjugation to BYI 08330-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 $\mu\text{g/g}$ and 190 $\mu\text{g/g}$ BYI 08330, respectively. Glucuronidation of the BYI 08330-enol in mice leads to much lower systemic levels of free BYI 08330-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.

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 ester group and O-demethylation of 8-

Toxicologically relevant compounds ‡
(animals and plants)

Toxicologically relevant compounds ‡
(environment)

methoxy group
Spirotetramat
Spirotetramat

4.2 Acute toxicity

Table 12: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute oral toxicity US-EPA-OPPTS, 870.1100; OECD Guideline No. 425. Deviation(s): none	Wistar Hanover Rat: f > 2000 mg/kg bw	No treatment-related findings.	<i>Eigenberg, 2004</i>
Acute dermal toxicity US-EPA-OPPTS, 870.1200; OECD Guideline No. 402, JMAFF Guideline No. 8268. Deviation(s): none	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.	<i>Eigenberg, 2004</i>
Acute inhalation toxicity OECD Guideline No. 403; 92/69/EEC; US-EPA OPPTS 870.1300. Deviation(s): none	Wistar Rat: LC50 > 4183 mg/m ³ . (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	<i>Pauluhn, 2002</i>

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 LD₅₀ 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, Borcheln, 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 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 gait. 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.
LC₅₀ 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 (CrI: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 LD₅₀ 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 LD₅₀ 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.

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.

4.4 Irritation

4.4.1 Skin irritation

Table 13: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation study OECD Guideline No. 404; EC Guideline B.4. Deviation(s): none	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 cm²). 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.

4.4.2 Eye irritation

Table 14: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye Irritation Study OECD Guideline No. 405; EC Guideline B.5. Deviation(s): none	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).

Justification for classification according to DSD and according to CLP:

Corneal opacity (grade 1) was observed in all animals 24 hours to 6 days after instillation, in animal

nos. 1 and 2 until 7 days after instillation.

Irritation of the **iris** (grade 1) was observed in all animals:

- in animal no. 1: 24 hours to 6 days after instillation,
- in animal no. 2: 72 hours to 5 days after instillation and
- in animal no. 3: 24 hours to 5 days after instillation.

Conjunctival redness (grade 1) was observed in all animals 1 hour to 72 hours after instillation, in animal nos. 2 and 3 until 4 days after instillation. **Conjunctival chemosis** (grade 1) was noted in animal no. 3 from 24 to 48 hours after instillation. There were no systemic intolerance reactions.

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.

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 sensitisation

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) OECD Guideline No. 406; EC Guideline B.6.; US EPA 712-C-98 197, OPPTS 870.2600.	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

Deviation(s): none			
Study for Skin Sensitization Effect of BYI 08330 in Guinea Pigs (Buehler Patch Test). OECD Guideline No. 406; EC Guideline B.6.; US EPA 712-C-98 197, OPPTS 870.2600. Deviation(s): none	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. OECD Guideline 429 (2002). Deviation(s): none	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, Borcheln) 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, spirotetramat 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 spirotetramat (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:

1st to 3rd 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, spirotetramat exhibits no dermal sensitization potential.

Evaluation of Potential Dermal Sensitization in the Local Lymph Node Assay

In a dermal sensitization study with spirotetramat (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 of ³H 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, spirotetramat exhibited dermal sensitization potential.

In comparison with the positive control substance, spirotetramat 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. No further details are given in the Report on medical surveillance on 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 1A for dermal sensitization and labeled with signal word “Warning” and hazard statement H317 (May cause an allergic skin reaction).

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) OECD 409 (MAY 1981); US-EPA-OPPTS 870.3150 (1998)	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) No specific test guideline	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) OECD 408 (1995), US-EPA-OPPTS 870.3100 (1998), JMAFF 12-Nou-san-No. 8147, 2-1-9 (2000). Deviations: none	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 [♂/♀]) OECD 452 (1981), US-EPA OPPTS 870.4100 (1998), JMAFF 12-Nou-san No. 8147; Deviations: none	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) OECD 409 (SEP 1998), US-EPA-OPPTS 870.3150 (1998), JMAFF, Ref. No. 12 Nousan No. 8147, (NOV 2000). Deviations: none	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) U.S. EPA Health Effects Test Guidelines, OPPTS 870.4100, OECD Guidelines for Testing of Chemicals, Section 4, Guideline 452. Deviations: none	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) U.S. EPA-OPPTS Guideline No. 870.3200. Deviations: none	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 to two male and two female Beagle dogs per group 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, (CrI:WI [GlX/BRL/Han] IGS BR) doses of 0, 150, 600, 2500, and 10000 ppm were chosen (Wahle, 2005) and administered to 10 male and 10 female rats. In order to determine the reversibility of possible effects, additional recovery groups (0 and 10,000 ppm) with an equal number of rats were observed for additional 4 weeks without treatment.

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 (9 animals affected) and hypospermia (5 animals affected) 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 (5 animals affected). 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 (9 males and 7 females affected) 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 statistically 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 T₄ in the 1200 and 2500 ppm male and female dose groups. There was a slight, but statistically significant decrease in T₃ 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, T₃ levels were comparable to the control mean. Despite percentage declines in T₄ 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 T₄ 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 (T₄) was statistically decreased in males at 1800 ppm for all post-treatment time points and at 600 ppm at termination of study. In females, T₄ was statistically decreased for most time points at 600 and 1800 ppm. Triiodthyronine (T₃) decreases were less pronounced and occurred in males at 1800 ppm for all time points. There was a tendency for T₄ values to fall below the historical control range at 1800 ppm in males and females; the same tendency was seen in T₃ 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 prothromboplastin 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: full inhibition of de novo synthesis of lipids in aphids. As a consequence, the total lipids in target pests feeding on leaves treated with spirotetramat are significantly decreased and this leads in the end to the death of the insect) 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. According to Annex VI of Directive 91/414, a substance is relevant for classification with “R48” - “Danger of serious damage to health by prolonged exposure” if serious damage (clear functional disturbance or morphological change which has toxicological significance) is likely to be caused by repeated or prolonged exposure by an appropriate route. Substances and preparations are classified at least as harmful when these effects are observed at levels of the order of < 50 mg/kg (bodyweight)/day (rat, oral).

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. According to Regulation No 1272/2008, substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. The guidance value range for oral rat toxicity is $10 < C \leq 100$ mg/kg bw/d.

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.

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
<i>In vitro studies</i>				
Salmonella/microsome test	<i>Salmonella typhimurium</i> (TA98, TA100, TA102, TA1535, TA1537)	0 - 5000 µg/plate	Negative	Herbold, 2006
Salmonella/microsome test	<i>Salmonella typhimurium</i> (TA98, TA100, TA102, TA1535, TA1537)	0 - 5000 µg/plate	Negative	Herbold, 2002

<i>In vitro</i> 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
<i>In vivo studies</i>				
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.

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 (CrI: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; \pm SD)	Main Study 18 months	
			Male	Female
Control	0	0	55	55
Low (LDT)	70	m: 10.9 \pm 1.4 f: 13.7 \pm 2.2	55	55
Mid (MDT)	1,700	m: 263 \pm 35 f: 331 \pm 49	55	55
High (HDT)	7,000 (1)	m: 1022 \pm 196 f: 1319 \pm 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.

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 OECD 415, OECD 421, US-EPA-FIFRA §83-4, MAFF (Japan, 1985)	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.	<u>Parental</u> : 500 ppm (28 mg/kg bw ♂, 31 mg/kg bw ♀) <u>Reproductive/</u> <u>Developmental</u> : 500 ppm (33 mg/kg bw)	<u>parental</u> : -body weight ↓ -body weight gain ↓ <u>reproductive</u> : -abnormal sperm cells -sperm motility ↓ -sperm progression ↓ <u>developmental</u> : -body weight ↓ -body weight gain ↓
Multigeneration, rats Young A. D.; 2006 OECD 416, OPPTS 870.3800, MAFF 12-Nousan-No. 8147	0, 250, 1000, 6000 ppm/diet (equivalent to 0, 17, 70 and 419 mg/kg bw (♂) and 0, 20, 82, 485 mg/kg bw (♀))	<u>Parental/reproductive/</u> <u>developmental</u> : 1000 ppm (70 mg/kg bw ♂, 82 mg/kg bw ♀)	<u>parental</u> : -body weight ↓ -body weight gain ↓ -food consumption ↓ -kidney weight ↓ -tubular dilation <u>reproductive</u> : -abnormal sperm cells -reproductive performance ↓ <u>developmental</u> : -body weight ↓ -body weight gain ↓
Developmental toxicity, rats Klaus A. M.; 2004 OECD 414 (2001), 88/302/EEC (1988), US-EPA- OPPTS 870.3700 (1998), JMAFF 12-Nousan-No. 8147 (2000, amended 2001); Deviations: none	0, 20, 140, 1000 mg/kg bw	<u>maternal/ developmental</u> : 140 mg/kg bw	<u>maternal</u> : -food consumption ↓ -body weight loss <u>developmental</u> : -fetal weight ↓ -skeletal variations ↑ -retarded ossification ↑ -skeletal malformations ↑
Supplementary developmental toxicity, rats Klaus A. M.; 2004 OECD 414 (2001), 88/302/EEC (1988), US-EPA- OPPTS 870.3700 (1998), JMAFF 12-Nousan-No. 8147 (2000, amended 2001); Deviations: none	0, 10, 35, 140 mg/kg bw	<u>maternal/ developmental</u> : 140 mg/kg bw	No toxicologically significant changes at the highest dose tested
Developmental toxicity, rabbits Klaus A. M.; 2001 OECD 414 (2001), 88/302/EEC (1988), US-EPA- OPPTS 870.3700 (1998), US EPA 712-C-98-207, JMAFF 12-Nousan-No. 8147 (2000, amended 2001); Deviations: none	0, 10, 40, 160 mg/kg bw	<u>maternal</u> : 10 mg/kg bw <u>developmental</u> : 160 mg/kg bw	<u>maternal</u> : -food consumption ↓ -body weight loss -abortion ↑ -clinical signs <u>developmental</u> : no effects

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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 = F₀). As this test was a preliminary dose finding study the actual feed consumption of the F₁ 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 F₁ 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 F₁ 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 F₁ 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 F₁ 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 F₁ 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 F₁ 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. F₁ 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 F₁ eight-to-nine week old interim animals in the 6000 ppm group. There were no abnormal sperm cells observed in F₁ eight-to-nine week old interim animals at 500 ppm and below.

Sexual maturation:

There were no treatment-related findings observed in the F₁ 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 F₁-generation animals. The target organ is the testes with progressive effects over successive generations: the F₁ 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 (F₁ 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 F₁ 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 F₁ 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 F₁-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 F₁-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 F₁-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 F₁-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 F₁-generation rats. Significant declines in terminal body weight were observed in both F₁ 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 F₁ 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 F₁-generation male and female rats of the 6000-ppm group and the epididymis of a single F₁ male from the 6000-ppm dose group. In F₁-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 F₁-generation male rat at a dose level of 6000 ppm 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 F₁ 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 F₁-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 F₁-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 F₁-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 F₁ and F₂ pups. Pup weights at birth were not affected (F₁ and F₂). On lactation day 21, the F₁ 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 F₂ 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 F₁ and F₂ weanlings no treatment-related macroscopic alterations were observed at any dietary level tested. Absolute brain weights were statistically significantly decreased (F₂ males, F₂ combined sexes) and relative brain weights were increased (F₁ and F₂ males and females; F₁ and F₂ combined sexes) at 6000 ppm. At 6000 ppm, absolute spleen weights were decreased in combined F₁ weanlings and in F₂ male and female weanlings. F₂ 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 F₁ and F₂ weanlings of either sex at any dietary level tested.

Maturation of external sexual organs (balanopreputial separation, vaginal opening) was not affected in F₁ 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 F₁-generation males and females, kidney histopathology, and decreased kidney weight in the F₁ males and females. Reproductive performance was affected in one F₁ male that had an increase in abnormal sperm and reduced fertility at 6000 ppm. Offspring survival was not affected by treatment. However, F₁ and F₂ 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 (F₁ males and females), weight gain (P males, F₁ males and females), and kidney histopathology and decreased kidney weights (F₁ 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 F₁ 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 F₁ and F₂ 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 orally by gavage of 0, 20, 140 and 1000 mg/kg bw (♀) from day 6 to day 19 post coitum.

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, dysplasia 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-arcuation 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-arcuation 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, sternbrae, vertebrae, skull bones) were noted at the 1000 mg/kg bw/day dose level in the osseous and/or cartilaginous parts. Increased incidences of skeletal variations (wavy ribs, 14th ribs, combined osseous and cartilaginous 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				
-1 st	65 (19) ^b	112** (24)	89 (20)	133** (22)
-2 nd	64 (19)	103* (24)	81 (20)	120** (22)
-3 rd	20 (12)	45* (15)	29 (10)	78** (18)
-4 th	39 (16)	77** (23)	61 (18)	111** (22)
-5 th	108 (20)	149 (24)	124 (23)	142** (22)
Distal phalanx digits, left				
-1 st	77 (19)	121* (24)	100 (23)	134** (22)
-2 nd	74 (19)	112 (24)	90 (22)	126** (22)
-3 rd	25 (13)	59** (18)	37 (12)	87** (20)
-4 th	50 (18)	85 (23)	76* (20)	118** (22)
-5 th	109 (20)	149 (24)	123 (23)	142** (22)
Distal phalanx toes, right				
-1 st	57 (19)	101** (23)	87** (23)	111** (22)
-2 nd	34 (14)	67* (22)	52 (16)	95** (22*)
-3 rd	21 (9)	44 (18)	39 (12)	84** (22**)
-4 th	45 (17)	75 (22)	75** (20)	101** (22)
Distal phalanx toes, left				
-1 st	74 (19)	123** (24)	99* (23)	110** (22)
-2 nd	41 (16)	75* (22)	62* (19)	93** (22)
-3 rd	26 (12)	53 (18)	48* (16)	82** (22**)
-4 th	45 (16)	84* (22)	75** (20)	101** (22)
Sternebra				
-2 nd	28 (16)	35 (16)	29 (16)	97** (21)
-3 rd	2 (2)	2 (2)	3 (2)	14* (10*)
-4 th	2 (2)	5 (4)	4 (3)	21** (12**)
-5 th	28 (14)	33 (17)	32 (17)	85** (22*)
-6 th	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				
-3 rd	1 (1)	7 (3)	4 (2)	12* (7)
-4 th	20 (11)	35 (14)	37 (18)	77** (20*)
Sacral vertebral arch(es), left				
-3 rd	1 (1)	7 (3)	4 (2)	13** (8)
-4 th	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				
-3 rd	82 (19)	120 (23)	98 (21)	139** (22)
-4 th	87 (19)	132* (24)	107 (22)	144** (22)
Proximal phalanx digits, left				
-3 rd	92 (20)	129 (24)	107 (23)	143** (22)
-4 th	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				
-1 st	5 (5)	7 (4)	2 (2)	19* (7)
-2 nd	5 (5)	11 (7)	5 (2)	22** (8)
-3 rd	5 (5)	11 (7)	4 (2)	23** (8)
-4 th	5 (5)	12 (7)	4 (2)	28** (9)
-5 th	7 (6)	11 (7)	4 (2)	28** (10)
Distal phalanx toes, left				
-1 st	3 (3)	8 (4)	4 (3)	16* (6)
-2 nd	4 (4)	11 (7)	3 (2)	23** (9)
-3 rd	3 (3)	11 (7)	3 (2)	24** (10)
-4 th	5 (5)	12 (7)	4 (2)	29** (10)
-5 th	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	28 (14)	21 (12)	19 (12)	69** (20)
-11 th	32 (14)	27 (12)	21 (12)	75** (21)
-12 th	15 (12)	5* (5*)	11 (9)	49** (19)
-13 th				
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.

Historical data range are given in table 22 and 23:

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

Observations	Dose (mg/kg/d)				% of Historical control data
	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 observations at the ribs in comparison with historical controls

Observations	Dose (mg/kg/d)				Historical control data (range)
	0	20	140	1000	
Wavy ribs	5.5	17**	17.8**	57.5**	(2.7 – 15.1) [1996 – 2001]
14 th 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-arcuation 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 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 (♀) from day 6 to 28 post coitum by gavage.

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 cartilaginous 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 spirovetramat 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:

Findings on the progeny only occurred at dose levels with signs of distinct (40 mg/kg bw) to severe maternal toxicity (160 mg/kg bw). 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.

Fetal external and visceral evaluations revealed a possibly increased incidence of fetuses with distinct liver lobulation in the 160 mg/kg group.

Table 24: Summary of fetal malformations

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 sternal segment or at 8th rib cervical rib, supernumerary ossification center (with cartilage) above the 1st sternal segment fused with it	1	3 (3)	1	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

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 (CrI: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 $756-1109 \times 10^6$ spermatozoa/g tissue for controls and $690-929 \times 10^6$ 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 F₁-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 F₁ males 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 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. 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 $\mu\text{g/g}$ and 190 $\mu\text{g/g}$ 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-arcuation 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 spirozetramat 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 spirozetramat 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 spirozetramat.

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 spirozetramat 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 spirozetramat 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 spirozetramat.

4.11.5 Conclusions on classification and labelling

Due to abnormal sperm cells, decreased sperm motility and progression and decreased reproductive performance, spirozetramat 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, spirozetramat 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, Spirotetramat 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).

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.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 25: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis Guideline: 94/37/EC, 95/36/EC, OECD Guideline 111 (2002)	<p><u>Spirotetramat, [3-¹⁴C] and [5-¹⁴C]-label:</u> DT50 (pH 4, 25 °C): 32.5 d (SFO, r² = 1) DT50 (pH 7, 25 °C): 8.6 d (SFO, r² = 1) DT50 (pH 9, 25 °C): 0.32 d (SFO, r² = 1)</p> <p><u>Metabolites:</u> Only Spirotetramat-enol (max. 93 % at pH 9, 25 °C) Spirotetramat-enol, [3-¹⁴C] and [5-¹⁴C]-label: Hydrolytically stable at pH 4, 7 and 9</p>	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)	<p><u>Spirotetramat, [3-¹⁴C] and [5-¹⁴C]-label:</u> 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)</p> <p>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</p> <p><u>Photolysis-metabolites (all rearrangement products):</u> Spirotetramat-photo-cyclopentyl: 42.9 % (7 d) Spirotetramat-photo-2-hydroxymethyl: 22.9 % (6 d) Spirotetramat-photo-2-formyl: 11.5 % (3 d) Spirotetramat-photomethyl-2-carbonate: 19.3 % (6 d)</p>	radiochemical purity: > 99 % and > 98 % (HPLC)	Stupp, H.-P., (2005a) Document No: M-266695-01-2, MEF-05/206
Soil Photolysis	<p><u>[3-¹⁴C]-label:</u> Spirotetramat-enol: 5.3 % after 7 days (n = 1) Spirotetramat-ketohydroxy: 12.3 % after 7 days (n = 1)</p> <p><u>[5-¹⁴C]-label:</u> Spirotetramat-enol: 10.1 % after 1 days (n = 1)</p>		

CLH REPORT FOR SPIROTETRAMAT

Method	Results	Remarks	Reference
	Spirotetramat-ketohydroxy: 20.9 % after 2 days (n = 1) 4-Methoxy-cyclohexanone: 10.0 % after 2 days (n = 1)		
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
Water/Sediment Study Guideline: 95/36/EC, OECD 308 (2002)	<u>Active substance Spiroteramat</u> Water: DT ₅₀ : 1.02 d DT ₉₀ : 3.4 d Whole system: DT ₅₀ : 1.06 d DT ₉₀ : 3.52 d <u>Metabolites:</u> Spirotetramat-enol Distribution (max in water: 78.8 % after 7 d, max. sediment: 36.6 % after 60 d, mean of both labels) Spirotetramat-ketohydroxy Distribution (max in water: 12.7 % after 120 d, max. sediment: 27.8 % after 120 d, mean of both labels)	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)	<u>Active substance Spiroteramat</u> 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) <u>Metabolites:</u> Spirotetramat-enol Whole system (geometric mean): DT ₅₀ : 47.3 d DT ₉₀ : 157 d Spirotetramat-ketohydroxy Whole system (geometric mean): stable		Röpke, B., 2006b M-277415-01-1, MEF-06/279
US EPA, Subdivision N, Section 162-1 (1982);	<u>Aerobic degradation in soil</u>		Babczynski, P., 2005

Method	Results	Remarks	Reference
<p>OECD 307 (2002); 95/36/EC amending 91/414/EEC; Japanese MAFF, 12 Nohsan 8147 (2000, 2001)</p> <p>Supportive non-guideline study, but approached to US EPA, Subdivision N, Section 162-1 (1982); OECD 307 (2002); EC-Directive 91/414/EEC Annex I Part 7 and Annex II Part 9; SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides.</p> <p>EC-Directive 91/414/EEC Annex I Part 7 and Annex II Part 9, OECD Guideline 307, EPA-Guidelines for Aerobic Soil Metabolism Studies, § 162-1, Japanese MAFF Guidelines</p>	<p>Mineralization after 100 days: 12.1 % after 126 days, [3-14C]-label (n = 1) 12.2 – 19.4 % after 50 days, [3-14C]-label (n= 3)</p> <p>Non-extractable residues after 100 days 27.6 % after 126 days, [3-14C]-label (n = 1) 21.5 – 31.0 % after 50 days, [3-14C]-label (n = 3)</p> <p><u>Rate of degradation in soil</u> Lab studies Spirotetramat DT₅₀ = 0.19 d (SFO, geometric mean)</p> <p><u>Field studies</u> Spirotetramat DT₅₀ = 0.7 d (SFO, geometric mean)</p>		<p>Babczinski, P., 2006a</p> <p>Babczinski, P., 2006b</p>

StabilityHydrolysis

Reference:	[Azaspirodecenyl-3-¹⁴C]- and [Azaspirodecenyl-5-¹⁴C]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:	<ul style="list-style-type: none"> [Azaspirodecenyl-3-¹⁴C]-spirotetramat, 3.71 MBq mg⁻¹, > 99 % radiochemical purity (HPLC), batch BECH 0980 and BECH 1518 [Azaspirodecenyl-5-¹⁴C]-spirotetramat, 4.03 MBq mg⁻¹, > 98 % radiochemical purity (HPLC), batch BECH 0981 and BECH 1519
Reference substances:	Spirotetramat (unlabelled), Spirotetramat-enol, Spirotetramat-ketohydroxy
Test systems:	<ul style="list-style-type: none"> 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) <p>All buffers sterilized (steam pressure sterilization) and checked for sterility throughout the test duration. Oxygen content reduced by nitrogen bubbling.</p>
Labels regarded as replicates	
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 ¹⁴CO₂ 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 5-¹⁴C 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 26: Hydrolysis of spirotetramat in sterile water buffered at pH 4, 7 and 9 [% of AR].

pH	4									7									9					
	50 °C			25 °C			20 °C			50 °C			25 °C			20 °C			30 °C			25 °C		
Compound/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 27: Calculated hydrolytic DT₅₀ and DT₉₀ 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

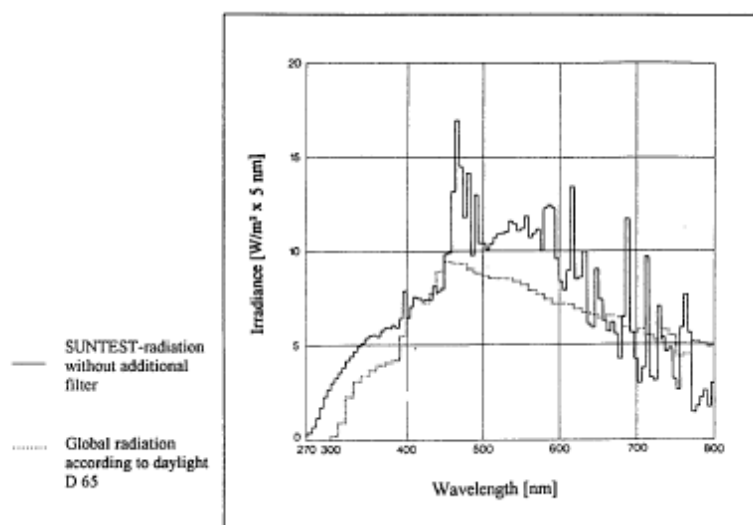
- sterile buffered water (pH 5.0), [3-¹⁴C] and [5-¹⁴C] label
- sterile natural water (pH 7.9), [3-¹⁴C] and [5-¹⁴C] label

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:	<ul style="list-style-type: none"> • [Azaspirodecenyl-3-¹⁴C]-spirotetramat, 3.67 MBq mg⁻¹, > 98 % radiochemical purity (HPLC), batch BECH 0950 • [Azaspirodecenyl-5-¹⁴C]-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 ¹⁴CO₂ were released from the [3-¹⁴C] labelled parent indicating mineralization at this part of the molecule. Almost no ¹⁴C was released from [5-¹⁴C] 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 28: Calculated photolytic DT₅₀ and DT₉₀ [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.

5.1.1 Biodegradation**5.1.1.1 Biodegradation estimation**

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

5.1.1.2 Screening testsReadily 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:	<ul style="list-style-type: none"> • 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)
Analysis:	pH of the test vessel at the end of test: 7.3 Biochemical oxygen demand (corrected for oxygen consumption by nitrification)
Incubation conditions:	22 ± 2 °C, 28 days

FINDINGS:**Table 29: 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.

5.1.1.3 Simulation testsBiodegradation 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:	<ul style="list-style-type: none"> [Azaspirodecenyl-3-¹⁴C]-spirotetramat, 3.67 MBq mg⁻¹, > 98 % radiochemical purity (HPLC), batch BECH 0955 [Azaspirodecenyl-5-¹⁴C]-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:	<u>Water</u> phase radio-assayed by LSC and concentrated using a speedvac concentrator. <u>Sediment</u> 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. <u>Non extractable residues</u> 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 ¹⁴CO₂ using 3-¹⁴C accounted for maximum of 11.0 % ('Hönniger Weiher') and 24.0 % ('Anglerweiher') of AR at study termination. Formation of ¹⁴CO₂ from 5-¹⁴C labelled spirotetramat was approx. half indicating that this part of the molecule is less available to biodegradation than the 3-¹⁴C 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 30: 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 ¹⁴CO₂ (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).

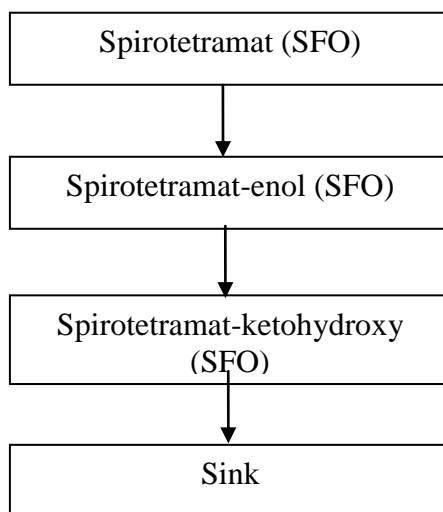


Figure 5.1.2.3-1: Multi-compartment model used for fitting residues of spirotetramat and metabolites in water/sediment studies (entire system).

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 ‘Hönniger Weiher’ was considered an outlier, amounts of spirotetramat-ketohydroxy were set to < LOQ.

FINDINGS:

Table 31: 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} → M _{SFO}
		‘Anglerweiher’	37.9	126	0.93	15.6	P _{SFO} → M _{SFO}
Spirotetramat-ketohydroxy	Entire system (Degradation)	‘Hönniger Weiher’	Not evaluable, no decline			P _{SFO} → M1 _{SFO} → M2 _{SFO}	
		‘Anglerweiher’	Not evaluable, no decline			P _{SFO} → M1 _{SFO} → 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.

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 ([3-¹⁴C] 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 CO₂. 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 CO₂ 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, CO₂ 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 CO₂ in the spirotetramat-enol degradation study was in a range of 16.7 – 43.0 % of AR by study termination (119 DAT). Significant higher ¹⁴CO₂ amounts were released from spirotetramat-enol labelled in the [3-¹⁴C] 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 [3-¹⁴C] 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 [3-¹⁴C] labelled spirotetramat or [5-¹⁴C] and [3-¹⁴C] labelled spirotetramat-enol as parent.

- pH (CaCl₂) 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 CaCl₂), 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 (DegT₅₀) values of spirotetramat were in a range of 0.09 – 0.30 days (chi² error ≤ 6.1 %); respective DegT₉₀ was in a range of 0.34 – 1.26 days. Since most environmental models are not capable to handle DFOP kinetics, a conservative SFO-DegT₅₀ for modelling may be derived from the DFOP-DegT₉₀ divided by 3.32. This procedure results in a DegT_{50recalc} in the range of 0.10 – 0.38 days.

Applying SFO kinetics (chi² error ≤ 21.8 % for one soil, for three soils chi² error ≤ 9.5 %), non-normalized DegT₅₀ values of spirotetramat were in a range of 0.08 – 0.33 days, respective DegT₉₀ 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 intended 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 32: 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
	80 th %ile (PMRA)	nc	nc	0.33	nc	nc	nc	0.51	nc	0.27	0.90	nc
	90 th %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 $[\text{arithmetic mean} + (t_{90,n-1} \times \text{SD}) / n^{0.5}]$, whereby $t_{90,n-1}$ = one-sided students t value at $\alpha = 0.1$, 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 33: 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)	Organi c C [%]	Clay [%]	DT ₅₀ [days]	DT ₉₀ [days]	R ²	χ ² error [%]	Kinetics
'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
	Tomat oes	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			
80 th %ile (PMRA)							1.0	3.4			
90 th %ile of C.I. on mean ^a (US-EPA)							0.9	3.0			

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

Soil Photolysis:

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 this (supplemental) study had too few data points to calculate a half-life. Four major (> 10 % of AR) transformation products were found in the irradiated soil samples on moist soils, i.e. spirotetramat-enol, spirotetramat-ketohydroxy, 4-methoxy-cyclohexanone (only [5-14C] label) and dimethylbenzoic acid (only [3-14C] label). Minor transformation products determined were spirotetramat-glyoxylic amide and spirotetramat-enol dimer.

5.1.2 Summary and discussion of degradation

Summary: Biotic degradation					Test guideline / design	GLP (y/n)	Reliability
Ready biodegradability Spirotetramat is considered to be not readily biodegradable					ECCD 92/69/EEC C.4-D, OECD 301 F	y	y
Water/sediment system (simulation test) active substance spirotetramat							
Substance	Compartment	Test system	DT50 [days]	Kinetics			
Spirotetramat	Water (Dissipation)	'I'	0.82	SFO			
		'II'	0.74	SFO			
	Entire system (Degradation)	'I'	0.86	SFO			
		'II'	0.70	SFO			
Spirotetramat-enol	Entire system (Degradation)	'I'	59.0	PSFO → MSFO			
		'II'	37.9	PSFO → MSFO			
I = Hönniger Weiher II = Anglerweiher 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, R2 ≥ 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. have decreased to amounts in a range of 9.9 – 25.6 % of AR..					95/36/EC, OECD Guideline 308 (2002), US-EPA N 162-4 (1982)	y	y
Degradtion in soil: 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 (DegT50) values of spirotetramat were in a range of 0.09 – 0.30 days (chi2 error ≤ 6.1 %); respective DegT90 was in a range of 0.34 – 1.26 days. Since most environmental models are not capable to handle DFOP kinetics, a conservative SFO-DegT50 for modelling may be derived from the DFOP-DegT90 divided by 3.32. This procedure results in a DegT50recalc in the range of 0.10 – 0.38 days. 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). Field Dissipation studies Dissipation of spirotetramat in field trials was rapid with a dissipation half-life (DT50) in a range of 0.3 – 1.0 day (SFO kinetics), respective DT90 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					US EPA, Subdivision N, Section 162-1 (1982); OECD 307 (2002); Supportive non-guideline study, but approached to US EPA, Subdivision N, Section 162-1 EC-Directive 91/414/EEC Annex I Part 7 and Annex II Part 9, OECD Guideline 307,	y	n

Summary: Biotic degradation	Test guideline / design	GLP (y/n)	Reliability
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.			

Summary: Abiotic degradation	Test guideline / design	GLP (y/n)	Reliability
<p>Hydrolysis: At 25 °C calculated half-life times were 32.5, 8.6 and 0.32 days (all SFO, R2 > 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. Owing to the complete mass balance 14CO2 formation is considered to be negligible.</p>	94/37/EC, 95/36/EC, OECD Guideline 111 (2002), US-EPA N 161-1 (1982), Canada PMRA DACO 8.2.3.2 (1987),	y	y
<p>Photolysis: Under irradiation in sterile buffer solutions at pH 5.0, spirotetramat degraded with a half-life of 2.7 days (following SFO kinetics, R2 = 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). Only small amounts (maximum 2.6 % of AR) of 14CO2 were released from the [3-14C] labelled parent indicating mineralization at this part of the molecule. 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.</p>	95/36/EC, 94/37/EC, SETAC (1995), US-EPA N 162-1 (1982), Canada PMRA DACO 8.2.3.3.2 (1987)	y	y
<p>Soil Photolysis: 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 this (supplemental) study had too few data points to calculate a half-life. Four major (> 10 % of AR) transformation products were found in the irradiated soil samples on moist soils, i.e. spirotetramat-enol, spirotetramat-ketohydroxy, 4-methoxy-cyclohexanone (only [5-14C] label) and dimethyl-benzoic acid (only [3-14C] label). Minor transformation products determined were spirotetramat-glyoxylic amide and spirotetramat-enol dimer.</p>	Directive 91/414/EEC, 1995 Pesticide Assessment Guidelines, Subdivision N, Environmental Fate, US EPA, 161-3: Photodegradation Studies on Soil, 1982 Canada PMRA, DACO No. 8.2.3.3.1 Considering Draft (2004-02) OECD Guideline for the Testing of Chemicals: Photo-transformation of Chemicals on Soil Surfaces	y	n

Conclusion

Spirotetramat is **not readily biodegradable** under test conditions within 28 days.

Ultimate degradation could be excluded in all discussed abiotic and biotic degradation studies as mineralization rate is too low. **Fast primary degradation was indicated** in abiotic degradation and simulation tests.

To conclude a rapid or non rapid degradation it has to be shown that the degradants are not classifiable or classifiable.

No aquatic toxicity data are available for photolytic degradants, therefore photolytic degradation is not considered relevant for classification.

The major metabolite detected in hydrolysis and simulation tests was the metabolite spirotetramat-enol. Aquatic toxicity data for fish, invertebrates, algae and aquatic plants are available. The most sensitive species was *Lemna Gibba* with an ErC50 (7 d) = 19.3 mg/L and a NOEC_{YIELD} (7d) = 0.95 mg/L reported as nominal concentration. The logPOW of metabolite BYI 08330-enol is 0.3 (at pH 7),

Based on these data and due to the proposed non rapid degradation ($DT_{50} = > 16$ d (stable in Hydrolysis study / $DT_{50} = 37.9 - 59$ d in Water/sediment system) spirotetramat-enol has to be classified as R52/53 (DSD) or as H411 toxic to aquatic life with long lasting effects (CLP).

No other metabolites have been considered as spirotetramat-enol has to be classified and therefore **a non rapid degradation** for the active substance spirotetramat is proposed

5.2 Environmental distribution

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 (CaCl₂): 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 CaCl₂). 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 [3-¹⁴C] 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 FAO² 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 degrade owing to reaction with OH radicals (atmospheric half-life < 2 hrs based on Atkinson calculation).

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.

5.2.3 Distribution modelling

No information available.

5.3 Aquatic Bioaccumulation

Table 34: Summary of relevant information on aquatic bioaccumulation

Spirotetramat						
Method	Results				Remarks	Reference
Partition coefficient n-octanol/water Guideline: EC A.8, OECD 117 (HPLC-method)	pH	4.0 (20 °C)	7.0 (20 °C)	9.0 (20 °C)	Test substance: BYI 08330, mix-batch 08045/0003, purity 99.1 %	Lemke, G., Muehlberger, B.; 2003 M-103244-01-1
	logP _{ow}	2.51	2.51	2.50		
Metabolite BYI 08330-enol (metabolite formed in hydrolysis- and water/sediment study)						
Partition coefficient n-octanol/water	pH		7.0			Draft Assessment Report
	logP _{ow}		0.3			

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.

The logP_{ow} of the environmental relevant metabolite BYI 08330-enol is 0.3 (at pH 7) indicating 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 *Skeletonem costatum* (E_bC₅₀: 0.32 mg/L).

Table 35: 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	<i>Oncorhynchus mykiss</i> 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	<i>Cyprinus carpio</i> 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	<i>Lepomis macrochirus</i> 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	<i>Cyprinodon variegatus</i> 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	<i>Pimephales promelas</i> 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	<i>Daphnia magna</i> 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	<i>Daphnia magna</i> 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	<i>Chironomus riparius</i> 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

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Method	test organism	test condition	exp. time	test conc.	Results			Reference
					endpoint	NOEC (mg ai/L)	EC50/LC50 (mg ai/L)	
OECD 219	<i>Chironomus riparius</i> 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	<i>Americamysis bahia</i> 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	<i>Crassostrea virginica</i> 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)	<i>P. subcapitata</i> 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	<i>Navicular pellicullosa</i> 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	<i>Anabaena flos-aqua</i> 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	<i>Skeletonema costatum</i> 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	<i>Lemna gibba</i> Duckweed	semi static	7 d	mm	yield growth rate	1.54	4.49 6.21	Dorgerloh, 2005j Document: DOM 24019, Edition Number: M-255296-01-2, 29.07.2005

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

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Metabolite BYI 08330-enol								
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	<i>Oncorhynchus mykiss</i> Rainbow trout	static, limit	96 hr	nom	mortality		>100	Nieden, D., 2005 a Report No.: EBFNM013, Edition Number: M-251850-01-2, 18.05.2005
OECD 202, EPA 72-2, OPPTS 850.1010, EU Directive 92/69/EEC C.2	<i>Daphnia magna</i>	static, limit	48 h	nom	mortality		>100	Dorgerloh, M., 2005 d EBFNM012, Edition Number: M-254487-01-2, 14.07.2005
No specified guideline, the study is following the procedure of OECD 202	<i>Chironomus riparius</i>	Static, water only	48 h	nom	mortality	LC10=17.1 NOEC: not determined	74.9	Dorgerloh, M., 2005 h EBFNX073, Edition Number: M-262638-01-2, 08.12.2005
Draft proposal for updating OECD 201, OPPTS 850.5400	<i>Pseudokirch subcap.</i>	static	72 h	mm	Biomass: NOEC/EbC50 Growth rate: NOEC/ErC50	31 mg/L (biomass and growth rate)	> 100 > 100	Dorgerloh, M. 2005 f EBFNM010, Edition Number: M-252531-01-2, 06.06.2005
OECD 221, OPPTS 850.4400	<i>Lemna gibba</i>	static	7 d	mm	Yield: NOEC/EyC50 Growth rate: NOEC/ErC50	0.95 3.05	5.4 19.3	Dorgerloh, M., 2005 k EBFNX065, Edition Number: M-252412-01-2, 07.06.2005

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 (<i>Oncorhynchus mykiss</i>)
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 CaCO₃

Temperature: 11.3 – 12.3 °C

pH: 7.0 – 7.1 (0 h, new medium), 7.0 (96 h, aged medium)

O₂ 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 36: 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, Chi²: 1.25
 Degrees of freedom: 3
 p (Chi²): 0.74

Conclusion: LC₅₀ (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

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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 CaCO₃
 Temperature: 20.5 – 23.2 °C
 pH: 7.1 – 7.2 (0 h, new medium), 7.1 (96 h, aged medium)
 O₂ 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 37: 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, Chi²: 17.69
 Degrees of freedom: 3
 p (Chi²): < 0.001

Conclusion: LC₅₀ (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 CaCO₃
 Temperature: 17.9 – 20.5 °C
 pH: 7.4 – 7.5 (0 h, new medium), 7.3 (96 h, aged medium)
 O₂ 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 38: 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, Chi²: 8.93
 Degrees of freedom: 3
 p (Chi²): 0.0302

Conclusion: LC₅₀ (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

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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
<u>Findings:</u>	
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 39: Cumulative mortality of sheephead 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 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
<u>Conclusion:</u>	LC ₅₀ (96 h): 1.96 mg/L NOEC: 0.52 mg/L based on mean measured concentration

Reference:	Acute toxicity of BYI 08330-enol to fish (<i>Oncorhynchus mykiss</i>)
Author(s), year:	Nieden, D., 2005 a
Report/Doc. number:	Report No.: EBFNM013, Edition Number: M-251850-01-2, 18.05.2005
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:	BYI 08330-enol (metabolite of spirotetramat), purity: 99.1 %, batch: 692-101-09-0005
Material and methods:	
Test species:	Rainbow trout (<i>Oncorhynchus mykiss</i>)
Number of organisms:	2 x 15 fish per concentration and control
Weight, length:	1.3 ± 0.6 g (mean ± SD) and 4.9 ± 0.7 cm (mean ± SD)
Loading:	0.49 g fish/L test medium
Type of test, duration:	Static limit test, 96 hours
Applied concentrations:	
Nominal:	0 (control), 100 mg/L
Measured (mean):	-- (control), 98 mg/L
Test conditions:	
Water quality:	Reconstituted water (according to ISO), hardness: 40 - 60 mg/L as CaCO ₃ . After the addition of test substance the pH was adjusted to about 7 with NaOH.
Temperature:	11.1 – 13 °C
pH:	6.7 – 7.2 (0 h), 6.8 – 6.9 (96 h)
O ₂ content:	86 – 102 % 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. At 0, 48 and 96 hours samples were taken for chemical analysis (HPLC-MS/MS and HPLC-UV) of BYI 08330-enol in test solutions.
Statistics:	None, because a limit test was performed
Findings:	
Analytical data:	Over the whole test period the mean measured concentration was 98.5 % (replicate A) and 97.7 % (replicate B) of nominal.
Behavioural effects:	No effects were observed in control and 100 mg/L treatment replicates.
Mortality:	No mortality was reported in control and 100 mg/L treatment replicates.
Conclusion:	LC ₅₀ (96 h): > 100 mg/L NOEC (96 h): 100 mg/L based on nominal 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 (<i>Pimephales promelas</i>)
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
<u>Test substance:</u>	Spirotetramat (BYI 08330), purity: 97.1 %, batch: 08045/0014
<u>Material and methods:</u>	
Test species:	Fathead minnow (<i>Pimephales promelas</i>)
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 <i>ad libidum</i> . Feeding was started shortly after hatching. Uneaten food and 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 40: 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 41: 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 <i>Daphnia magna</i> 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
<u>Test substance:</u>	Spirotetramat (BYI 08330), purity: 97.2 %, batch: 08045/0014
<u>Material and methods:</u>	
Test species:	Waterflea (<i>Daphnia magna</i>)
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 <i>Daphnia magna</i> 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
<u>Findings:</u>	
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-enol to the waterflea *Daphnia magna* in a static laboratory test system - limit test**
 Author(s), year: Dorgerloh, M., 2005 d
 Report/Doc. number: EBFNM012, Edition Number: M-254487-01-2, 14.07.2005
 Guideline(s): OECD 202, EPA 72-2, OPPTS 850.1010, EU Directive 92/69/EEC C.2
 GLP: Yes
 Deviations: None of relevance
 Validity: Acceptable

Test substance: BYI 08330-enol (metabolite of spirotetramat), purity: 99.1 %, batch: 692-101-09-0005, water solubility (at pH 7): 2.7g/L

Material and methods:

Test species: Waterflea (*Daphnia magna*)
 Number of organisms: 6 replicates each with 5 daphnids per treatment and control
 Age: First instar < 24 hours old
 Type of test, duration: Static limit test, 48 h
 Applied concentrations:
 Nominal: 0 (control), 100 mg/L
 Measured (mean): -- (control), 111.5 mg/L
 Test conditions:
 Water quality: M7 medium (according to “original draft” of an EEC *Daphnia magna* pilot ring test), hardness: 196 mg/L as CaCO₃, adjusted to pH 7 before use for dilution procedure
 Temperature: 20.6 – 21.2 °C
 pH: 7.0 – 6.9 (0 h), 7.5 – 7.9 (48 h)
 O₂ content: 89 – 96 %, 8.1 – 8.7 mg O₂/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-enol in the test media samples were taken at test initiation (0 h) and termination (48 h). Test samples were analysed by direct injection into a HPLC-UV instrument without a centrifugation.
 Statistics: EC50: None, because at the limit concentration (100 mg/L nominal) no immobilisations were observed, NOEC: directly from the raw data

Findings:

Analytical data: The measured concentrations at the start and end of the test were 113 and 110 % of nominal, overall mean measured concentration was 111.5 % of nominal.
 Effects: After 48 hours no immobility and no sublethal effects were observed in the control, and in the limit test concentrations of 100 mg/L. Thus the NOEC was determined to be 100 mg/L and the EC50 was > 100 mg/L.

Conclusion: EC50 (48 h): > 100 mg/L
NOEC: 100 mg/L
based on nominal 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)
O₂ content: > 80 %, (8.6 – 8.9 mg O₂/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 42: 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, Chi²: 6.62
Degrees of freedom: 4
p (Chi²): 0.158

Conclusion: LC₁₀ (48 h): 0.699 mg/L
LC₅₀ (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: **Acute toxicity of BYI 08330-enol to larvae of *Chironomus riparius* in a 48 h water-only study**

Author(s), year: Dorgerloh, M., 2005 h

Report/Doc. number: EBFNX073, Edition Number: M-262638-01-2, 08.12.2005

Guideline(s): No specified guideline, the study is following the procedure of OECD 202

GLP: Yes

Deviations: None of relevance

Validity: Acceptable

Test substance: BYI 08330-enol (metabolite of spirotetramat), purity: 99.1 %, batch: 692-101-09-0005, water solubility (at pH 7): 2.7g/L

Material and methods:

Test species: Midge (*Chironomus riparius*)

Number of 4 replicates each with 10 larvae per treatment and control

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organisms:

Age: L1 (first instar) larvae
 Type of test, duration: Static (water-only) test, 48 h

Applied

concentrations:

Nominal: 0 (control), 1.0, 2.0, 4.0, 8.0, 16, 32, 64 mg/L
 Measured (mean): -- (control), 0.95, 1.93, 4.06, 8.3, 15.65, 32.25, 66.45 mg/L

Test conditions:

Water quality: M7 medium (according to OECD 202), hardness: not stated
 Temperature: 20.8 – 21 °C
 pH: 7.8 – 8.2 (0 h), 8.1 – 8.4 (48 h)
 O₂ content: > 90 %, (8.5 – 8.7 mg O₂/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-enol in the test media samples were taken at test initiation (0 h) and termination (48 h). Test samples were analysed by direct injection into a HPLC-UV instrument without a centrifugation.

Statistics: LC₁₀ and LC₅₀: Probit analysis, NOEC: not determined

Findings:

Analytical data: The mean measured concentrations at the start and end of the test were in the range of 98 – 105 % and 88 – 103 % of nominal, respectively. Overall mean measured concentrations ranged from 95 to 104 % of nominal.

Effects: See table B.9.2.1.2-2

Table 43: Effects on midge (*C. riparius*) exposed to BYI 08330-enol

BYI 08330-enol [mg/L]		Exposed chironomids (= 100 %)	Mortality after 48 hours	
nominal	mean measured		[n]	[%]
Control		40	0	0
1.0	0.95	40	0	0
2.0	1.93	40	0	0
4.0	4.06	40	0	0
8.0	8.28	40	0	0
16.0	15.68	40	5	12.5
32.0	32.16	40	11	27.5
64.0	67.59	40	16	40.0
LC10 (48 h):		17.07 mg/L (95% C.I. 12.15 – 23.98 mg/L) based on nominal 11.62 mg/L (95% C.I. 4.74 – 28.48 mg/L) based on mean measured		
LC50 (48 h):		74.85 mg/L (95% C.I. 48.86 – 114.64 mg/L) based on nominal 97.78 mg/L (95% C.I. 43.15 – 221.60 mg/L) based on mean measured		

Details of the Probit: Slope b: 1.38
 95 % CI of b: not stated
 Variance of b: 0.248
 Intercept a: 2.246
 Goodness of fit, Chi²: 0.175

<u>Conclusion:</u>	Degrees of freedom: 1 p (Chi ²): 0.676 LC ₅₀ (48 h): 74.9 mg/L LC ₁₀ (48 h): 17.1 mg/L NOEC (48 h): not determined based on nominal concentration
<u>Comments:</u>	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 (<i>Americamysis bahia</i>) 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.

<u>Test substance:</u>	Spirotetramat (BYI 08330), purity: 97.2 %, batch: 08045/0014
<u>Material and methods:</u>	
Test species:	Saltwater mysid (<i>Americamysis bahia</i>)
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
<u>Findings:</u>	
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 44: 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, Chi²: 13.25
 Degrees of freedom: 3
 p (Chi²): 0.0041

Conclusion: LC₅₀ (96 h): 5.5 mg/L
 NOLC: < 0.73 mg/L
 based on mean measured concentration

Reference:	BYI 08330 - Acute toxicity to eastern oysters (<i>Crassostrea virginica</i>) 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:	<ul style="list-style-type: none"> • 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
 O₂ content: 3.8 – 7.3 mg O₂/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:

EC₅₀: 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 45: 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:

EC₅₀ (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 <i>Daphnia magna</i> 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
<u>Test substance:</u>	Spirotetramat (BYI 08330), purity: 97.1 %, batch: 08045/0014
<u>Material and methods:</u>	
Test species:	Waterflea (<i>Daphnia magna</i>)
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 <i>Daphnia magna</i> 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 <i>Desmodesmus subspicatus</i> 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 Bartlett's test and Kolmogoroff-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 considering nominal concentrations are based on data of pooled controls.
 Statistical analyses considering mean measured concentrations are using only negative control.

Findings:

Analytical data: The mean measured concentrations ranged from 87.2 – 110.7 %

Effects:

Table 46: 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
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)} Statistics 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:	<i>Chironomus riparius</i> 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
<u>Material and methods:</u>	
Test species:	Midge (<i>Chironomus riparius</i>)
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.
<u>Findings:</u>	
Analytical data:	

Table 47: 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 48: Effects of spirotetramat on midge (*C. riparius*) in a water-spiked test

Spirotetramat [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, EC₅₀: 0.46 mg/L
 Development rate of pooled sexes, males and females:
 NOEC: 0.8 mg/L, LOEC: 1.6 mg/L, EC₅₀: > 0.8 mg/L
 Endpoints based on initial nominal concentrations.

Comments: While no major deviations are noted from guideline 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:	<i>Pseudokirchneriella subcapitata</i> - 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: <ul style="list-style-type: none"> • The temperature is less than the EPA-specified temperatures (24-25°C) 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 10⁴ 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,

Solvent: 63.2 mg/L
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 49: 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_bC₅₀ (0-72 h): 6.58 mg/L
E_rC₅₀ (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 <i>Navicula pelliculosa</i>
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 <i>Navicula</i> 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; EC₅₀: 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 50: 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_bC₅₀ (0-96 h): 4.05 mg/L
 E_rC₅₀ (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 (24-25°C).
 Validity: Acceptable, even if the inhibition for growth rate is lower than recommended the endpoints 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 10⁴ 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; EC₅₀: 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 51: 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_bC₅₀ (0-96 h): 15.2 mg/L
 E_rC₅₀ (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 <i>Skeletonema costatum</i>
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 <i>Skeletonema costatum</i> 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

<u>Test substance:</u>	Spirotetramat (BYI 08330), purity: 97.99 %, batch: 08045/0014
<u>Material and methods:</u>	
Test species:	Marine diatom (<i>Skeletonema costatum</i>)
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.
<u>Findings:</u>	
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 52: 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_bC₅₀ (0-96 h): 0.36 mg/L
 E_rC₅₀ (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

Reference: *Pseudokirchneriella subcapitata* - Growth inhibition test with BYI 08330-enol
 Author(s), year: Dorgerloh, M. 2005 f
 Report/Doc. number: EBFNM010, Edition Number: M-252531-01-2, 06.06.2005
 Guideline(s): Draft proposal for updating OECD 201, OPPTS 850.5400
 GLP: Yes
 Deviations: US EPA:
 • The temperature is less than the EPA-specified temperatures (24-25°C) 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.
Validity: Acceptable

Test substance: BYI 08330-enol (metabolite of spirotetramat), purity: 99.1 %, batch: 692-101-09-0005

Material and methods:
 Test species: Green alga (*Pseudokirchneriella subcapitata*)
 Number of organisms: 1 x 10⁴ cells/mL; 3 replicates for each concentration, 6 replicates for the medium and solvent control
 Type of test, duration: Static test, 72 h

Applied

concentrations:

Nominal: 0 (medium control and solvent control), 1.0, 3.1, 10, 31, 100 mg/L
 Measured (mean): -- (medium control and solvent control), 1.02, 2.9, 10.1, 29.4, 102 mg/L
 Solvent: 0.5 mL/L Dimethylformamide (DMF)

Test conditions:

Water quality: Nutrient medium (according to OECD guideline)
 Temperature 22.3 – 23.5 °C
 pH 7.1 – 7.9 (0 h), 7.6 – 8.5 (96 h)
 Incubation: Continuous “cool white” illumination with fluorescent light (5560 - 7350 lux), orbital shaking at 100 rpm

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. The test samples were analysed by direct injection into an HPLC-MA/MS instrument without a centrifugation. LOEC: ANOVA Procedure, t-test; EC₅₀: Not applicable because the inhibition at highest test concentration was clearly below 50 %, comparison of control groups: Student- t-test for homogenous variances
 All statistical analyses are based on data of pooled control.

Statistics:

Findings:

Analytical data: Mean measured concentrations were in the range of 94 – 104 % and 94 – 102 % of nominal concentrations on day 0 and day 3, respectively.

Effects:

Morphological effects: None

Biomass & growth rate: See table B.9.2.1.3-4

Table 53: Effects of BYI 08330-enol (metabolite of spirotetramat) on the green alga *P. subcapitata*

BYI 08330 [mg/L] (nominal)	Inhibition after 72 hours	
	Biomass (AUC)	Growth rate
0 (pooled control) ¹⁾	--	--
1.0	-9.2	-1.0
3.1	-15.1	-3.4
10	0.2	0.5
31	1.9	1.5
100	32.9*	13.0*
NOEC	31 mg/L	31 mg/L
LOEC	100 mg/L	100 mg/L
EC ₅₀	> 100 mg/L)	> 100 mg/L)

¹⁾ 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

Conclusion: E_bC₅₀ (0-72 h): > 100 mg/L

E_rC_{50} (0-72 h): > 100 mg/L,
 NOEC (0-72 h): 31 mg/L (biomass and growth rate)
 based on nominal concentrations

Effects on aquatic plants

Reference:	<i>Lemna gibba</i> 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
<u>Test substance:</u>	Spirotetramat (BYI 08330), purity: 97.2 %, batch: 08045/0014
<u>Material and methods:</u>	
Test species:	Duckweed (<i>Lemna gibba</i>)
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 ($\alpha = 0.05$, one sided) and Dunnett's multiple t-tests
<u>Findings:</u>	
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 54: Analytical results of spirotetramat

Day	Nominal concentration of BY1 08330 [mg/L]	Actual concentration of BY1 08330				Time weighted mean [mg/L]*
		Detection 1 [mg/L]	Detection 2 [mg/L]	Mean [mg/L]	% of nominal	
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	0.14
3 aged		0.122	0.120	0.121	40	
3 new		0.258	0.256	0.257	86	
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	0.47
3 aged		0.362	0.361	0.362	38	
3 new		0.779	0.779	0.779	82	
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	1.54
3 aged		1.08	1.08	1.08	35	
3 new		2.41	2.40	2.40	79	
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	6.06
3 aged		4.76	4.70	4.73	48	
3 new		7.66	7.62	7.64	78	
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	20.5
2 aged		15.3	15.3	15.3	49	
2 new		26.5	26.5	26.5	85	
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	71.4
2 aged		48,3	48,5	48,4	48	
2 new		87,0	85,8	86,4	86	
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 55: 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	% inhibition in 7 d 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*

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	inhibition in 7 d based on total frond area
100 ^{a)}	71.4	97.6*	113.5*	116.1*

* Significant difference (alpha = 0.05) from the control

Table 56: 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_yC₅₀ (7 d): 4.62 mg/L
 E_rC₅₀ (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

Reference: *Lemna gibba* G3 - Growth inhibition test with BYI 08330-enol under static test conditions
 Author(s), year: Dorgerloh, M., 2005 k
 Report/Doc. number: EBFNX065, Edition Number: M-252412-01-2, 07.06.2005
 Guideline(s): OECD 221, OPPTS 850.4400
 GLP: Yes
 Deviations: OECD: None of relevance
 US EPA: The light intensity was higher than the EPA recommended light intensity.
 Validity: Acceptable

Test substance: BYI 08330-enol (metabolite of spirotetramat), purity: 99.1 %, batch: 692-101-09-0005

Material and methods:

Test species: Duckweed (*Lemna gibba*)
 Number of organisms: 12 fronds per vessel; 3 replicates for each concentration, control and solvent control
 Type of test, duration: Static test, 7 d
 Applied concentrations:
 Nominal: 0 (medium control), 0.09, 0.30, 0.95, 3.05, 9.77, 31.3, 100 mg/L
 Measured (mean): -- (medium control), n.a., n.a., 1.02, 3.41, 11.7, 37.6, 119 mg/L
 Test conditions:

Water quality: 20X-AAP growth medium (according to OECD guideline), the pH was adjusted to 7.5 ± 0.1

Temperature: 22.3 – 24.0 °C

pH: 7.2 – 7.8 (day 0), 8.2 – 8.8 (day 7)

Incubation: Continuous illumination with white fluorescent light (mean intensity: 8.96 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 weight of plants (needed for yield calculations) was measured at the end of the study (final biomass). For chemical analysis (HPLC-MS/MS, HPLC-UV) of test the substance samples of test solution were taken on day 0 and on day 7 (test termination). Test samples were analysed by direct injection into an HPLC instrument without centrifugation.

Statistics: LOEC: ANOVA ($\alpha = 0.05$, one sided) and Dunnett’s multiple tests

Findings:

Analytical data: Overall mean measured concentrations ranged from 114 % to 120 % on day 0 and from 97 % to 120 % on day 7 of nominal.

Effects:

Morphological effects: None visual effects were observed in concentration levels up to 0.95 mg.L. At ≥ 3.5 mg/l chlorosis was noted.

Biomass & growth rate: Doubling time T_d of the control = 1.9 d (fulfilled the validity criterion), see also table B.9.2.1.4-3

Table 57: Effects of BYI 08330-enol on duckweed *Lemna gibba*

BYI 08330-enol [mg/L] (nominal)	Yield inhibition [%]	Growth rate inhibition [%]	
		based on frond number	based on total frond area
0.09	0.1	-0,9	-2,9
0.30	0.1	-2,7	-2,4
0.95	3.1	0,1	0,1
3.05	27.8*	8,3	9,5
9.77	80.8*	38,5*	55,2*
31.3	84.4*	50,3*	60,8*
100	88.6*	45,8*	67,0*
NOEC	0.95 mg/L	3.05 mg/L	3.05 mg/L
LOEC	3.05 mg/L	9:77 mg/L	9:77 mg/L
EC ₅₀ (95% C.I.)	5.4 (3.3 – 9.1) mg/L	60.8 (21.6 - 1643) mg/L	19.3 (6.9 – 74.2) mg/L

* Significant difference ($\alpha=0.05$) from the control

Conclusion: E_yC_{50} (7 d): 5.4 mg/L
 E_rC_{50} (7 d): 19.3 mg/L
 NOEC (7d): 0.95 mg/L (yield); 3.05 mg/L (growth rate) based on nominal concentrations

Comments: BYI 08330-enol is a metabolite of spirotetramat which is an insecticide and therefore a study on higher aquatic plants is not required according to directive 91/414/EEC. However, in general an exposition of *Lemna gibba* is possible and therefore the study is considered in the aquatic risk assessment.

Summary and discussion: Acute (short-term) aquatic toxicity

Table 58: Acute (short-term) aquatic toxicity of the active substance Spirotetramat (Generally expressed in terms of LC50 or EC50 (mg/L))

Group	Endpoint	L(E)C ₅₀ [mg/L]	Test guideline / design	GLP (y/n)	Reliability
Fish (96 hr LC ₅₀):					
<i>Cyprinodon variegatus</i> Sheephead minnow	mortality	1.96 (mm)	OECD 203, US EPA OPPTS 850.1075, FIFRA 72-2	y	y
Aquatic invertebrate(48hr EC ₅₀):					
<i>Daphnia magna</i>	mortality	> 42.7 (mm)	OECD 202, EPA 72-2, OPPTS 850.1010, EU Directive 92/69/EEC C.2	y	y
<i>Crassostrea virginica</i> Eastern oyster	shell deposition	0.85 (mm)	EPA OPPTS 850.1025, FIFRA 72-3	y	y
Sediment dwelling organisms (48hr EC ₅₀):					
<i>Chironomus riparius</i> Midge	mortality	1.30 (mm)	No specified guideline, the study is following the procedure of OECD 202	y	y
Algae (72 or 96 hr E _r C ₅₀):					
<i>Skeletonema costatum</i> Diatom (marine)	growth rate	0.96 (mm)	Draft proposal for updating OECD 201 (2004), OPPTS 850.5400, FIFRA 123-2	y	y
Aquatic plants (72 or 96 hr E _r C ₅₀):					
<i>Lemna gibba</i> Duckweed	growth rate	6.21 (mm)	OECD 221, EPA OPPTS 850.4400	y	y
<p>Conclusion: relevant endpoints for classification ErC₅₀ = 0.96 mg/l <i>Skeletonema costatum</i> EC₅₀ = 0.85 mg/l <i>Crassostrea virginicae</i></p>					

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

Table 59; Acute (short-term) aquatic toxicity of the metabolite Spirotetramat-enol (BYI 08330-enol main metabolite detected in hydrolysis and water/sediment study)

(Generally expressed in terms of LC50 or EC50 (mg/L))

Group	Endpoint	L(E)C ₅₀ [mg/L]	Test guideline / design	GLP (y/n)	Reliability
Fish (96 hr static, limit, LC ₅₀):					
<i>Oncorhynchus mykiss</i> Rainbow trout	mortality	> 100 _(nom)	OECD 203, EPA 72-1, OPPTS 850.1075, EU Directive 92/69/EEC C.1	y	y
Aquatic invertebrate(48 hr, static, limit, EC ₅₀):					
<i>Daphnia magna</i> Waterflea	mortality	> 100 _(nom)	OECD 202, EPA 72-2, OPPTS 850.1010, EU Directive 92/69/EEC C.2	y	y
Sediment dwelling organisms (48 hr, static, water only, EC ₅₀):					
<i>Chironomus riparius</i> Midge	mortality	> 100 _(nom)	No specified guideline, the study is following the procedure of OECD 202	y	y
Algae (72 hr static E _r C ₅₀):					
<i>Pseudokirch subcap.</i>	growth rate	> 100 _(mm)	Draft proposal for updating OECD 201, OPPTS 850.5400	y	y
Aquatic plants (7 d static E _r C ₅₀):					
<i>Lemna gibba</i> Duckweed	growth rate	19.3 _(mm)	OECD 221, OPPTS 850.4400	y	y
Conclusion: relevant endpoint for classification (only for DSD)					
ErC50 = 19.3 mg/l <i>Lemna gibba</i>					

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

Table 60: Comparison of acute aquatic toxicity data between active substance Spirotetramat and the degradation product Spirotetramat-enol

(Generally expressed in terms of LC₅₀ or EC₅₀ (mg/L))

Group/species	duration	Endpoint	Spirotetramat	Spirotetramat-enol
<i>Cyprinodon variegatus</i>	(96 hr)	mortality	LC50= 1.96 (mm)	
<i>Oncorhynchus mykiss</i>	(96 hr)	mortality		LC50= > 100 (nom)
<i>Chironomus riparius</i>	(48 hr)	mortality	EC50= 1.30 (mm)	EC50= > 100 (nom)
<i>Daphnia magna</i>	(48 hr)	mortality	EC50= > 42.7 (mm)	EC50= > 100 (nom)
<i>Lemna gibba</i>	7 d (static)	Yield: Growth rate:	EyC50=4.49 (mm) ErC50=6.21 (mm)	EyC50=5.4 (mm) ErC50=19.3 (mm)
Based on available data Spirotetramat-enol shows a much lower toxicity (fish and chironomus) or similar toxicity (daphnids and aquatic plants), when compared to the active substance Spirotetramat.				

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

Table 61: Chronic (long-term) aquatic toxicity of the active substance Spirotetramat
(Generally expressed in terms of NOEC (mg/L))

Group	Endpoint	NOEC [mg/L]	Test guideline / design	GLP (y/n)	Reliability
Fish (33 d NOEC):					
<i>Pimephales promelas</i> Fathead minnow	hatchability fry survival growth	1.16 (mm) 0.534 (mm) 1.16 (mm)	EPA 72-4a, EPA OPPTS 850.1400, OECD 210	y	y
Aquatic invertebrate (21 d NOEC):					
<i>Daphnia magna</i> Waterflea	mortality adults reproduction growth	2.0 (mm) 5.0 (mm) 5.0 (mm)	OECD 211, EEC Directive C.20, US EPA 72-4, OPPTS 850.1300	y	y
Sediment dwelling organisms (28 d NOEC):					
<i>Chironomus riparius</i> Midge	emergence development	0.1 (mm) 0.8 (mm)	OECD 219	y	y
Algae (96 hr NOEC):					
<i>Skeletonema costatum</i> Diatom (marine)	biomass growth rate	0.124 (mm)	Draft proposal for updating OECD 201 (2004), OPPTS 850.5400, FIFRA 123-2	y	y
Aquatic plants (7 d NOEC):					
<i>Lemna gibba</i> Duckweed	yield growth rate	1.54 (mm)	OECD 221, EPA OPPTS 850.4400	y	y
Conclusion: relevant endpoint for classification NOEC _{EMERGENCE} = 0.1 mg/l <i>Chironomus riparius</i>					

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

Table 62: Chronic (long-term) aquatic toxicity of the metabolite Spirotetramat-enol (BYI 08330-enol, main metabolite detected in hydrolysis and water/sediment study)
(Generally expressed in terms of NOEC (mg/L))

Group	Endpoint	NOEC [mg/L]	Test guideline / design	GLP (y/n)	Reliability
Algae (72 hr NOEC):					
<i>Pseudokirch subcap.</i>	biomass growth rate	31 mg/L (biomass and growth rate)	Draft proposal for updating OECD 201, OPPTS 850.5400	y	y
Aquatic plants (7 d NOEC):					
<i>Lemna gibba</i> Duckweed	yield growth rate	0.95(mm) 3.05 (mm)	OECD 221, EPA OPPTS 850.4400	y	y
Conclusion: relevant endpoint for classification NOEC _{YIELD} = 0.95 mg/l <i>Lemna gibba</i>					

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

5.4.4 Other aquatic organisms (including sediment)

None.

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

Endpoint	Classification Criteria		Evidence for Spirotetramat
	CLP (2 nd ATP)	DSD	
Degradation Spirotetramat	<p>Spirotetramat is not readily biodegradable under test conditions within 28 days.</p> <p>Ultimate degradation could be excluded in all discussed abiotic and biotic degradation studies as mineralization rate is too low. Fast primary degradation was indicated in abiotic degradation and simulation tests. To conclude a rapid or non rapid degradation it has to be shown that the degradants are not classifiable or classifiable.</p> <p>No aquatic toxicity data are available for photolytic metabolites.</p> <p>The major metabolite (for more information see below) detected in hydrolysis and simulation tests is metabolite spirotetramat-enol (BYI 08330-enol). Aquatic toxicity data for fish, invertebrates, algae and aquatic plants are available. The most sensitive species was <i>Lemna gibba</i> with an ErC50 (7 d) = 19.3 mg/L and a NOEC_{YIELD} (7d) = 0.95 mg/L. The logPOW of metabolite BYI 08330-enol is 0.3 (at pH 7).</p> <p>Based on these data and due to the proposed non rapid degradation (DT50 = > 16 d, stable in hydrolysis study / DT50 = 37.9 – 59 d in water/sediment system) spirotetramat-enol has to be classified as R52/53 (DSD) or as H411 toxic to aquatic life with long lasting effects (CLP).</p> <p>No other metabolites have been considered as degradants, because spirotetramat-enol has to be classified and hence a non rapid degradation for the active substance spirotetramat is proposed.</p>		<p>The classification as R53 according to Directive 67/548/EEC is based on the fact that the active substance is not considered as ready biodegradable/rapid degradable.</p>
Bioaccumulation Spirotetramat	Log K_{ow} is < 4 (2.51 at pH 4 to 9)	Log K_{ow} is < 3 (2.51 at pH 4 to 9)	<p>The Log Kow does not exceed the classification criteria for DSD as well as for CLP indicating a low potential for bioaccumulation.</p>
Acute aquatic toxicity Spirotetramat	<p>LC/EC₅₀ ≤ 1 mg/L</p> <p>ErC50 = 0.96 mg/l <i>Skeletonema costatum</i></p> <p>EC50 = 0.85 mg/l <i>Crassostrea virginicae</i></p>		<p>Spirotetramat is of high acute toxicity to marine algae <i>Skeletonema costatum</i> with a ErC50 = 0.96 mg/L and to marine invertebrate <i>Crassostrea virginicae</i> with an EC50 = 0.85 mg/l. Therefore Spirotetramat fulfills the criteria for the proposed classification as R50 according to Directive 67/548/EEC and the criteria for the proposed classification as H400 according to Regulation EC 1272/2008.</p>

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Endpoint	Classification Criteria		Spirotetramat
	CLP (2 nd ATP)	DSD	
Chronic aquatic toxicity Spirotetramat	For nonrapidly degradable substances: 0.01 <NOEC ≤0.1 mg/l		Spirotetramat is not rapidly degradable and of high chronic toxicity to sediment dwelling organism (<i>Chironomus riparius</i>) with a NOEC _{EMERGENCE} = 0.1 mg/L. Therefore Spirotetramat fulfills the criteria for the proposed classification as H410 according to Regulation EC 1272/2008.
	<i>Chironomus riparius</i> NOEC _{EMERGENCE} = 0.1 mg/l		
SUMMARY	H400 M-factor =1 H410 M-factor =1	R50/53	PROPOSED CLASSIFICATION


Endpoint	Classification Criteria		Spirotetramat -enol (main metabolite detected in hydrolysis and simulation test)
	CLP (2 nd ATP)	DSD	
Degradation Spirotetramat-enol	The major metabolite detected in hydrolysis and simulation tests was the metabolite spirotetramat-enol. Ultimate degradation could be excluded in all discussed abiotic and biotic degradation studies as mineralization rate is too low. Fast primary degradation was indicated. Based on available degradation data DT50 = > 16 d (stable in Hydrolysis study and DT50 = 37.9 – 59 d in water/sediment system) a non rapid degradation is proposed.		The classification as R53 according to Directive 67/548/EEC. is based on the fact that the degradant is not considered as rapid degradable.
Bioaccumulation Spirotetramat-enol	Log K_{ow} is < 4 (0.3 at pH 7)	Log K_{ow} is < 3 (0.3 at 7)	The Log Kow does not exceed the classification criteria for DSD as well as for CLP indicating a low potential for bioaccumulation.
Acute aquatic toxicity Spirotetramat-enol	10 < L(E)C50 ≤ 100 mg/L		Spirotetramat-enol is of moderate acute toxicity to aquatic plants <i>Lemna gibba</i> with an ErC50 = 19.3 mg/L, spirotetramat-enol therefore has to be classified as R52 (DSD) . No classification is necessary according to CLP regulation.
	<i>Lemna gibba</i> ErC50 (7 d) =19.3 mg/L		
Chronic aquatic toxicity Spirotetramat-enol	For non rapidly degradable substances: 0.1 <NOEC ≤1 mg/l		Spirotetramat-enol is chronic toxic to aquatic plants <i>Lemna gibba</i> with an NOEC _{YIELD} (7d)= 0.95 mg/L , spirotetramat-enol therefore has to be classified as H411 according to Regulation EC 1272/2008.
	<i>Lemna gibba</i> NOEC _{YIELD} (7d)= 0.95 mg/L		
SUMMARY	H411	R52/53	PROPOSED CLASSIFICATION

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

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

- N Dangerous for the Environment
- R50 Very toxic to aquatic organisms
- R53 May cause long term effects in the environment
- S 56 Dispose of this material and its container to hazardous or special waste collection point.
- S 57 Use appropriate container to avoid environmental contamination.
- S 60 This material and its container must be disposed of as hazardous waste.
- S 61 Avoid release to the environment. Refer to special instructions/safety data sheets.

Conclusion of environmental classification according to Regulation EC 286/2011 (2nd ATP to EC 1272/2008)

Classification categories	<p>aquatic environmental hazard acute category 1</p> <p>aquatic environmental hazard chronic category 1</p>
GHS Pictogram	
Signal Word	Warning
Hazard Statement	<p>'Very toxic to aquatic life',</p> <p>'Very toxic to aquatic life with long lasting effects'</p>
M-factor (acute)	1
M-factor (chronic)	1
Precautionary statements — Prevention	<p>Avoid release to the environment</p> <p>Collect spillage</p> <p>Dispose of contents/container to</p>

6 OTHER INFORMATION

Environmental fate properties and environmental hazard assessments of this CLH report were based on studies and summaries of the Draft Assessment Report and its addenda.

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
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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
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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
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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
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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

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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

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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

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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.; Hurt, 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

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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

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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

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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

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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 ¹⁴ CO ₂ 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

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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

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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
Petriere, 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

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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

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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

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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
Wirmitzer, 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 BYI 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

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

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 (<i>Cyprinodon variegatus</i>) 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 <i>Skeletonema costatum</i> 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 (<i>Americamysis bahia</i>) 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 (<i>Crassostrea virginica</i>) 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	Springbor n
Dorgerloh, M.	2004a	Acute toxicity of BYI 08330 (tech.) to fish (<i>Oncorhynchus mykiss</i>) 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 (<i>Cyprinus carpio</i>) Bayer CropScience AG, Report No.: DOM24022, Edition Number: M-128667-01-2 Date: 25.11.2004 GLP unpublished	Yes	BCS

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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.	2004c	<i>Pseudokirchneriella subcapitata</i> - 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 (<i>Lepomis macrochirus</i>) 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 (<i>Pimephales promelas</i>) Bayer CropScience AG, Report No.: EBFN0305, Edition Number: M-260676-01-2 Date: 16.11.2005 GLP unpublished	Yes	BCS
Dorgerloh, M.	2005c	Acute toxicity of BYI 08330 (tech.) to the waterflea <i>Daphnia magna</i> 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.	2005d	Acute toxicity of BYI 08330-enol to the waterflea <i>Daphnia magna</i> in a static laboratory test system -limit test Bayer CropScience AG, Report No.: EBFNM012, Edition Number: M-254487-01-2 Date: 14.07.2005 GLP unpublished	Yes	BCS
Dorgerloh, M.	2005e	Influence of BYI 08330 (tech.) on development and reproductive output of the waterflea <i>Daphnia magna</i> 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

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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.	2005f	<i>Pseudokirchneriella subcapitata</i> - Growth inhibition test with BYI 08330-enol Bayer CropScience AG, Report No.: EBFNM010, Edition Number: M-252531-01-2 Date: 06.06.2005 GLP unpublished	Yes	BCS
Dorgerloh, M.	2005g	Acute toxicity of BYI 08330 (tech.) to larvae of <i>Chironomus riparius</i> 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.	2005h	Acute toxicity of BYI 08330-enol to larvae of <i>Chironomus riparius</i> in a 48 h water-only study Bayer CropScience AG, Report No.: EBFNX073, Edition Number: M-262638-01-2 Date: 08.12.2005 GLP unpublished	Yes	BCS
Dorgerloh, M.	2005i	<i>Chironomus riparius</i> 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
Dorgerloh, M.	2005j	<i>Lemna gibba</i> 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
Dorgerloh, M.	2005k	<i>Lemna gibba</i> G3: Growth inhibition test with BYI 08330-enol under static conditions Bayer CropScience AG, Report No.: EBFNX065, Edition Number: M-252412-01-2 Date: 07.06.2005 GLP unpublished	Yes	BCS

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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
Kern, M.E.; Lam, C.V.	2005	Toxicity of BYI 08330 to the freshwater diatom <i>Navicula pelliculosa</i> 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 <i>Anabaena flos-aquae</i> 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
Nieden, D.	2005a	Acute toxicity of BYI 08330-enol to fish (<i>Oncorhynchus mykiss</i>) Bayer CropScience AG, Report No.: EBFNM013, Edition Number: M-251850-01-2 Date: 18.05.2005 GLP unpublished	Yes	BCS

ANNEXES