Document Title

Tier 2 Summary of the Fate and Behavior in the Environment of the Active Substance

Spirotetramat (BYI08330)

Data Requirements

Directive 91/414/EEC
Regulatory Directive 2003-01/Canada/PMRA
OPPTS guidelines/US/EPA

Annex IIA
Section 5, Point 7
Document M

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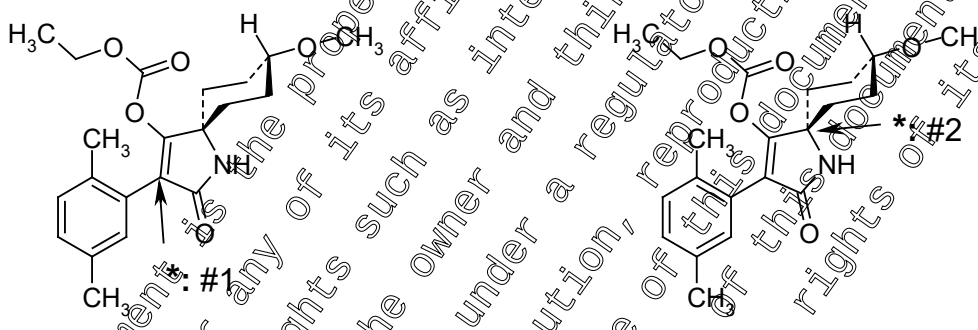
OWNERSHIP STATEMENT

TABLE OF CONTENTS

IIA 7 Fate and Behavior in the Environment

Information is provided in the pages that follow with respect to the fate and the behavior in soil, water and air of spirotetramat (BYI08330). This active substance is a novel ketoenol (tetramic acid) systemic broad spectrum insecticide against sucking pests (aphids, white flies, scales, trips, mealy bugs, etc.) in many target crops like vegetables, orchard crops, citrus, cotton, tobacco and grapes. Representative uses for this dossier are citrus and lettuce. It is proposed that the plant protection product be applied as a spray application at a maximum rate of 288 g as/ha in EU regions and 176 g as/ha in the USA and Canada.

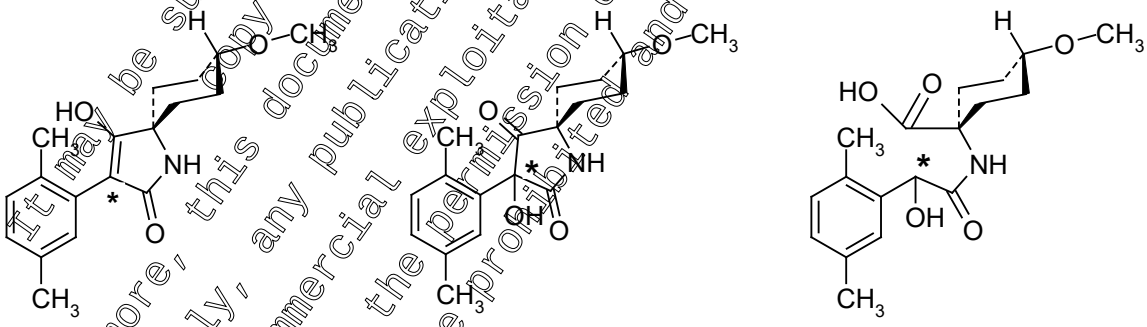
The studies concerning the fate and behavior of spirotetramat in the environment were conducted using one or two radiolabelled forms, the [azaspirodecenyl-3-^{14}C] and [azaspirodecenyl-5-^{14}C]BYI08330 as well as the non-labeled parent compound. Other radiolabels were not investigated since the pathway of degradation is well understood using those two radiolabels. In the Tier II summaries that follow, reference is made to simply label #1 (= azaspirodecenyl-3-^{14}C) or label #2 (= azaspirodecenyl-5-^{14}C). The structure of spirotetramat and the positions of the radiolabels were as follows:



[Azaspirodecenyl-3-^{14}C]BYI08330 (*: label #1) [Azaspirodecenyl-5-^{14}C]BYI08330 (*: label #2)

* indicates position of radiolabel

The results of appropriate studies are included in the following chapters. The proposed metabolic pathways in soil and water are given in Figure IIA 7.1-1 and Figure IIA 7.8.3-2. In addition, studies with the following radiolabelled or non-radiolabelled metabolites were performed:



BYI08330-enol BYI08330-ketohydroxy BYI08330-MA-amide

Further, one study on degradation in soil was performed with the [carbonyl-^{14}C] labeled BYI08330-methoxy cyclohexanone.

In original reports study authors may have used different names for certain degradation products of spirotetramat. In this summary, a single name and a single code number for each metabolite is used, always. In Document N of this dossier a list of metabolites contains the structural formula, various names, short forms and code numbers attributed to the metabolites. This list of metabolites is also

included at the end of this chapter. The matrices and reports in which the metabolites were identified are also included in this list.

IIA 7.1 Route of degradation in soil - laboratory studies

IIA 7.1.1 Aerobic degradation

Report:	**KIIA 7.1.1/01, [redacted], 2005 (MEF-04/169)**
Title:	**Aerobic Degradation/Metabolism of BYI8330 in Four Different Soils**
Report No & Document No	**MEF-04/169 M-256849-02-2**
	OECD: TG 307; Aerobic and Anaerobic Transformation in Soil, April 24, 2002; Commission Directive 95/36/EC amending Council Directive 91/414/EEC (Annexes I and II, Fate and Behavior in the Environment), July 14, 1995; US EPA Subdivision N, Section § 162-1; Japanese MAFF Guidelines.
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of March 2003 to March 2004. Study completion date, inclusive amendment no.1: 2005-07-12

EXECUTIVE SUMMARY

The biotransformation of cis-[azaspirodecenyl-3-^{14}C]BYI08330 was studied in three EU soils and one US soil for 50 days (EU soils) or 360 days (US soil) under aerobic conditions in the dark at 20 ± 1 °C and 50% WHCmax (EU soils) or 75% of 1/3 bar moisture (US soil).
The soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero (acidic extraction conditions are needed for the test item). However, under these conditions, the major metabolite BYI08330-enol is expected to be partly unstable and degrades to BYI08330-ketohydroxy. Therefore, the degradation/metabolism of BYI08330-enol in soils was investigated in a separate study (see later), and those results have to be included in the proposed overall metabolic pathway of spirotetramat in soil (see Figure IIA 7.1-1).
The parent compound was quickly degraded: Already after 1-2 days more than 90% of the test item dissipated and declined. At study termination, evolved $^{14}CO_2$ (no volatile organics occurred) accounted up to 19.4% of AR at DAT-50 (EU soils), and accounted for 15.3% of AR for the US soil after 360 days. During the course of the study a number of degradates was observed in all four soils. Five major degradates were present in all soils and were identified. Besides the two main soil metabolites BYI08330-enol (max. 24.3% of AR at DAT-3) and BYI08330-ketohydroxy (max. 16.3%, DAT-1), BYI08330-MA-amide (max. 6.4%, DAT-179) and two BYI08330-enol-dimers were found. In addition, two minor degradates were identified as BYI08330-desmethyl-enol and BYI08330-oxo-enol amounting to maximum 3.7% and 1.2% of AR, respectively. The route of oxidative BYI08330-enol dimerization leading to dimer 1 or dimer 2, and re-entry of the BYI08330-enol after their cleavage is considered as of minor importance for use according to the GAP, because the formation of dimers is regarded as an artificial process mainly caused by the hot spot application in this test.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat: Code = BYI08330;
Label #1 = [Azaspirodecenyl-3-^{14}C]BYI08330 (sample ID: BECH 0755)
Specific activity 3.71 MBq/mg (100.2 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)
Identity and purity of test item in the application solution were checked

2. Soil: The biotransformation of BYI08330 was studied in three EU soils and one US soil for 50 days (EU soils) or 360 days (US soil*)* under aerobic conditions in the dark at 20 ± 1 °C. Fresh samples (about 40 days prior to the start of the incubation) of all the soils were taken from the respective fields and after removing the stones and plant material, the soils were at ambient conditions and sieved with a 2 mm sieve. Finally, the soil batches were each mixed thoroughly for optimal batch homogeneity.

Table IIA 7.1.1-1: Soil physicochemical properties (MEF-04/169)

Designation	Source	Soil Type (USDA)	pH ($CaCl_2$)	Organic Carbon [%]	Texture Analysis [% sand/silt/clay]
[redacted]	[redacted], Florida, USA	sandy loam	5.4	0.93	77.3 / 12.7 / 10.0
[redacted]	[redacted], Germany	sandy loam	6.3	1.02	72.4 / 22.6 / 5.0
[redacted]	[redacted], Germany	silt loam	6.5	0.83	36.9 / 51.1 / 12.0
[redacted]	[redacted], Germany	silt	6.7	2.11	8.5 / 81.3 / 10.2

Cation exchange capacity (CEC) ranged between 6 to 15 meq/100 g DM.
Measurement of initial & final soil biomass (mg microbial C/kg soil DM) indicated that the soils were viable throughout the study. The selected soils have been used in several environmental fate studies and meet the guidelines' requirements.

B. STUDY DESIGN

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 20 ±1 °C. The test system consisted of Erlenmeyer flasks (300 mL) attached with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds.

Aliquots of 100 g of dry soil were weighed into the test flasks. For the [redacted] soil (metabolism soil) replicates were set up for each sampling (13 sampling dates including time 0). For the EU soils, only for time-0 duplicates were prepared, all other sampling dates were analyzed via single test flasks (8 sampling dates including time 0).

BYI08330 was applied to the soils at nominal rates of 0.128 mg/kg soil (DM) (US soil) or 0.768 mg/kg soil (DM) (EU soils), equivalent to a single maximum use rate of 288 g/ha (calculation based on homogeneous distribution for 15 cm soil depth and 2.5 cm soil depth to be used for foliar application in EU soils). Moisture adjustment after application to either 50% MWHC (EU soils) or 75% of 1/3 bar moisture (US soil) was carried out for each individual flask by addition of deionized water, and the vessel initial weights were recorded.

Test Item Stock Solution: The entire amount of the supplied [azaspirodecenyl-3-^{14}C]BYI8330 was dissolved in 2 mL acetonitrile.

Test Item Application Solutions: Two application solutions were prepared from the stock solution. For the application to the US soil, 3 mL of application solution Ia was prepared by transferring 67.5 µL (i.e. 45 µL and 22.5 µL) of the stock solution into a screw capped vial and diluting with 2932.5 µL (i.e. 1955

µL and 977.5 µL) acetonitrile. The projected amount of 53.5 µL application solution Ia/flask results in a nominal application rate of 12.8 µg/100 g soil (DM).
For the application to the EU soils, 3.7 mL of application solution IIa were prepared by transferring 686 µL (i.e. 486 µL and 200 µL) of the stock solution into a screw capped vial and diluting with 3032 µL acetonitrile. The projected amount of 37.7 µL application solution Iia/flask results in a nominal application rate of 76.8 µg/100 g soil (DM).
Mode of Application: On 2003-05-05, 100 g dry soil were weighed into 300 mL-Erlenmeyer flasks and dosed with small acetonitrile volumes of applications solutions Ia (53.5 µL per flask) and IIa (37.7 µL per flask). Treatment was made as small droplets manually applied directly to the soil surface using an Eppendorf pipette, thus simulating a soil spray application. Each flask was then gently shaken to incorporate the radiochemical into the test soil and to evaporate the organic solvent. Care was taken not to form clods during the mixing process.
Remark: Within ^{14}C-laboratory studies on metabolism in soil it is always a technical problem and a challenge to apply test items with a low water solubility like Spirotetramat. Organic solvents or co-solvents are needed to get the test soil homogeneously treated with the amount needed for simulation of the field rates. On the other hand, higher volumes of organic solvents must not be used due to interference with the viability of soil, neither decreasing nor enhancing the viable biomass in the test soil. In cases where the test item further has a rather low stability in soil (i.e. it would significantly degrade during a tumbling and mixing application procedure), the only adequate way is to dose the soil with a comparatively low volume of higher concentrated organic test solution by a so-called "hot spot application". As a consequence, in an environment where higher BYI08330-enol concentrations occur, the probability of dimer formation is much higher than in a more diluted environment.

2. Sampling: Microbial biomass was determined prior to commencement of the test (soils sampled at the day of application), after the end of the study using the EU soils (DAT-59), and for the US soil at DAT-136 and at the end of the study (DAT-360). Entire test flasks were taken for processing and analysis at approximately 1 minute, 6 hours, and 1, 3, 7, 14, 30, 50 days after treatment (DAT) in the case of the EU soils. Study termination for EU soils was justified by verification of the respective OECD triggers, i.e. > 90% degradation, > 5% mineralization of the test item. For the US soil experiment, additional samplings were conducted at DAT-86, 126, 179, 270 and 360.

3. Description of analytical procedures: The soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero (acidic extraction conditions are needed for the test item). However, under these conditions, the major metabolite BYI08330-enol is expected to be partly unstable and degrades to form BYI08330-ketohydroxy and others. This fact lead to the overall conclusion that a valid quantitative metabolite profiling can accurately derived only from the laboratory study performed with the major metabolite BXI08330-enol, applied as test item in a relatively large aqueous volume (see report KIIA 7.1.1/03). There, the enol-dimers were found to be minor metabolites, always.
The 100-g dry (DM) soil samples of each sampling interval were extracted using an ambient procedure by shaking at room temperature (3 times acetonitrile/water 1:1 [containing 0.5% formic acid], pH 2.5), followed by a stepwise aggravated extraction by shaking at room temperature (twice acetonitrile/1 N hydrochloric acid, pH 1) and a terminal acetonitrile extraction. The BYI08330-residues were radio-assayed by LSC and analyzed separately in the combined ambient and combined aggravated extracts by HPLC on reversed phase (C-18). Solid samples (i.e. soil and paper filters) were combusted and ^{14}C levels were measured using LSC. In order to identify parent compound and the transformation products co-chromatography and spectrometric methods were used.
For the time-0 samples, soil was weighed directly into centrifuge beakers. The moisture of these soil samples was not adjusted, since they were extracted immediately after application (within ca. 1 min).

II. RESULTS AND DISCUSSION

A. DATA

The respective data for the four soils are shown in Table IIA 7.1.1-2 to Table IIA 7.1.1-5. The data show that (even by performing the same kind of hot spot application) in the US soil (that received a much lower concentrated treatment solution) much lower portions of dimers (always below 5% of AR) were formed than in the 3 EU soils.

B. MASS BALANCE

The material balances ranged from 85.7% to 100.5% of AR. Only two out of 37 mass balance values were slightly below 90% (85.7 and 86.8%). A decreasing trend with the incubation time was not to be observed.

C. BOUND AND EXTRACTABLE RESIDUES

Unextractable (bound) ^{14}C-residues increased from ≤0.4% of AR at DAT-0 to maximum 35.2% at DAT-3 (in case of [redacted] soil) and then declined to 27.0% at the end of the US soil study (DAT 360).

Extractable ^{14}C-residues were all >90% of the applied amount at DAT-0 (range between 94.4 and 98.2% of AR) and decreased from a mean of 96.6% of AR at DAT-0 to a mean of 55.6% of AR at DAT-50 (all four soils) and to 51.8% at DAT 360 (US soil study). DAT-0 test item recoveries were all >90% and ranged from 91.6 to 96.1% of AR.

Table IIA 7.1.1-2: Biotransformation of BYI08330 in sandy loam soil [redacted] under aerobic conditions; mean values expressed as % of AR (MEF-04/169)

Compound	Days After Treatment (DAT)												
	0	0.25	1	3	7	14	30	50	86	126	179	270	360
BYI08330	96.1	52.2	15.3	7.0	6.5	4.5	5.1	4.0	3.7	2.9	3.5	3.2	3.5
Desmethyl-enol	-	-	1.1	1.0	3.1	1.7	3.7	2.5	3.3	3.2	3.7	3.1	1.4
Oxo-enol	-	-	-	0.3	1.0	0.7	-	1.2	-	-	0.4	-	-
Enol	-	5.0	9.2	13.2	10.5	8.6	6.2	7.4	5.5	7.4	4.6	4.3	2.7
Enol-Dimer 1	-	-	0.6	0.7	1.6	2.8	3.1	2.8	1.6	2.1	1.8	1.1	0.5
Enol-Dimer 2	-	-	1.4	0.5	0.4	1.5	1.8	1.9	2.2	1.0	1.3	0.8	0.8
Ketohydroxy	-	8.0	8.7	12.1	11.8	7.7	9.4	9.9	10.6	14.2	11.0	12.7	13.6
MA-amide	-	0.5	2.5	1.9	4.2	4.2	5.4	5.0	5.4	6.0	6.4	6.2	6.2
Unidentified *	1.6	14.6	24.2	19.1	17.6	20.1	22.8	21.5	22.5	21.7	20.1	23.7	23.2
Total extracted	97.7	80.3	63.0	55.7	56.2	51.9	57.5	55.6	54.9	58.5	52.8	55.1	51.8
$^{14}CO_2$	-	0.5	1.7	3.7	5.9	7.6	8.4	9.7	15.5	12.1	15.4	14.8	15.3
Volatile org.	-	-	-	-	-	-	-	-	0.1	-	-	-	-
Unextractable	0.4	19.7	31.8	35.2	33.0	30.5	31.5	30.3	29.9	27.6	27.8	27.2	27.0
Total recovery	98.1	100.5	96.5	94.6	95.1	90.1	97.4	95.6	100.4	98.2	96.0	97.1	94.2

*: Up to 18 HPLC peaks/DAT; maximum value for a single peak: 4.0% of AR.
Diffuse RA: maximum 14.3% of AR/extract (spread over > 25 HPLC-minutes)

Table IIA 7.1.1-3: Biotransformation of BYI08330 in sandy loam soil [redacted] under aerobic conditions, values expressed as % of AR (MEF-04/169)

Compound	Days After Treatment (DAT)							
	0 **	0.25	1	3	7	14	30	50
BYI08330	91.8	38.3	8.3	3.1	3.5	2.6	2.2	2.5
Desmethyl-enol	-	0.2	1.9	1.5	2.7	1.6	2.6	1.8
Oxo-enol	-	-	-	1.0	-	0.4	-	-
Enol	-	6.8	18.8	19.0	11.9	10.2	8.8	9.5
Enol-Dimer 1	-	2.3	2.4	3.5	7.0	7.0	7.4	7.5
Enol-Dimer 2	-	3.1	6.2	5.0	2.7	5.7	5.5	6.9
Ketohydroxy	-	10.6	16.3	14.8	12.8	7.4	5.1	5.9
MA-amide	-	0.4	2.3	3.0	6.0	4.4	3.5	3.1
Unidentified *	4.3	8.4	9.7	16.9	15.8	13.7	19.5	19.6
Total extracted	96.0	70.1	65.7	69.7	62.3	53.4	54.6	56.9
$^{14}CO_2$	-	0.3	1.8	3.1	6.3	8.9	10.0	12.2
Volatile org.	-	-	-	-	-	-	-	-
Unextractable	0.1	16.4	27.2	25.4	26.2	24.3	27.9	25.5
Total recovery	96.2	86.8	94.8	96.1	94.8	85.7	92.5	94.7

*: Up to 17 HPLC peaks/DAT; maximum value for a single peak: 3.1 % of AR.
**: Mean of duplicates
Diffuse RA: Maximum 8.0% of AR/extract (spread over > 25 HPLC-minutes)

Table IIA 7.1.1-4: Biotransformation of BYI08330 in silt loam soil [redacted] under aerobic conditions, values expressed as % of AR (MEF-04/169)

Compound	Days After Treatment (DAT)							
	0 **	0.25	1	3	7	14	30	50
BYI08330	91.6	41.2	8.9	3.4	3.5	2.8	3.0	3.4
Desmethyl-enol	-	-	0.4	-	2.7	1.9	2.1	2.3
Oxo-enol	-		-	-		-	-	-
Enol	-	9.4	20.8	24.3	15.1	15.5	11.4	9.9
Enol-Dimer 1	-	4.4	3.3	3.5	12.7	9.4	9.8	7.7
Enol-Dimer 2	-	4.2	7.2	6.3	4.2	8.9	9.2	8.1
Ketohydroxy	-	10.5	15.0	14.3	12.9	8.4	6.6	5.6
MA-amide	-	0.2	1.9	5.1	5.4	3.9	3.3	3.3
Unidentified *	2.8	11.1	14.3	18.2	18.1	18.2	20.8	22.3
Total extracted	94.4	81.1	71.9	73.2	74.6	68.9	66.3	62.7
$^{14}CO_2$		0.2	1.5	3.0	2.1	6.9	10.7	15.4
Volatile org.	-	-		-	-	-	-	-
Unextractable	0.1	14.1	21.3	21.0	19.0	17.4	20.6	21.5
Total recovery	94.5	95.4	94.7	97.1	95.7	93.2	97.6	99.6

*: Up to 19 HPLC peaks/DAT; maximum value for a single peak: 2.9% of AR.
**: Mean of duplicates
Diffuse RA: Maximum 7.5% of AR/extract (spread over > 25 HPLC-minutes)

Table IIA 7.1.1-5: Biotransformation of BYI08330 in silt soil [redacted] under aerobic conditions, values expressed as % of AR (MEF-04/169)

Compound	Days After Treatment (DAT)							
	0 **	0.25	1	3	7	14	30	50
BYI08330	93.2	11.6	5.8	1.9	3.7	2.0	2.1	2.6
Desmethyl-enol	-	1.0	0.6	1.7	2.3	2.3	1.6	1.6
Oxo-enol	-	0.6	-	-	1.1	0.4	-	-
Enol	-	11.6	17.2	16.9	14.1	8.4	9.8	10.4
Enol-Dimer 1	-	4.3	2.1	2.5	5.7	4.2	5.5	4.9
Enol-Dimer 2	-	3.7	3.8	2.1	1.6	3.5	3.8	6.0
Ketohydroxy	-	14.7	9.4	10.8	11.0	4.3	3.8	2.4
MA-amide	-	2.4	2.8	4.8	5.3	2.8	2.0	1.1
Unidentified *	5.0	16.0	22.2	16.0	16.4	18.2	20.5	18.3
Total extracted	98.2	65.8	63.8	58.9	61.3	46.4	49.1	47.2
$^{14}CO_2$	-	0.3	1.3	3.2	4.8	11.4	13.3	19.4
Volatile org.	-	-	-	-	-	-	-	
Unextractable	0.3	32.3	30.9	34.5	32.8	33.8	34.6	31.0
Total recovery	98.5	98.4	96.0	95.6	99.0	91.2	97.0	97.6

*: Up to 20 HPLC peaks/DAT; maximum value for a single peak: 4.9% of AR.
**: Mean of duplicates
Diffuse RA: Maximum 8.5% of AR/extract (spread over > 25 HPLC-minutes)

D. VOLATILIZATION

At study termination (DAT-50 in case of EU soils), volatile radioactivity identified as $^{14}CO_2$ (no volatile organics were observed) accounted for 12.2 to 19.4% of AR at, and accounted for 9.7% (at DAT-50) and 15.3% (at DAT-360) of AR in case of the US soil, respectively.

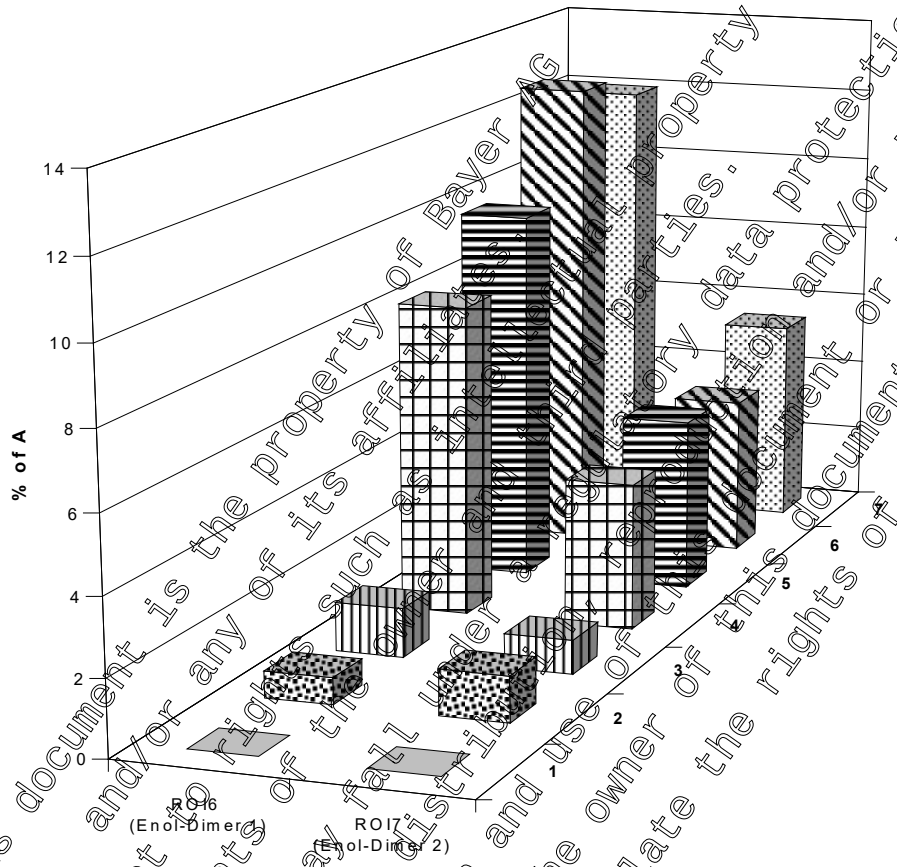
E. TRANSFORMATION OF TEST ITEM

The parent compound was quickly degraded (for synopsis of results see Table IIA 7.1.1-6). Already within 1-2 days >90% of the test item dissipated and declined from 96.1% of AR at DAT-0 to 3.5% at the end of the study in the US soil (DAT-360), and on average from 92.2% to 2.8% of AR in the three EU soils (DAT-50). The degradation behavior of the test item followed first order kinetics.
During the course of the study a number of degradates was observed in all four soils. Five major degradates were present in all soils and were identified. Besides the two main soil metabolites BYI08330-enol (max. 24.3% of AR at DAT-3) and BYI08330-ketohydroxy (max. 16.3%, DAT-1), BYI08330-MA-amide (max. 6.4%, DAT-179) and two BYI08330-enol-dimers were found. The route of oxidative BYI08330-enol dimerization leading to dimer 1 or dimer 2, and re-entry of the BYI08330-enol after their cleavage is considered as of minor importance for use according to the GAP, because the formation of dimers is regarded as an artificial process mainly caused by the hot spot application in this test.

By some additional treatment experiments it could be clearly shown that the amount and kind of application determines the degree of formation of the BYI08330-enol dimers. The highest amount of dimers was found, whenever a small volume of ACN with high concentrated pure BYI08330-enol or BYI08330 was applied. Rather low amounts were found whenever a higher volume or diluted aqueous solution or a simulated aqueous spray solution using formulated product was applied to soil. The latter is then more adequately simulating a practical spray onto soil. Also with the tumbling application procedure (treatment of a rather dry sub sample followed by a more or less complete mixing of test substance in the entire mass of soil [several kilograms] prior to rising soil moisture) very low amounts

of dimers were found. Results were visualized in Appendix 23 of report KIIA 7.1.1/01 and by the following graph.

Effect of the Soil Application Mode of BYI8330 or its Enol on the Degree of Enol-Dimer Formation



No.	Dosed compound	Concentration [mg/mL]	Solvent and volume	Mode of application
1	BYI08330	0.02*	ACN	Tumbling: spiking of 20 g dry soil subsample, then mix into 1.1 kg soil prior to moistening (fast degradation mainly to BYI08330-ketohydroxy was observed)
2	Enol	3	Water, 7 mL	Soil spiking by a pipette (high aqueous volume)
3	BYI08330 formulation OD100	753	425 L water/ha	Track sprayer
4	Enol	1671	Water, 0.2 mL	Soil spiking by a pipette
5	Enol	1139	Water, 1.4 mL	Soil spiking by a pipette
6	BYI08330	2037	ACN, 38 µL	"Hot spot" soil spiking by a pipette
7	Enol	1067	Water, 3.0 mL	Soil spiking by a pipette

The various soil treatments 1-7 were performed during the experimental phaseof various lab studies*), i.e. no. 6 represents the BYI08330 treatment in study KIIA 7.1.1/01, no. 2 represents the treatment in BYI08330-enol study KIIA 7.1.1/03, no. 3 represents the BYI08330 OD100 spray treatment in outdoor study KIIA 7.1.1/02.

*): partly supportive non GLP-tests

In addition, two minor degradates were identified as BYI08330-desmethyl-enol and BYI08330-oxo-enol amounting to maximum 3.7% (DAT-30 and DAT-179) and 1.2% of AR (DAT-50), respectively. The corresponding maximum concentrations in the four soils at DAT-50 (end of the three EU soil studies) were 10.4% (enol), 9.9% (ketohydroxy), 5.0% (MA-amide), 7.7% (enol-dimer 1) and 8.1% (enol-dimer 2). The corresponding maximum concentrations at DAT-360 (US soil) were 2.7% (enol), 13.6% (ketohydroxy), 6.2% (MA-amide), 0.5% (enol-dimer 1) and 0.8% (enol-dimer 2).

All other degradates were minor and were not considered further. All major degradates were transient during the study, but reached a plateau towards the end of the study in at least one of the four soils.

The maximum total unidentified radioactivity ranged from 19.6 to 24.2% of AR consisting of at maximum between 17 and 20 HPLC ROI's and diffuse radioactivity per sampling interval for the four soils. The maximum amount of an unidentified component ranged between <2.9 and <4.9% of AR for the four soils.

The before-mentioned results were included in the proposed overall pathway of degradation of spirotetramat in soil shown in Figure IIA 7.1-1.

Table IIA 7.1.1-6: Synopsis of results of biotransformation of BYI08330 in soils (MEF-04/169)

Soil	[redacted]	[redacted]	[redacted]	[redacted]
Soil type	Sandy loam	Sandy loam	Silt loam	Silt
Simple 1st order DT_{50} [days] of BYI08330	0.3	0.2	0.2	0.1
Major transformation products *)	Enol Ketohydroxy MA-Amide CO_2	Enol Ketohydroxy Enol-Dimer 1 Enol-Dimer 2 CO_2	Enol Ketohydroxy Enol-Dimer 1 Enol-Dimer 2 CO_2	Enol Ketohydroxy Enol-Dimer 2 CO_2
Minor transformation products	Desmethyl-Enol Oxo-Enol Enol-Dimer 1 Enol-Dimer 2	Desmethyl-Enol Oxo-Enol MA-Amide	Desmethyl-Enol Oxo-Enol MA-Amide	Desmethyl-Enol Oxo-Enol Enol-Dimer 1 MA-Amide

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs or steadily increasing until the end of the study

III CONCLUSIONS

Based on the results obtained within this study a degradation pathway of BYI08330 in aerobic soil is proposed. The compound is rapidly degraded in soil and thoroughly metabolized via three routes each starting with a hydrolytic cleavage of the BYI08330 carbonate ester into the BYI08330-enol. The major modification of the enol occurs via oxidation at the benzylic carbon position leading to BYI08330-ketohydroxy, which can be opened hydrolytically to BYI08330-MA-amide ("ring-opened BYI08330-ketohydroxy), and finally mineralized (presumably via the mandelic and benzoic acid derivatives) to CO_2.

A second (minor) degradation pathway occurs via a desmethylation of BYI08330-enol to BYI08330-desmethyl-enol, it's oxidation to the BYI08330-oxo-enol and final mineralization to CO_2.

A third route includes oxidative BYI08330-enol dimerization leading to BYI08330-enol-dimer 1 or BYI08330-enol-dimer 2, and re-entry of the BYI08330-enol after cleavage dimers into both the before-mentioned pathways. This route is considered as of minor importance for use according to the GAP, because the formation of dimers is regarded as an artificial process mainly caused by the hot spot application in this test.

For this basic route of degradation study the soil processing procedure was optimized for DAT-0 >90% recovery of the test item (acidic extraction conditions due to the test item's instability at pH>7). However, under these conditions the major metabolite enol is partly unstable and degrades via formation of ketohydroxy and others. Therefore, the degradation/metabolism of the enol in soils was necessarily investigated in a separate study (see below), and those results have to be included in the proposed overall pathway of degradation of spirotetramat in soil (see Figure IIA 7.1-1).

Supportive study: Route of degradation under outdoor conditions

Report:	**KIIA 7.1.1/02, [redacted], 2006 (MEF-06/041)**
Title:	**Outdoor Metabolism of [Azaspirodecenyl-3-^{14}C]BYI08330 in Two Soils**
Report No & Document No	**MEF-06/041 M-270597-01-2**
Guidelines:	Supportive Non-Guideline Study, guided by: US EPA Pesticide Assessment Guidelines, Subdivision N, Section 162-1, 1982 OECD: Guideline 307; Aerobic and Anaerobic Transformation in Soil, 2002 Commission Directive 95/36/EC amending Council Directive 91/414/EEC (Annexes I and II, Fate and Behavior in the Environment, 1995) SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides. 1995
GLP	Fully GLP compliant - laboratory certified by German “Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen“.
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of June2004 to March 2005. Study completion date; 2006-04-03

EXECUTIVE SUMMARY

The biotransformation of radiolabeled BYI08330 (spirotetramat) was investigated in two soils for 127 days under outdoor climatic conditions. The soils ([redacted]a, Germany, loam; and [redacted], Florida, USA, sandy loam) were poured (without sieving; “semi-disturbed” system) into a container of approx. 1 m^2 area which was divided into two halves by a separating plate. The container was filled with gravel up to a height of 40 cm, followed by the soils of 20 cm height each. The container was placed in the outdoor vegetation hall of the Agricultural Center in [redacted], Germany) and was protected from rainfall.

[Azaspirodecenyl-3-^{14}C]BYI08330 formulated as an OD 100 was applied at 94.6% of the highest recommended single use rate for field application (288 g/ha). Application was carried out on 2004-06-28 using a computer controlled track sprayer fitted with a flat fan nozzle. The soils were kept bare and free of weeds, and irrigation was performed for adequate moisture. The study was conceived as a supportive non-guideline study, but was following related aerobic soil degradation guidelines as much as appropriate, and was conducted in compliance with current GLP regulations.

The parent compound was quickly and thoroughly degraded: Already 1 day after application, only 53.6 and 72.2% of the applied test item were detectable in soils [redacted]a and [redacted], respectively. After one week, the values were 13.4 and 17.8% AR, declining to 1.0% at the end of the study at DAT-127 in both soils.

During the course of the study a large number of degradates was observed in all four soils. Only two of them were major degradates – BYI08330-ketohydroxy (max. 25.3% AR, DAT-14) and BYI08330-enol (max. 7.8% AR, DAT-7).

The results obtained confirmed the pathway already established in the guideline aerobic soil metabolism studies. Three new metabolites, which were not identified in the previously mentioned two laboratory aerobic degradation studies, were identified as glyoxylic amide, benzoic acid and ketohydroxy-carboxy. The former two metabolites were originally identified within a laboratory aerobic soil photolysis study and were confirmed to occur in the current study under outdoor conditions. For overall pathway see Figure IIA 7.1-1.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat: Code = BYI08330
Label = [Azaspirodecenyl-3-^{14}C]BYI08330 (sample ID: BECH 1569)
Specific activity 3.67 MBq/mg (99.1 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC) and >98% (acc. radio.-TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)
Identity and purity of test item in the application solution were checked

2. Soil: The study was carried out using two soils, chosen to cover two different representative scenarios in soil physicochemical properties. The soils were taken from the A horizon (ca. 0-20 cm depth) of their respective sampling areas. Stones and plant debris were removed, and the (non-sieved) soils were stamped into the container on top of the gravel ground (40 cm in height) layer by layer (total soil height 20 cm). In the current report, this soil system is called "semi-disturbed".

Table IIA 7.1.1-7: Soil physicochemical properties (MEF-06/041)

Designation	Source	Soil Type (USDA)	pH ($CaCl_2$)	Organic Carbon [%]	Texture Analysis [% sand/silt/clay]
[redacted]	[redacted], Florida, USA	Sandy loam	5.4	0.8	77.9 / 13.6 / 8.6
[redacted]	[redacted], Germany	Loam	6.5	1.0	44.7 / 38.3 / 17.1

Cation exchange capacity (CEC) was 4.2 and 9.3 meq/100 g DM for [redacted] and [redacted].
The soil microbial viability was characterized by determination of the microbial biomass (mg microbial C/kg soil DM) at DAT-2 shortly after application and after the end of the study at DAT-128.
The selected soils have been used in several environmental fate studies and meet the guidelines' requirements.

B. STUDY DESIGN

1. Experimental conditions: The study was conceived as a supportive non-guideline study, but was following related aerobic soil degradation guidelines as much as appropriate, and was conducted in compliance with current GLP regulations. The test system was a planting container (125 x 80 x 60 cm [L/W/H]) containing the two soils and placed in a radioactivity-controlled vegetation hall (outside of building 6682 of the BCS-RD-D-MEF Institute: 40 m above sea level, 51° 4' northern latitude, 6° 55' eastern longitude), which assured that soil temperature and irradiation by sunlight were comparable to real field situations. The container was located right at the edge under the roof where the glass-cover ends, changing into a roof made out of a wire netting. The soils were kept free from weeds and exposed to natural temperature, humidity, and light-dark cycles. Weather data were monitored at the meteorological station of Bayer CropScience Agricultural Center, [redacted] throughout the entire study. The data were reported in a monthly compilation. The overall climatic conditions in summer 2004 were warm and dry. Since the soils were protected from rainfall by a glass roof, soil moisture was regulated by overhead irrigation as appropriate for good agricultural conditions. The test system was exposed to natural daylight/night cycles.
For radiation protection and avoid cross-contamination of each soil, a plastic frame (approximately 28 cm in height) was mounted on top of the two parts of the soil-filled container (thus reducing the sprayed area to 0.9 m^2). The bottom of the container was open for drainage (however, no drainage water was obtained during the entire study).
An amount of 27.57 mg/m² of [azaspirodecenyl-3-^{14}C]BYI08330 formulated as an OD 100 was sprayed corresponding to 94.6% of the highest recommended single use rate for field application of 288 g/ha.

Application was carried out on 2004-06-28 using a computer controlled track sprayer fitted with a flat fan nozzle.

2. Sampling: Four soil cores (5 cm in diameter, 20 cm in depth) were randomly taken from each half of the soil container at 1, 7, 14, 28, 63, and 127 days after treatment (DAT), using a soil borer. After removal of the cores, the holes were filled with new respective (sieved) soil and marked to prevent another sampling at the same place. On DAT-1 the TRR of each soil core (upper 10 cm) was separately measured prior to combination for the determination of the homogeneity of the application.

The upper 10 cm of the sampled soil cores were air-dried at ambient temperature (overnight), homogenized using a planetary mill (Retsch P6000) and their dry weight determined. Then a tenth of the weight of each core was taken, combined and mixed, and the mixed sample ([redacted]a: 82.7 to 94.6 g; [redacted]: 89.8 to 100.5 g) used for extraction. The remaining sample was used for TRR determination by combustion/LSC. The 10-20 cm layers of the soil cores were kept frozen as a reserve. The soil samples were extracted with acidic acetonitrile/water mixtures, i.e. by an optimized method for high recovery of the test item.

3. Description of analytical procedures: At the collection date the soil was were dried, extracted and analyzed by LSC on the following day, and usually processed and analyzed by reversed phase HPLC for the primary profiling the day after. Until analysis the extracts were stored in a refrigerator. From DAT-28 on, the profiling HPLC method was changed (original HPLC method 2 [Kromasil] was replaced by HPLC method 1 [Purospher]). Therefore, all extracts from DAT-1, -7 and -14 were either re-analyzed (DAT-7) or newly processed after storage of the original extract in a freezer (DAT-1, DAT-14), and the BYI08330 residues were analyzed by HPLC. Radio-TLC was used for confirmatory identification of isolated metabolites only (i.e. HPLC cuts spiked with non-radiolabelled referenced substances). Identification of the transformation products was performed by co-chromatography with reference substances and LC/MS spectrometry. Since the study was conducted under outdoor conditions, collection of $^{14}CO_2$ and volatile organic compounds was not possible, and therefore, the total recovered radioactivity was lower than the total applied radioactivity (100% AR).

II. RESULTS AND DISCUSSION

A. DATA

The respective data for the four soils are shown Table IIA 7.1.1-8 and Table IIA 7.1.1-9.

B. MASS BALANCE

The TRR ranged from 73.8 to 24.9% AR for loam soil [redacted]a at DAT-1 and DAT-127, respectively. The corresponding values for soil [redacted] were 94.6 to 22.8% AR after the two respective sampling intervals. Thus, the loss in mass balance within the entire study period was greater than 75 %. Since the study was conducted under outdoor conditions in an "open system" (collection of $^{14}CO_2$ and volatile organic compounds was not possible and just the treated soil was investigated), the total recovered radioactivity was expected to be lower than the total applied radioactivity (100% AR).

C. BOUND AND EXTRACTABLE RESIDUES

Non-extractable residues increased up to 13.2 and 16.8% AR in the two soils at DAT-28 and -63, respectively, and then decreased towards the end of the study to 8.5 and 5.4% AR in the two soils, respectively. The total amount of extractable ^{14}C-residues from the top soil layer (0-10 cm) ranged between 69.2 and 87.2% AR at DAT-1, and then decreased to between 16.4 and 17.4% AR at DAT-127

for soils [redacted]a and [redacted], respectively. Already 1 day after application, only 53.6 and 72.2% of the applied test item were detectable in soils [redacted]a and [redacted], respectively. After one week, the values were 13.4 and 17.8% AR, declining to 1.0% at the end of the study at DAT-127 in both soils.

D. VOLATILIZATION

Since $^{14}CO_2$ released into the air was not measurable as implied by the outdoor study design, and organic volatiles were not to be observed in the basic soil metabolism study mentioned before, possible mineralization could be estimated by calculating the losses of radioactivity. Ignoring possible translocation of RA into the deeper soil layer (since no drainage water was obtained during the entire study), a portion of >75% of AR dissipated within DAT-127, indicating a high degree of degradation and rate of mineralization of the test item.

E. TRANSFORMATION OF TEST ITEM

The degradation behavior of the test item followed first order kinetics. Kinetics analyses using the Simple First Order (SFO) routine of the software tool "ModelManager showed that spirotetramat degraded rapidly with a DT50 of 1.2 and 2.9 days in soils [redacted]a and [redacted], respectively (mean: 2.1 days). The calculated DT90 values were reached in both soils (4.1 and 9.6 days, mean 6.9 days). The behavior of all degradates within the study was transient; no degradate accumulated towards the end of the study.

During the course of the study a large number of degradates was observed in both soils (at maximum 39 HPLC regions of interest [ROIs] were set at a single sampling interval). The total unidentified radioactivity consisted on maximum of 14 and 29 HPLC ROIs and diffuse radioactivity per sampling interval for soils [redacted]a and [redacted], respectively.

Two major degradates were detected in the soils: BYI08330-ketohydroxy (max. 25.3% AR, DAT-14) and enol (max. 7.8% AR, DAT-7). As minor degradates were identified: BYI08330-MA-amide (max. 6.2% AR, DAT-28), BYI08330-benzoic acid (max. 3.3% AR, DAT-7), BYI08330-glyoxylic amide (max. 2.3% AR, DAT-7), BYI08330-enol-dimer 1 (max. 1.5% AR, DAT-28), BYI08330-enol-dimer 2 (max. 0.9%, DAT-7) and BYI08330-ketohydroxy-carboxy (max. 1.3% AR). The corresponding maximum concentration in the two soils at DAT-127 (end of study) were 0.5% BYI08330-enol, 8.0% BYI08330-ketohydroxy, 2.8% BYI08330-MA-amide and 0.5% BYI08330-benzoic acid; BYI08330-glyoxylic amide, BYI08330-enol-dimer 1 and -dimer 2 were either < LOQ or not detected. All other degradates were minor as well and were not considered further. The before-mentioned results were included in the proposed overall pathway of degradation of spirotetramat in soil shown in Figure IIA 7.1-1.

III CONCLUSIONS

The outdoor metabolism study demonstrated that spirotetramat quickly degrades in soils under the formation of numerous degradates. Following first order kinetics, a mean DT50 in two soils of 2.1 days was calculated. Metabolites generated from spirotetramat further degraded and are expected not to accumulate in the environment. When applied under nearly practical use conditions, the enol-dimers 1 and 2 do not play a significant role in the metabolic pattern. Other metabolites known from the laboratory soil photolysis study were detected as well, but only occurring at minor amounts under outdoor conditions.

Based on the results obtained within this study (for synopsis see Table IIA 7.1.1-10) the degradation

pathway of spirotetramat already established in the laboratory aerobic soil metabolism studies is confirmed and supplemented. The proposed overall pathway of degradation of spirotetramat in soil is shown in Figure IIA 7.1-1.

Table IIA 7.1.1-8: Biotransformation of BYI08330 in loam [redacted] a under outdoor conditions; mean values expressed as % of AR (MEF-06/041)

Compound	Days After Treatment (DAT)					
	1	7	14	28	63	127
BYI08330	53.6	13.4	3.8	2.5	2.5	1.0
Enol	2.5	5.9	3.3	1.5	1.0	0.5
Ketohydroxy	6.6	22.7	23.6	10.5	14.2	1.9
MA-amide	-	2.6	3.6	6.2	2.0	2.8
Glyoxylic amide	0.8	2.3	1.6	1.1	-	-
Benzoic acid	1.0	3.3	3.0	2.1	0.4	0.5
Enol-Dimer 1	1.1	1.3	1.4	1.5	0.4	-
Enol-Dimer 2	-	0.9	0.6	0.7	0.3	-
Unidentified *	3.6	11.6	13.7	20.8	14.1	9.2
Total extracted	69.2	63.9	54.6	46.8	34.9	16.4
$^{14}CO_2$	N/A	N/A	N/A	N/A	N/A	N/A
Volatile org.	N/A	N/A	N/A	N/A	N/A	N/A
Unextractable	4.6	9.8	7.9	13.2	8.4	8.5
Total recovery	73.8	73.7	62.5	60.0	43.3	24.9

*: Up to 14 detected regions/DAT; maximum value for a single non-identified region was 2.1% of AR at DAT-7.
- : Not detected; N/A: not applicable for this kind of study

Table IIA 7.1.1-9: Biotransformation of BYI08330 in sandy loam [redacted] under outdoor conditions; mean values expressed as % of AR (MEF-06/041)

Compound	Days After Treatment (DAT)					
	1	7	14	28	63	127
BYI08330	72.2	17.8	10.2	4.5	1.4	1.0
Enol	3.8	7.8	5.4	1.9	0.9	0.4
Ketohydroxy	5.9	20.4	25.3	20.5	3.1	8.0
MA-amide	0.2	1.8	1.9	2.0	4.9	1.0
Glyoxylic amide	0.2	0.6	0.7	0.6	0.7	<LOQ
Benzoic acid	0.3	1.2	2.1	0.9	1.0	0.3
Enol-Dimer 1	0.8	1.1	0.8	1.5	0.2	<LOQ
Enol-Dimer 2	0.3	0.9	0.4	0.6	0.2	<LOQ
Unidentified *	4.2	13.0	15.4	18.3	16.7	6.6
Total extracted	87.2	64.5	62.2	50.6	29.1	17.4
$^{14}CO_2$	N/A	N/A	N/A	N/A	N/A	N/A
Volatile org.	N/A	N/A	N/A	N/A	N/A	N/A
Unextractable	7.3	15.2	10.6	14.6	16.8	5.4
Total recovery	94.6	79.7	72.8	65.2	46.0	22.8

*: Up to 29 detected regions/DAT; max. value for a single non-identified region was 1.7% of AR at DAT-28.
N/A: not applicable for this kind of study
LOQ: < 0.1% of AR

Table IIA 7.1.1-10: Synopsis of results of biotransformation of BYI08330 in soils under outdoor conditions (MEF-06/041)

Soil	[redacted] AIII	[redacted]
Soil type	Loam	Sandy loam
Major transformation products*	Enol Ketohydroxy	
Minor transformation products	MA-amide Glyoxylic amide Benzoic acid Ketohydroxy-carboxy Enol-dimer 1 Enol-dimer 2	
Kinetics Evaluation: Simple 1st order regression (SFO)		Mean
M_0 (% AR)	98.6	97.0	97.8
k (1/days)	0.558	0.241	0.4
DT_{50} (days)	1.2	2.9	2.1
DT_{90} (days)	4.1	9.6	6.9
R^2	0.981	0.990	0.986

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs

Supportive study: Route of degradation of metabolite BYI08330-enol

Chemical name (CAS): cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one; CAS #: 203312-38-3

Report:	**KIIA 7.1.1/03, [redacted], 2006 (MEF-05/157)**
Title:	**[Azaspirodecenyl-3-^{14}C]- and [Azaspirodecenyl-5-^{14}C]-Labeled BYI08330-*cis*-Enol: Comparative Aerobic Soil Metabolism/Degradation in Four Soils**
Report No & Document No	**MEF-05/157 M-269304-01-2**
Guidelines:	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.
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of January 2004 to June 2005. Study completion date: 2006-01-17

EXECUTIVE SUMMARY

The biotransformation of cis-[azaspirodecenyl-3-^{14}C]BYI08330-enol and cis-[azaspiro-decenyl-5-^{14}C]BYI08330-enol was studied in three EU soils and one US soil for 119 days under aerobic conditions in the dark at 20 ±1 °C and at approx. 80% of 1/3 bar moisture (US soil) or 60% WHCmax (EU soils). The application rate of BYI08330-enol to the soils was calculated based on the highest recommended single use rate of spirotetramat for field application (288 g/ha) and an assumed worst case amount of its first soil metabolite BYI08330-enol. Following the respective required conversion factors a rate of 0.13 and 0.309 mg test item per kg DM of soil was calculated for the US soil and the EU soils. The soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero (i.e. slightly alkaline extraction conditions were needed for the test item).

The material balances demonstrated that no significant RA dissipated from the flasks or was lost during processing. Using an aqueous extraction step (desorption) in advance of the conventional organic/aqueous extraction a significant part of AR could only be dissolved at the very early DAT, despite a solubility of 78 mg BYI08330-enol per liter of water, indicating a significant increase of sorption with time. Extractable ^{14}C-residues at study termination had decreased to < 25% of AR in all four soils. For all soils lower extraction efficiency was observed at study termination when using the 3-^{14}C-labeled compared to the 5-^{14}C-labeled test item. This was complementary to the amount of ^{14}C mineralized to $^{14}CO_2$.

Non-extractable ^{14}C-residues increased from 4.2 to 28.4% of AR at DAT-0 (approximately 2 min after application) to 44.8 to 62.5% of AR at DAT-119 in the four soils. As observed for the extractable ^{14}C-residues, the non-extractable ^{14}C-residues were significantly lower using the 3-^{14}C-labeled test item as compared to the 5-^{14}C-test item. At study termination, $^{14}CO_2$ accounted for 16.7 to 27.8% of AR at DAT-119 using the 5-^{14}C-labeled test item, and accounted for 28.2 to 43.0% of AR using the 3-^{14}C-test item. No volatile organics evolved from the systems.

The test item dissipated following pronounced biphasic kinetics with an extremely quick first phase. Within a second slower degradation phase, the test item declined to 2.7 to 6.1% of AR in the four soils at the end of the study at DAT-119.

During the course of the study a large number of degradates was observed in all four soils. The maximum amount of any unidentified component was ≤ 4.9% of AR for the four soils. Label-specific degradates were not observed throughout the entire study, and the degradation pathway found in the before-mentioned study on spirotetramat was confirmed. In addition, a metabolite previously not found, the BYI08330-oxo-ketohydroxy was identified. However, it was a very minor component and was not quantifiable. In all four soils, BYI08330-ketohydroxy was detected at levels > 10%. BYI08330-enol-dimer 1 amounted once to 5.0% (DAT-60) in one soil, and BYI08330-enol-dimer 2 was maximum 3.6% (DAT-14). BYI08330-MA-amide amounted once > 5% (5.2% at DAT-4), and BYI08330-desmethyl-enol was max. 1.8% at DAT-4. All degradates (for overall pathway see Figure IIA 7.1-1) were transient during the study and did not increase towards the end of the study (with the exception of BYI08330-enol-dimer 2 which exhibited scattering results).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BYI08330-enol, within the study also called "enol"
Identity and purity of test item in the application solution were checked

Label #1, label position = [Azaspirodecenyl-3-^{14}C] (sample ID: BECH 00992)
Specific activity 4.54 MBq/mg (122.8 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

Label #2, label position = [Azaspirodecenyl-5-^{14}C] (sample ID: BECH 00995)
Specific activity 4.99 MBq/mg (134.94 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

2. Soil: The biotransformation of BYI08330-enol was studied in three EU soils and one US soil for 119 days under aerobic conditions in the dark at 20 ± 1 °C. All soils were taken freshly from the respective field within a month (EU soils) or within 70 days (US soil) prior to the start of the incubations. They were taken from the A horizon (ca. 0-20 cm depth) of their respective sampling area. Stones and plant material were removed, and soil moisture was partially reduced by spreading the soil at ambient temperature to allow for sieving to a particle size of 2 mm (approximately two weeks prior to the start of the pre-incubation for the degradation tests). Finally, the soil batches were each mixed thoroughly for optimal batch homogeneity. For physicochemical properties of soils see Table IIA 7.1.1-1 of the aerobic degradation study with spirotetramat.

B. STUDY DESIGN

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 20 ± 1 °C. The test system consisted of Erlenmeyer flasks (300 mL) attached with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds. Dry soil aliquots of 100 g were weighed into the test flasks. For all four soils and all sampling intervals, the two test flasks containing the soils treated with the two differently labeled test items were considered as replicates. In addition, for DAT-0 separate replicates were set up with the 5-^{14}C-labeled test item.
An attempt was made to approach more realistic GAP application conditions (compared to the aerobic laboratory degradation study with spirotetramat) by using a higher volume (7.4 to 11 mL) of aqueous application solution for the treatments. The actual rate (100% AR) of [azaspirodecenyl-3-^{14}C]BYI08330-enol applied to the US soil was 53.5 kBq/flask corresponding to 118 µg/kg soil (DM).

The actual amount (100% AR) applied to the EU soils was 133.8 kBq/flask corresponding to 295 µg/kg soil (DM). The actual amount (100% AR) of [azaspirodecenyl-5-14C]BYI08330-enol applied to the US soil was 62.9 kBq/flask corresponding to 126 µg/kg soil (DM), and that applied to the EU soils was 139.9 kBq/flask corresponding to 280 µg/kg soil (DM), respectively. The calculations were based on homogeneous distribution in 15 cm depth of US soil, and (as it is required for EU foliar spray uses) in 2.5 cm depth of EU soils. Moisture adjustment after application to either 60% MWHC (EU soils) or approx 80% of 1/3 bar moisture (US soil) was carried out for each individual flask by addition of deionized water, and the flasks' initial weights were recorded.

2. Sampling: Microbial biomass was determined prior to commencement of the test (21 days before application), around the application date (DAT-4, at an intermediate date (DAT-62) and at the end of the study (DAT-119). Entire test flasks were taken for processing and analysis at 1-2 minutes, 6 hours, and 1, 4, 7, 14, 32, 60, 90 and 119 days after treatment (DAT).

3. Description of analytical procedures: The soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero. Thereby, the 100-g dry (DM) soil samples of each sampling interval were extracted using a slightly alkaline aqueous extraction procedure (aqueous ammonia, resulting in pH values of approx. 7 to 8.5) and a slightly alkaline conventional organic/aqueous procedure (3 times ACN/H_2O/NH_4Cl/NH_3 (10:10:0.02:0.05 [v:v:w:v]; pH approx. 8.5), all by shaking at room temperature. This was followed by an "aggressive" microwave extraction at 70 °C (10 min) using the same medium as for the conventional extractions. The BYI08330-enol residues were radio-assayed by LSC and analyzed separately in the aqueous extract, the combined ambient extracts and the "aggressive" extract by reversed phase (C-18) HPLC. Solid samples (i.e. soil and paper filters) were combusted and ^{14}C levels were measured using LSC. For identification of test item and transformation products, co-chromatography and spectrometric methods were used.

II. RESULTS AND DISCUSSION

A. DATA

The respective results for the four soils are shown in Table IIA 7.1.1-11 to Table IIA 7.1.1-14. The data for individual replicates were presented, if calculation of a mean of both radiolabels was not applicable (i.e. for unidentified RA, total extracted RA, $^{14}CO_2$, volatile org. RA and unextractable RA).

B. MASS BALANCE

The average material balance ranged from 84.8 to 108.4% of AR, with two out of 80 values ranged slightly below 90% at 88.9 and 89.2%, and one at 84.8%; one flask was at 76.4% most probably due to volatile leakage and was rejected. A decreasing trend with the incubation time was not observed.

C. BOUND AND EXTRACTABLE RESIDUES

Unextractable (bound) ^{14}C-residues ranged from 4.2 - 28.4% of AR at DAT-0 (approximately 1-2 min after application) to 44.8 - 62.5% of AR at DAT-119 in the four soils. As with the extractable ^{14}C-residues, the non-extractable ^{14}C-residues were significantly lower in the 3-^{14}C-label experiments as compared to the 5-^{14}C-label.

Using an aqueous extraction step (desorption) prior to the conventional organic/aqueous extraction a significant part of AR could only be dissolved at the very early DAT, despite a solubility of 78 mg BYI08330-enol per liter of water. Extractable ^{14}C-residues were >90% of AR at DAT-0 for soils [redacted] and [redacted] (range between 92.0 and 103.2% of AR), and between 76.0 and 77.6% of AR

for soils [redacted] and [redacted]. A decrease to < 25% of AR in all four soils was observed, ranging from 9.2% of AR (soil [redacted]) to 24.7% of AR (soil [redacted]) at study termination (DAT-119). For all soils lower extraction efficiency was observed at study termination when using the 3-^{14}C-labeled compared to the 5-^{14}C-labeled test item.

D. VOLATILIZATION

At study termination (DAT-119), $^{14}CO_2$ accounted for 16.7 to 27.8% AR when using the 5-^{14}C-labeled test item, and accounted for 28.2 to 43.0% AR when using the 3-^{14}C-test item, respectively. No volatile organics were evolved from the systems.

E. TRANSFORMATION OF TEST ITEM

The test item dissipated following pronounced biphasic kinetics. In three soils more than 82% of the test item dissipated within six hours (DAT-0.25), i.e. indicating an extremely quick first phase. In the fourth soil (silt loam soil [redacted]) this happened in the same manner but slightly later at DAT-1. Within a second slower degradation phase, the test item declined to 2.7 to 6.1% of AR in the four soils at the end of the study at DAT-119.

The details of respective kinetic modeling of test item and degradates by using MatLab® (application KinGUI) are described in the next section (rate of degradation) of this document. In Table IIA 7.1.1-15 only the best fit DT50 (days) of test item are the shown, i.e. that resulting by using the bi-exponential model DFOP (double first order in parallel). This model yielded BYI08330-enol DT_{50} values ranging between 0.02 and 0.09 days for the four soils (a mean DT50 of 0.08 days; chi^2 statistics mean value of 7.7).

During the course of the study a large number of degradates was observed (at maximum 27 HPLC integration regions of interest [ROI] at a single sampling interval) in all four soils. The maximum amount of any unidentified component was ≤ 4.9% of AR for the four soils. Label-specific degradates were not observed throughout the entire study, and the degradation pathway found in before-mentioned study on spirotetramat was confirmed.

Besides the test item BYI08330-enol, BYI08330-ketohydroxy, the two BYI08330-enol dimers 1 and 2, BYI08330-MA-amide and BYI08330-desmethyl-enol were identified. In addition, a metabolite previously not found, the BYI08330-oxo-ketohydroxy was identified. However, it was a very minor contaminant and was not quantifiable. In all four soils, BYI08330-ketohydroxy was analyzed at levels > 10%. BYI08330-enol-dimer 1 amounted once to 5.0% (DAT-60) in one soil, and BYI08330-enol-dimer 2 was maximum 3.6% (DAT-14). BYI08330-MA-amide amounted once > 5% (5.2% at DAT-4), and BYI08330-desmethyl-enol was max. 1.8% at DAT-4. All other degradates were minor and were not considered further. All major degradates were transient during the study and did not increase towards the end of the study (with the exception of BYI08330-enol-dimer 2 which exhibited scattering results).

The proposed pathway of degradation of BYI08330-enol in soil is included in that of spirotetramat shown in Figure IIA 7.1-1.

Table IIA 7.1.1-11: Biotransformation of BYI08330-enol in sandy loam [redacted] under aerobic conditions (values expressed as % of AR) (MEF-05/157)

Compound	Days After Treatment (DAT)									
	Means of Both Radiolabels (#1 + #2)									
	0	0.25	1	4	7	14	32	60	90	119
BYI08330-enol (t.i.)	78.0	17.3	13.4	10.9	14.7	10.7	7.7	6.1	7.1	4.8
- Desmethyl-enol	n.d.	0.2	0.5	1.0	1.1	0.8	0.9	0.7	0.7	0.4
- Enol-Dimer 1	0.2	2.5	1.8	2.9	1.2	3.4	3.6	2.3	2.3	1.8
- Enol-Dimer 2	n.d.	1.6	1.7	1.8	1.4	2.2	1.6	1.3	1.3	1.8
- Ketohydroxy	11.7	17.0	17.4	15.7	12.0	8.2	5.4	3.7	3.8	2.5
- MA-amide	n.d.	0.9	1.4	2.4	2.9	2.9	2.2	1.5	1.3	0.9
Total RA recovery	104.8	100.0	100.6	98.3	97.7	99.2	98.8	96.6	98.5	98.8
RA	Values of Individual Replicates (A and B)									
Unidentified * (A)	5.4	9.4	10.9	12.5	14.7	14.4	16.1	16.6	21.9	11.4
(B)	6.5	10.6	12.5	6.4	7.9	10.3	12.2	12.0	15.5	9.4
Total extracted (A)	92.0	50.3	46.1	44.0	45.7	39.9	36.8	31.5	37.2	24.7
(B)	99.7	48.6	49.8	44.2	43.7	41.0	34.3	28.2	33.2	20.3
$^{14}CO_2$ (A)	n.a.	0.5	1.0	1.9	2.8	4.2	7.6	10.9	14.0	16.7
(B)	n.a.	1.5	3.3	6.1	7.9	11.2	17.1	20.1	24.8	28.2
Volatile org. (A)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
(B)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unextractable (A)	9.3	48.5	50.7	50.2	48.4	52.6	52.4	52.7	45.2	55.3
(B)	8.7	50.5	50.3	50.3	46.9	49.6	49.4	49.8	42.7	52.3

Replicate A: treated with radiolabel #2; replicate B: treated with radiolabel #1
*: Up to 18 HPLC peaks/DAT; maximum value for a single peak: 4.7% of AR.
Diffuse RA: Maximum 6.9% of AR/extract (spread over > 20 HPLC-minutes)

Table IIA 7.1.1-12: Biotransformation of BYI08330-enol in sandy loam [redacted] under aerobic conditions (values expressed as % of AR) (MEF-05/157)

Compound	Days After Treatment (DAT)									
	Means of Both Radiolabels (#1 + #2)									
	0	0.25	1	4	7	14	32	60	90	119
BYI08330-enol (t.i.)	53.7	14.1	14.7	14.6	17.0	11.9	8.7	6.7	7.6	6.1
- Desmethyl-enol	0.2	0.1	0.5	1.2	1.6	0.8	0.4	0.2	0.3	<LOQ
- Enol-Dimer 1	0.9	4.0	2.4	3.5	1.8	4.4	4.9	5.0	4.1	3.7
- Enol-Dimer 2	n.d.	2.7	3.0	2.4	2.5	3.6	2.8	2.3	2.0	3.3
- Ketohydroxy	13.8	16.6	16.2	9.3	6.0	3.0	2.2	1.4	1.8	0.5
- MA-amide	n.d.	1.3	2.6	2.7	2.0	1.4	0.1	0.1	0.2	0.1
Total RA recovery	102.8	99.0	100.7	99.1	98.6	100.2	98.9	89.8	94.1	96.3
RA	Values of Individual Replicates (A and B)									
Unidentified * (A)	7.2	10.4	10.9	12.9	13.8	14.4	14.4	14.2	20.4	7.4
(B)	9.4	10.8	8.8	9.5	10.5	8.5	9.0	10.4	10.8	6.5
Total extracted (A)	76.0	48.8	48.9	46.0	44.9	38.9	33.9	30.9	35.9	22.9
(B)	77.6	50.2	49.4	43.8	41.1	34.5	27.9	24.9	27.3	18.5
$^{14}CO_2$ (A)	n.a.	0.4	1.0	2.4	3.7	5.5	10.2	13.4	16.8	19.0
(B)	n.a.	1.3	3.5	8.2	12.3	17.4	23.4	26.5	31.1	32.5
Volatile org. (A)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
(B)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unextractable (A)	28.4	49.7	50.2	46.2	51.1	52.9	54.7	44.9	36.3	54.3
(B)	23.6	47.6	48.5	51.6	44.0	51.1	47.6	38.9	40.8	45.5

Replicate A: treated with radiolabel #2; replicate B: treated with radiolabel #1
*: Up to 17 HPLC peaks/DAT; maximum value for a single peak: 3.7% of AR.
Diffuse RA: Maximum 5.9% of AR/extract (spread over > 20 HPLC-minutes)

Table IIA 7.1.1-13: Biotransformation of BYI08330-enol in silt loam [redacted] under aerobic conditions (values expressed as % of AR) (MEF-05/157)

Compound	Days After Treatment (DAT)									
	Means of Both Radiolabels (#1 + #2)									
	0	0.25	1	4	7	14	32	60	90	119
BYI08330-enol (t.i.)	83.3	38.0	17.2	4.8	11.6	7.7	5.7	3.7	4.0	2.7
- Desmethyl-enol	n.d.	n.d.	0.6	2.0	1.6	1.5	0.4	0.2	0.1	n.d.
- Enol-Dimer 1	0.3	1.4	0.9	1.2	0.7	1.5	1.8	2.0	1.0	1.0
- Enol-Dimer 2	n.d.	1.0	0.9	1.0	1.2	1.3	1.3	0.8	0.6	1.2
- Ketohydroxy	11.2	22.3	24.0	19.8	11.1	5.7	2.2	1.1	0.9	0.6
- MA-amide	n.d.	0.9	1.6	5.2	4.9	3.6	0.9	0.1	0.2	0.2
Total RA recovery	105.6	99.9	100.5	99.5	99.3	99.2	97.8	98.7	98.1	97.6
RA	Values of Individual Replicates (A and B)									
Unidentified * (A)	7.9	10.3	11.0	16.0	13.8	18.7	16.0	13.1	14.6	6.3
(B)	5.0	7.9	12.0	13.4	14.6	13.7	13.5	9.5	7.9	4.9
Total extracted (A)	103.2	72.0	57.6	48.8	47.3	38.9	29.3	21.4	21.4	12.8
(B)	99.6	73.3	55.8	48.4	43.2	36.2	24.5	16.9	14.6	9.9
$^{14}CO_2$ (A)	n.a.	0.2	0.8	2.1	2.9	6.0	11.6	19.2	23.4	27.8
(B)	n.a.	0.3	2.3	5.8	9.1	16.0	25.8	34.9	40.3	43.0
Volatile org. (A)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
(B)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unextractable (A)	4.2	27.9	43.4	49.6	50.4	56.3	57.8	58.3	53.3	57.0
(B)	4.4	26.1	40.9	44.2	45.7	45.0	46.5	46.6	43.1	44.8

Replicate A treated with radiolabel #2; replicate B treated with radiolabel #1
*: Up to 21 HPLC peaks/DAT; maximum value for a single peak: 4.9% of AR.
Diffuse RA: maximum 5.6% of AR/extract (spread over > 20 HPLC-minutes)

Table IIA 7.1.1-14: Biotransformation of BYI08330 in silt [redacted] under aerobic conditions (values expressed as % of AR) (MEF-05/157)

Compound	Days After Treatment (DAT)									
	Means of Both Radiolabels (#1 + #2)									
	0	0.25	1	4	7	14	32	60	90	119
BYI08330-enol (t.i.)	51.6	10.7	10.5	12.0	11.8	8.6	6.3	4.6	6.0	3.8
- Desmethyl-enol	n.d.	0.6	1.3	1.3	1.0	0.2	0.2	<0.1	0.1	n.d.
- Enol-Dimer 1	n.d.	1.6	1.1	1.8	0.9	1.8	1.9	2.1	1.0	1.1
- Enol-Dimer 2	n.d.	1.2	1.4	1.7	1.5	1.6	0.9	0.9	0.6	1.4
- Ketohydroxy	18.4	15.7	13.3	4.4	1.9	1.3	1.4	0.5	0.6	0.3
- MA-amide	n.d.	1.5	2.5	1.8	0.7	0.3	0.2	n.d.	0.1	n.d.
Total RA recovery	101.2	97.6	98.5	96.4	95.7	88.8	96.6	96.0	87.6	96.7
RA	Values of Individual Replicates (A and B)									
Unidentified * (A)	5.9	8.9	8.2	10.7	10.4	9.5	9.8	9.2	10.8	4.6
(B)	7.1	7.5	10.7	7.3	7.9	7.1	6.2	4.3	6.3	3.3
Total extracted (A)	77.1	38.8	40.0	33.8	29.3	24.6	20.9	17.1	19.8	11.8
(B)	76.0	40.3	39.1	30.1	24.8	19.7	17.1	12.7	13.9	9.2
$^{14}CO_2$ (A)	n.a.	0.3	1.3	3.8	5.7	8.6	12.6	17.2	20.3	22.0
(B)	n.a.	1.5	5.3	12.4	17.1	8.6	27.2	31.9	35.2	36.9
Volatile org. (A)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
(B)	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unextractable (A)	25.6	59.6	57.9	60.1	60.9	59.3	63.0	62.0	44.8	62.5
(B)	23.8	54.5	53.3	52.7	53.7	56.7	52.5	51.1	41.3	51.0

Replicate A treated with radiolabel #2; replicate B treated with radiolabel #1
*: Up to 15 HPLC peaks/DAT; maximum value for a single peak: 3.7% of AR.
Diffuse RA: maximum 5.0% of AR/extract (spread over > 20 HPLC-minutes)

Table IIA 7.1.1-15: Synopsis of results of transformation of BYI08330-enol in soil (MEF-05/157)

Soil	[redacted]	[redacted]	[redacted] AIII	[redacted]	Tested Soils
Soil type	Sandy loam	Sandy loam	Silt loam	Silt	Mean
DT_{50} [days] of BYI08330-enol (DFOP)	0.02	0.20	0.02	0.09	0.08
DT_{90} [days] of BYI08330-enol (DFOP)	74.0	22.9	106.9	53.7	64.4
Major transformation products *)	-Ketohydroxy CO_2 and Bound Residues				
Minor transformation products	-MA-amide -Desmethyl-enol -Enol-dimer 1 -Enol-dimer 2 -Oxo-ketohydroxy				

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs

III CONCLUSIONS

Based on the results obtained within the present laboratory investigation in four aerobic soils a degradation pathway of BYI08330-enol, the major degradate of spirotetramat, is proposed and then included in the overall metabolic pathway of spirotetramat in soil (see Figure IIA 7.1-1). It was demonstrated that BYI08330-enol quickly dissipates in soil following pronounced biphasic kinetics and is thoroughly mineralized. A positive correlation of the BYI08330-enol dissipation and the formation of soil degradates with the soil biomass was, at least in part, evident. No correlation was found between BYI08330-enol dissipation and the soil pH values.

A fast decrease of extractable ^{14}C-residues to < 25% of and a lower extraction efficiency using the 3-^{14}C- compared to the 5-^{14}C-labeled test item were observed until study termination with all four soils. Using an aqueous extraction step (desorption) prior to the conventional organic/aqueous extraction, a significant part of the AR could be extracted at the very early time points. However, already after six hours this portion had decreased significantly, and the water-extractable portion was 0.9 to 3.5% of AR at study termination in all four soils, only.

As expected from the BYI08330 soil metabolism study the major modification of the enol occurs via oxidation at the benzylic carbon position leading to the ketohydroxy, which can be opened hydrolytically to the MA-amide ("ring-opened ketohydroxy), and finally mineralized (presumably via the mandelic and benzoic acid derivatives) to CO_2. In addition, BYI08330-oxo-ketohydroxy, a metabolite previously not found, was identified. However, it was a very minor component and was not quantifiable. The significantly higher mineralization rate with the 3-^{14}C- compared to the 5-^{14}C-cyclohexyl-labeled test item could be explained by the intermediate formation of ^{14}C-labeled benzoic acid derivatives which should be more accessible to metabolic decarboxylation compared to the 5-^{14}C-cyclohexyl-labeled breakdown products.

A second (minor) degradation pathway occurs via a desmethylation of the enol to the desmethyl-enol and final mineralization to CO_2. A third (minor) route includes oxidative enol dimerization leading to enol-dimer 1 or enol-dimer 2, and re-entry of the enol after cleavage of the enol-dimers into both the before-mentioned pathways. All observed degradates were transient during the study and did not increase towards the end of the study (with the exception of BYI08330-enol-dimer 2 which exhibited scattering results). Radiolabel-specific degradates were not observed throughout the entire study.

IIA 7.1.2 Anaerobic degradation

Report:	**KIIA 7.1.2/01, [redacted], 2006 (MEF-05/515)**
Title:	**BYI08330: Anaerobic Soil Metabolism**
Report No & Document No	**MEF-05/515 M-270739-01-2**
Guidelines:	Official Journal of the EC, Commission Directive, 95/36/EC, amending Council Directive 91/414/EEC: Annexes II+III, Fate and Behavior in the Environment; OECD Guideline for Testing of Chemicals, Guideline 307, Aerobic and Anaerobic Transformation in Soil, Adopted Document; US EPA Subdivision N, Section 162-2; CAN PMRA DACO 8.2.3.4.4; Japanese MAFF New Test Guidelines for Supporting Registration of Chemical Pesticides, 12 Nohsan 8147
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and dates	Bayer CropScience AG, RD, Metabolism and Environmental Fate, D-[redacted], Germany, conducted the study during the period April 2003 to March 2006. Study completion date: 2006-05-03

EXECUTIVE SUMMARY

The aerobic/anaerobic biotransformation of BYI08330 (spirotetramat) in soil under dark laboratory conditions has been investigated in one sandy loam soil using [azaspirodecenyl-3-^{14}C]BYI08330 (label covering the most stable and representative part of the molecule). Samples were incubated for approx one half-life (i.e. 4.8 hours simulating the half-life in aerobic soil) under aerobic conditions in the dark at 20 °C and about 50 % of maximum water holding capacity. Following the short aerobic phase, the samples were flooded with oxygen-depleted de-ionized water (3 cm layer above soil level), set under nitrogen atmosphere, and maintained in the dark under anaerobic conditions for 180 days at 20 °C.

The complete material balance found at all sampling intervals demonstrated that no significant portion of radioactivity dissipated from the vessels or was lost during processing. Only little release of volatile radioactivity occurred in this study. It was found that the amount of test item BYI08330 in the entire system was quickly dropping to values of below 2 % AR within a week.

Based on the degradate profiles obtained within this study a degradation pathway was proposed which is almost identical to the pathway obtained in the aerobic degradation study for the test item. Based on these study results, it is concluded, that applied BYI08330 will not persist in a flooded anaerobic soil situation, and will not form degradates different to those observed in soil under aerobic conditions (for overall pathway of degradation in soil see Figure IIA 7.1-1).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat: Code = BYI08330
Label = [azaspirodecenyl-3-^{14}C]BYI08330 (sample ID: BECH 0755)
Specific activity 3.71 MBq/mg (100.2 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)
Identity and purity of test item in the application solution were checked

2. Soil: The aerobic/anaerobic biotransformation of BYI08330 was studied in soil "[redacted], Field Plot AIIIa" representing a light-textured agricultural soil of European origin. The soil was taken freshly from the respective field. Stones and plant material were removed, and soil moisture was partially reduced by soil spreading at ambient temperature to allow for sieving to a particle size of 2 mm. Finally, the soil batch was mixed thoroughly for optimal batch homogeneity.

Table IIA 7.1.2-1: Soil Physicochemical Properties (MEF-05/515)

Designation	Source	Soil Type (USDA)	pH in 0.01 M ($CaCl_2$) (1:5)	Organic Carbon [%]	Texture Analysis [% sand/silt/clay]
[redacted]a Batch #030515	[redacted] Germany	Sandy loam	6.8	0.4	52.45 / 30.90 / 16.65

Cation exchange capacity (CEC): 4.8 meq/100 g DM.
Measurement of initial soil biomass (mg microbial C/kg soil DM) indicated that the soil was aerobic viable at the beginning of the study. Anaerobic bacteria plate count tests indicated that the soil was anaerobic viable throughout the anaerobic incubation phase.
The selected soil has been used in several environmental fate studies and meets the guidelines' requirements.

B. STUDY DESIGN

1. Experimental conditions: The study was performed in static incubation test systems held for approx one half-life of test item under aerobic conditions in the dark at 20 ±1 °C. Thereby, the test system consisted of Erlenmeyer flasks (300 mL) attached with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds.

Aliquots corresponding to 100 g of dry soil were weighed into the test flasks. Replicates were set up for each sampling interval. [Azaspirodecenyl-3-^{14}C]BYI08330, a radiolabel covering the most stable part of the molecule, was applied at a rate of 80.43 µg/100 g soil (dry matter). Assuming homogeneous distribution in 2.5 cm topsoil layer, this rate was equivalent to 105% of the intended maximum field application rate of 288 g/ha. Identity verification of the test item in application solution was accomplished by NMR, LC-MS and LC-MS/MS analysis. Moisture adjustment after application to 50% of maximum water holding capacity was carried out for each individual flask by addition of de-ionized water.

Following the short aerobic incubation phase (just 4.8 hours), the samples were flooded with oxygen-depleted de-ionized water (3 cm layer above soil level), set under nitrogen atmosphere, and maintained in the dark under anaerobic conditions for 180 days at 20 °C. At start of the anaerobic study phase, the trap systems were replaced by sealable two-valve glass stoppers connected with plastic gas sampling bags, closing the flasks to maintain their nitrogen atmosphere.

2. Sampling: Microbial biomass was determined from treated and non-treated soil samples at the beginning of the aerobic phase. Anaerobic bacteria plate count tests were performed throughout the anaerobic incubation phase. Duplicate test flasks were processed for analysis after 4.8 hours of aerobic incubation, and at 0, 0.6, 1, 4, 6, 14, 32, 60, 90, 120 and 180 days after flooding the test systems (anaerobic incubation). Soil and water layer were separated by decanting, to allow for individual analysis. The water layer was analyzed directly, without extraction. The soil was extracted two times with a mixture of acetonitrile/water/formic acid (50/50/0.5, v/v/v) and once with acetonitrile (combined as "organic extract") and twice with acetonitrile/1 M HCl (1/1, v/v; combined as "acid extract"). All extractions were performed at room temperature.

3. Description of analytical procedures: BYI08330 residues in water layers and soil extracts were concentrated and subsequently assayed by reversed phase HPLC with flow-through radioactivity detection. Identification of BYI08330 residues and of its metabolites was achieved by co-elution with certified reference items and by LC-MS and LC-MS/MS of eluted fractions from the HPLC. A limit of quantification (LOQ) of ≤ 0.19 % AR for the entire system was calculated for HPLC flow-through radioactivity detection within the sample matrix.

II. RESULTS AND DISCUSSION

A. DATA

The respective data for all sampling intervals are shown in Table IIA 7.1.2-2.

B. MASS BALANCE

During the study the total recovery of radioactivity (RA) in individual test flasks ranged from 95.6 to 102.8% of AR (mean 99.3%; ±1.7%). A decreasing trend with the incubation time was not observed. The complete material balance found at all sampling intervals demonstrated that no significant portion of radioactivity dissipated from the test systems or was lost during processing.

C. BOUND AND EXTRACTABLE RESIDUES

In the aerobic incubation phase (just 0.2 days long), non-extractable residues (NER) in soil increased quickly from 1.7% of AR at test start to 11.7% of AR. During the subsequent anaerobic incubation, NER quickly increased further to 17.5% of AR at DAT-0.6 and then slowly decreased throughout the study to 7.9% of AR at end of study (mean values).

The total RA extractable from the soil decreased with incubation interval from 99.8% of AR (day of application) to 32.1% of AR at the end of the study. In contrast, the portion of total RA present in the water layer increased from 24.6% of AR on the day of flooding to a percentage of 58.7% of AR at end of study (mean values).

D. VOLATILIZATION

Only minimal release of volatile radioactivity occurred in this study. $^{14}CO_2$ during the 0.2 days of aerobic incubation accounted for 0.1% of AR at maximum. Mineralization to $^{14}CO_2$ furthermore was low after flooding of the soil during the anaerobic incubation phase, with a maximum of 0.3% of AR at DAT-90 (90 days after flooding, single value). Organic volatiles were not observed in the aerobic or in the anaerobic study phase (<0.1% of AR at all sampling intervals).

E. TRANSFORMATION OF TEST ITEM

The amounts of test item BYI08330 in the entire system were quickly dropping to values of below 2 % AR within one week (for synopsis of results see Table IIA 7.1.2-3, for further kinetics evaluation see section 7.2.4).

In the course of the study several HPLC peaks were detected and quantified besides unaltered BYI08330. The most prominent metabolite in both, soil and water layer, was BYI08330-enol increasing in the entire system until DAT-6 and then staying at a plateau of about 50% of AR until the end of the study. The levels of BYI08330-ketohydroxy detected in the entire system were steeply increasing to

19.3% of AR at DAT-1 and then decreased slowly to 7.7% of AR towards the end of the study (mean values).

BYI08330-MA-amide amounted to levels of up to 7.2% of AR in the entire system at the end of the study. Similarly high levels of up to 7.0% of AR (at DAT-6 and declining to 0.3% of AR at the end of the study) were reached by BYI08330-enol-dimer 1. BYI08330-enol-dimer 2 reached its highest levels of up to 4.6% of AR at the end of the study (mean values were given in all cases). BYI08330-di-hydroxy was detected at minor levels of up to 3.2% of AR and BYI08330-oxo-enol of up to 1.4% of AR (mean values were given in all cases).

Furthermore a metabolite designated R30 was detected with levels of 8.3% of AR at DAT-0.6 and of 4.7% of AR at DAT-1 and of 2.0% of AR at DAT-4. Afterwards it was detected with levels of below 1.0% of AR (mean values). Attempts to fully elucidate its structure by LC-MS, LC-MS/MS and NMR were not successful. From the results of the LC-MS/MS spectrometric analysis it could be concluded that the major component was an unknown enol based compound possibly originating from polymeric structures (like the enol-dimers).

The amount of unidentified radioactivity (individual regions of <3% of AR) reached mean values of up to of 6.0% of AR (DAT-180) in the water layer and up to 10.0% of AR (DAT-32) in the soil layer. In the entire system up to 14.9% of AR were left as unidentified RA consisting of about 28 different regions in the HPLC chromatogram. Largest individual unknown metabolites reached up to 1.8% of AR in the soil layer and 1.7% of AR in the water layer (single values).

Table IIA 7.1.2-2: Biotransformation of BYI08330 in sandy loam soil [redacted]a under aerobic#), then turning to anaerobic conditions (MEF-05/515)

Compound	Days After Treatment (DAT)											
	-0.2#)	0.0	0.6	1	4	6	14	32	60	90	120	180
BYI08330	95.5	59.0	9.4	6.7	3.2	1.4	1.6	1.5	1.5	0.9	1.0	<LOQ
Dihydroxy	<LOQ	<LOQ	0.1	0.6	1.1	1.0	2.0	3.2	2.7	3.2	3.1	2.7
MA-amide	<LOQ	0.8	3.6	4.9	3.0	2.1	1.8	3.9	4.5	5.3	6.5	7.2
Enol	1.8	8.5	26.7	32.5	38.0	45.3	51.6	40.4	46.6	50.1	47.3	54.6
Ketohydroxy	0.5	9.1	16.5	19.3	17.2	15.6	14.6	16.1	15.3	12.6	11.5	7.7
R30	<LOQ	0.5	8.3	4.7	2.0	0.7	0.4	0.5	0.2	0.5	0.3	0.8
Enol-Dimer 1	<LOQ	3.5	6.2	4.1	6.7	7.0	4.1	3.9	0.9	1.1	0.6	0.3
Enol-Dimer 2	<LOQ	1.0	2.8	0.9	1.3	2.3	3.1	4.0	3.1	3.4	4.1	4.6
Unidentified *)	2.0	2.9	7.5	7.9	8.5	8.3	8.3	14.9	13.5	10.2	13.7	13.0
Total in water & soil extracts	99.8	85.2	81.1	81.6	81.1	83.7	87.6	88.3	88.2	87.3	88.1	90.8
$^{14}CO_2$ total **)	N/A	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1
Volatile org.	N/A	N/A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unextractable	1.7	11.7	17.5	17.2	16.3	15.5	12.4	12.5	12.2	11.6	10.7	7.9
Total recovery	101.5	97.0	98.7	99.0	97.6	99.4	100.1	101.0	100.5	99.1	98.9	98.8

All figures are means of duplicates expressed as % of applied radioactivity (AR)
*: Up to 28 different regions in the HPLC chromatogram/DAT; maximum value for a single peak: 1.8% of AR.
**): Sum of $^{14}CO_2$ trapped from gas phase and portion dissolved in water

Table IIA 7.1.2-3: Synopsis of biotransformation of BYI08330 in sandy loam [redacted]a under short aerobic, then turning to anaerobic conditions (MEF-05/515).

	Data for the entire test system
Best fit: FOMC Model *): DT_{50} [days] DT_{90} [days] Chi^2 Error [%]	 0.06 1.33 5.27
Major transformation products **)	Enol Ketohydroxy Enol-Dimer 1 MA-Amide
Minor transformation products	Di-hydroxy Enol-Dimer 2 R30 (characterized as similar to enol-dimers) CO_2

*): Model input dataset was the abundance of residual BYI08330 found in the entire test system during the anaerobic study phase (starting from DAT-0) (single values). All data points were weighted equally. For optimal goodness of fit, the initial value was also allowed to be estimated by the model.

**): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs

III CONCLUSIONS

Based on the degradate profiles obtained within this anaerobic soil study, a degradation pathway was proposed which is almost identical to the degradation pathway obtained in aerobic soil. Thus, the results were included in the proposed overall pathway of degradation of spirotetramat in soil (see Figure IIA 7.1-1).

It is concluded that BYI08330 applied to soil will be degraded rapidly in a subsequently flooded anaerobic soil situation, and will not form degradates different from those observed in soil under aerobic conditions, and/or known from abiotic hydrolysis experiments. Table IIA 7.1.2-3 gives a synopsis of results.

IIA 7.1.3 Soil photolysis

Report:	**KIIA 7.1.3/01, [redacted], 2005 (MEF-04/481)**
Title:	**[Azaspirodecenyl-3-^{14}C]- and [Azaspirodecenyl-5-^{14}C]BYI08330: Phototransformation on Soil**
Report No & Document No:	**MEF-04/481 M-252907-01-2**
Guidelines:	Commission Directive 95/36/EC amending Council 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
GLP:	Fully GLP compliant - laboratory certified by German “Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen”.
Testing Laboratory and Dates:	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of March 2003 to March 2004. Study completion date: 2005-04-25

EXECUTIVE SUMMARY

The phototransformation of [azaspirodecenyl-3-^{14}C]- and -[azaspirodecenyl-5-^{14}C]-BYI08330 (labels #1 and #2) was studied on sandy loam soil (pH 5.4, organic carbon 0.93%) for seven days at 20 ± 1 °C and at a moisture of 75% of 1/3-bar water holding capacity.

BYI08330 was applied directly to the soil at an initial concentration of about 2 mg/kg soil (DM), equivalent to a single maximum use rate of 288 g/ha (calculation based on homogeneous distribution for 1 cm soil depth). The treated samples were continuously exposed to artificial irradiation (xenon lamp with <290 nm cut-off filter). In addition, dark controls were set up. Test vessels were connected to traps for the collection of CO_2 and organic volatiles. Samples were taken and processed at 0, 0.2, 1, 2, 3, 4, and 7 days for the determination of the parent compound and transformation products. The soil samples were extracted three times with acidic acetonitrile/water, two times with acetonitrile/1 M aqueous hydrochloric acid (1/1, v/v), and once with acetonitrile. The BYI08330 residues were analyzed by reversed phase HPLC with radioactivity detection. Identification and confirmation of the parent compound and transformation products was done by HPLC-MS, HPLC-MS/MS and/or co-chromatography.

Using label #1 the material balance was 101.7 and 99.1 % in the dark and irradiated soil samples, respectively. The mass balance for label #2 was 103.1 and 100.5 %.

The degradation in dark test systems was about four times faster than in irradiated test systems, indicating decreased biological activity in irradiated systems. This may also be a reason for the higher levels of products observed in the irradiated systems, since they were not further biodegraded as usual. Based on the experimental DT50 of 5.0 and 2.4 days of BYI08330 for the irradiated label #1 and #2 test systems, the mean DT50 of BYI08330 under environmental conditions is calculated to be 19.8 solar summer days at [redacted], Arizona, USA or 30.8 solar summer days at [redacted], Greece. Whereas in the dark, the DT50 was found to be equal or lower than 1.2 days, only.

Considering both labels #1 and #2, three major transformation product were found in the irradiated soil samples, *i.e.* BYI08330-enol, BYI08330-ketohydroxy and BYI08330-methoxy cyclohexanone. Minor

transformation products identified were BYI08330-benzoic acid, BYI08330-glyoxylic amide, and BYI08330-enol dimer. At study termination, the CO_2 amounted to 3.8% and 7.3% of AR, for label #1 and label #2 test systems, respectively. In the dark samples, the CO_2 amounted to 1.6% and 1.4% of AR, only. Organic volatile formation was negligible throughout the study (<1%). Non-extractable ^{14}C-residues increased to maximum 12.1% and 30.9% of AR at study termination for the irradiated and the dark samples, respectively.

In order to cover requirements written in the current draft OECD TG supplementary experiments (day 0 and day 7 sampling only) were conducted with a drier test soil and with one soil from another origin ([redacted]) to identify moisture and/or soil-specific effects. In these tests the route of BYI08330 degradation was similar, and it was found that degradation is slower in dried soil.

A transformation pathway of BYI08330 under the influence of simulated sunlight on soil is proposed, which is included in Figure IIA 7.1-1. However, a distinct phototransformation product is not to be expected after the use of spirotetramat under outdoor conditions.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat: Code = BYI08330
Identity and purity of test item in the application solutions were checked

Label #1: Label position = [azaspirodecenyl-3-^{14}C]BYI08330 (sample ID: BECH 1518)
Specific activity 3.71 MBq/mg (100.2 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

Label #2: Label position = [azaspirodecenyl-5-^{14}C] (sample ID: BECH 1519)
Specific activity 4.03 MBq/mg (108.8 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Chemical purity: >98% (HPLC, UV detection at 210 nm)

2. Soil: The phototransformation of BYI08330 was studied in a sandy loam soil collected from the Bayer CropScience Southern Field Technology Station, field plot V, located in [redacted], Florida, USA. It was soil from the same location as used for the other studies before (see Table IIA 7.1.3-1). Once received at the test facility, the soil was stored in a refrigerator until study commencement. Aliquots of soil sieved through 2 mm sieve were taken for pre- and main tests. While the pre-tests were being conducted, the aliquot for the main test again was stored in climatic chamber until being needed for the study. Prior to treatment with the test item, the soil moisture was determined. The soil was then acclimatized for about 30 min. to the study temperature, and weighed on a dry-weight basis for individual test systems. In addition, a supportive experiment was conducted with a second soil (([redacted], see Table IIA 7.1.3-1) in order to cover requests from the OECD guideline proposal.

Table IIA 7.1.3-1: Soil physicochemical properties (MEF-04/481)

Designation	Source	Soil Type (USDA)	pH ($CaCl_2$)	Organic Carbon [%]	Texture Analysis [% sand/silt/clay]
[redacted] (used for main test)	[redacted], Florida, USA	sandy loam	5.4	0.93	77.3 / 12.7 / 10.0
[redacted] (used for supportive test)	[redacted] Germany	silt loam	6.5	0.83	36.9 / 51.1 / 12.0

Cation exchange capacity (CEC) ranged between 6 to 15 meq/100 g DM.
Measurement of initial & final soil biomass (mg microbial C/kg soil DM) indicated that the soils were viable throughout the study.
The selected soils have been used in several environmental fate studies and meet the guidelines' requirements.

B. STUDY DESIGN

1. Experimental conditions: The tests were performed using individual static test systems held at aerobic conditions at 20 ±1 °C for a maximum period of 7 days. They consisted of glass flasks (approx. 10.2 cm² irradiated area) filled with 3 g dry weight of viable soil/replicate, closed by a quartz glass cover and attached with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds.
BYI08330 dissolved in 100 µL of acetonitrile/water (1:1; v:v) was applied directly to the surface of the soil using a variable Eppendorf pipette at an initial concentration of about 2 µg/g soil, equivalent to a single maximum use rate of 288 g./ha (calculation based on homogeneous distribution for 1 cm soil depth). The co-solvent was allowed to evaporate for 3-5 min. Moisture adjustment after application to 75% of 1/3 bar moisture was carried out for each individual flask by addition of deionized water, and the vessel initial weights were recorded in order to maintain the soil moisture.

Treated samples were either incubated in the dark as controls or continuously exposed to artificial irradiation (xenon lamp with <290 nm cut-off filter. The experimental light intensity (1115 W/m² and 1132 W/m² for labels #1 and #2) of continuous irradiation was in a way that 7 days of irradiation is equivalent to 37 solar days in June under extreme sunlight conditions at [redacted], AZ (USA) or to 59 days in June under extreme European conditions in Greece.

2. Sampling: Entire test flasks were taken at 0, 0.2, 1, 2, 3, 4, and 7 days for the determination of the parent compound and transformation products. For the supplemental experiment with the dry soil variant and the second soil ([redacted]) samplings were conducted at DAT-0 and DAT-7, only.

3. Description of analytical procedures: The 3-g dry (DM) soil of each sampling interval was extracted using an ambient procedure by shaking at room temperature. They were extracted three times with acetonitrile/water (1/1, v/v + 0.5% formic acid), two times with acetonitrile / 1 M aqueous hydrochloric acid (1/1, v/v), and once with acetonitrile. The BYI08330-residues were radio-assayed by LSC and analyzed separately in the combined normal and combined acidic extracts by HPLC on reversed phase (C-18) with radioactivity detection. Solid samples (i.e. soil and paper filters) were combusted and ^{14}C levels were measured using LSC. Identification and confirmation of the parent compound and transformation products was done by HPLC-MS, HPLC-MS/MS and/or co-chromatography.

C. Determination of Degradation Kinetics

A simple first-order (SFO) degradation rate constant (k) was determined by the software program (ModelManager® 1.1) using a nonlinear optimization method. The percentage of AR as BYI08330 was plotted against time. The equation for the simple first-order degradation relationship is:

$$C_t = C_0 \times e^{-kt}$$

Where C_0 and C_t are the BYI08330 concentrations at time 0 and t (days), respectively. Based on the above relationship the DT_{50} (or $T_{1/2}$) and DT_{90} (or $T_{1/10}$) values, in days, were calculated as follows:

$$T_{1/2} = \ln (0.5)/-k$$

$$T_{1/10} = \ln (0.1)/-k$$

II. RESULTS AND DISCUSSION

A. DATA

The data for the irradiated test systems and the dark controls are shown in Table IIA 7.1.3-2 to Table IIA 7.1.3-5.

B. MASS BALANCE

For irradiated test systems, the average material balance ranged from 95.2 to 101.7% (label #1) and from 95.9 to 104.7% (label #2) of the AR, respectively, with an overall mean ± standard deviation of 99.1 ± 2.1% for label #1 and 100.5 ± 3.0% for label #2.

For dark test systems, the average material balances ranged from 99.6 to 103.4% and from 101.6 to 104.5% of the AR respectively for label #1 and label #2.

C. BOUND AND EXTRACTABLE RESIDUES

For irradiated test systems label #1 and #2, the extractable radioactivity decreased from an average of 99.5%/103.0% of the AR at day 0 to 81.7%/76.5% by day 7, respectively. Not extractable (bound) residues) NER increased from 0.1%/0.1% of the AR at day 0 to 9.6%/12.1% of the AR at the end of the test, respectively.

For dark test systems label #1 and #2, the extractable radioactivity decreased from an average of 99.5%/103.0% of the AR at day 0 to 67.4%/73.7% by day 7, respectively. NER increased from 0.1%/0.1% of the AR at day 0 to 30.9%/27.5% of the AR at the end of the test, respectively.

D. VOLATILIZATION

In the irradiated test systems $^{14}CO_2$ formation increased up to 3.8%/7.3% by day 7, respectively. In the dark controls $^{14}CO_2$ formation increased up to 1.6%/1.4% by day 7, respectively.

Organic volatile formation was negligible throughout the study (<1%).

Table IIA 7.1.3-2: Phototransformation of BYI08330 in sandy loam [redacted], mean values of radiolabel #1 expressed as % of AR (MEF-04/481)

Compound	Days After Treatment (DAT)						
	0	0.2	1	2	3	4	7
BYI08330 (t.i.)	98.3	94.6	84.2	74.8	68.7	53.7	35.5
BYI08330-enol	<0.1	1.5	3.8	2.6	1.6	2.0	5.3
- Enol-Dimer 1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- Benzoic acid	<0.1	<0.1	1.4	2.7	3.5	4.0	4.8
- Glyoxylic amide	<0.1	<0.1	<0.1	<0.1	0.9	0.7	1.1
- Ketohydroxy	<0.1	1.3	5.8	8.8	8.4	11.4	12.3
- ROI 3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- ROI 8	<0.1	<0.1	<0.1	1.3	<0.1	3.6	<0.1
- ROI 9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	4.0
- ROI 10	<0.1	<0.1	0.7	2.6	2.4	3.2	4.0
- ROI 11	<0.1	<0.1	<0.1	<0.1	<0.1	3.9	4.2
Unidentified diffuse RA	<0.1	<0.1	<0.1	<0.1	2.9	4.0	8.6
Total extracted *	99.5	97.7	96.2	93.3	88.8	87.4	81.7
$^{14}CO_2$	n.a.	<0.1	0.1	0.3	1.0	2.1	3.8
Volatile org.	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unextractable	0.1	3.0	5.3	6.9	8.0	8.4	9.6
Total RA recovery	99.6	100.7	101.7	100.6	97.8	97.9	95.2

Radiolabel #1 = cis-[azaspirodecenyl-3-^{14}C]BYI08330
ROI #: Defined region of interest (peak zone) set for radio-detection
*: Including the final ACN extract that was not further considered for analyses due to its low RA content

Table IIA 7.1.3-3: Phototransformation of BYI08330 in sandy loam [redacted], mean values of radiolabel #2 expressed as % of AR (MEF-04/481)

Compound	Days After Treatment (DAT)						
	0	0.2	1	2	3	4	7
BYI08330 (t.i.)	101.4	82.1	57.3	37.7	35.9	33.2	30.8
BYI08330-enol	<0.1	10.0	10.1	8.3	7.9	3.4	4.0
- Enol-Dimer 1	<0.1	<0.1	1.6	1.9	<0.1	<0.1	0.8
- Glyoxylic amide	<0.1	0.7	3.0	4.1	3.5	2.2	3.4
- Ketohydroxy	<0.1	6.0	16.6	20.9	19.5	19.1	16.7
- Methoxy cyclohexanone	<0.1	<0.1	6.1	10.0	8.8	8.1	6.0
- ROI 1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- ROI 11	<0.1	<0.1	<0.1	0.7	1.3	<0.1	<0.1
- ROI 13	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- ROI 15	<0.1	<0.1	<0.1	0.8	2.3	3.5	3.3
- ROI 16	<0.1	<0.1	<0.1	2.5	2.8	5.6	2.9
- ROI 17	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.9
- ROI 18	<0.1	<0.1	<0.1	<0.1	3.6	4.7	4.0
Unidentified diffuse RA	<0.1	<0.1	<0.1	<0.1	<0.1	0.6	2.7
Total extracted *	103.0	99.5	95.2	87.6	86.5	81.4	76.5
$^{14}CO_2$	n.a.	<0.1	0.6	2.3	3.2	5.3	7.3
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unextractable	0.1	5.2	7.4	9.3	9.8	10.9	12.1
Total RA recovery	103.1	104.7	103.2	99.2	99.5	97.6	95.9

Radiolabel #2 = cis-[azaspirodecenyl-5-^{14}C]BYI08330
ROI #: Defined region of interest (peak zone) set for radio-detection
*: Including the final ACN extract that was not further considered for analyses due to its low RA content

Table IIA 7.1.3-4: Biotransformation of BYI08330 in dark controls of sandy loam [redacted], mean values of radiolabel #1 expressed as % of AR (MEF-04/481)

Compound	Days After Treatment (DAT)						
	0	0.2	1	2	3	4	7
BYI08330 (t.i.)	98.3	92.4	53.2	26.7	18.1	14.0	9.4
BYI08330-enol	<0.1	1.9	12.3	19.8	18.3	15.8	14.2
- Enol-Dimer 1	<0.1	<0.1	2.2	3.4	4.1	5.4	4.6
- Benzoic acid	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- glyoxylic amide	<0.1	<0.1	0.7	2.1	2.1	0.8	<0.1
- Ketohydroxy	<0.1	2.1	11.3	18.9	22.6	29.5	33.0
- ROI 3	<0.1	<0.1	<0.1	<0.1	0.3	0.8	1.9
- ROI 8	<0.1	<0.1	<0.1	0.5	<0.1	0.1	<0.1
- ROI 9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- ROI 10	<0.1	<0.1	<0.1	1.3	1.8	2.5	3.0
- ROI 11	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unidentified diffuse RA	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total extracted *	99.5	96.7	80.7	73.9	68.0	70.2	67.4
$^{14}CO_2$	n.a.	<0.1	0.3	0.5	1.0	1.0	1.6
Volatile org.	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unextractable	0.1	6.7	21.8	27.8	32.8	30.9	30.9
Total RA recovery	99.6	103.4	102.8	102.1	101.8	102.1	99.9

Radiolabel #1 = cis-[azaspirodecenyl-3-^{14}C]BYI08330
ROI #: Defined region of interest (peak zone) set for radio-detection
*: including the final ACN extract that was not further considered for analyses due to its low RA content

Table IIA 7.1.3-5: Biotransformation of BYI08330 in sandy loam [redacted], mean values of radiolabel #2 expressed as % of AR (MEF-04/481)

Compound	Days After Treatment (DAT)						
	0	0.2	1	2	3	4	7
BYI08330 (t.i.)	101.4	76.6	24.3	14.8	10.2	8.6	7.2
BYI08330-enol	<0.1	11.2	14.2	14.6	15.5	16.6	12.5
- Enol-Dimer 1	<0.1	1.0	6.3	7.8	8.6	6.5	8.0
- Glyoxylic amide	<0.1	<0.1	3.1	2.8	2.2	2.3	1.0
- Ketohydroxy	<0.1	5.2	24.8	29.2	30.1	32.6	33.9
- Methoxy cyclohexanone	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- ROI 1	<0.1	<0.1	2.4	2.9	2.5	2.6	2.6
- ROI 11	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- ROI 13	<0.1	<0.1	<0.1	<0.1	2.0	2.1	3.8
- ROI 15	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- ROI 16	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- ROI 17	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
- ROI 18	<0.1	<0.1	<0.1	<0.1	1.6	2.8	3.3
Unidentified diffuse RA*	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total extracted **	103.0	95.1	76.6	73.7	74.1	75.5	73.7
$^{14}CO_2$	n.a.	<0.1	0.3	0.7	0.9	1.0	1.4
Volatile org.	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Unextractable	0.1	9.1	25.8	27.3	27.9	28.0	27.5
Total RA recovery	103.1	104.2	102.6	101.6	103.0	104.5	102.7

Radiolabel #2 = cis-[azaspirodecenyl-5-^{14}C]BYI08330
ROI #: Defined region of interest (peak zone) set for radio-detection
*: Including the final ACN extract that was not further considered for analyses due to its low RA content

E. TRANSFORMATION OF TEST ITEM

The parent compound was quickly degraded (for synopsis of results see Table IIA 7.1.3-6). However, the biotransformation in the dark controls was approx. four times faster compared to the irradiated soil samples, indicating a decreased biological activity caused by a kind of photo-sterilizing effect. This may also be a reason for higher levels of intermediates observed in the irradiated systems. Based on the experimental DT50 of 5.0 and 2.4 days of BYI08330 for the irradiated label #1 and #2 test systems, the mean DT50 of BYI08330 under environmental conditions is calculated to be 19.8 solar summer days at [redacted], Arizona, USA or 30.8 solar summer days at [redacted], Greece. Whereas in the dark, the DT50 was found to be equal or lower than 1.2 days, only.
During the course of the study a large number of degradates was observed in the irradiated soil samples as well as the dark controls. Considering both labels #1 and #2, three major transformation product were found in the irradiated soil samples, *i.e.* BYI08330-enol, BYI08330-ketohydroxy and BYI08330-methoxy cyclohexanone. Minor transformation products identified were BYI08330-benzoic acid, BYI08330-glyoxylic amide and BYI08330-enol dimer. The before-mentioned results were included in the proposed overall pathway of degradation of spirotetramat in soil shown in Figure IIA 7.1-1.

In order to cover requirements written in the current draft OECD TG supplementary experiments (day 0 and day 7 sampling only) were conducted with dried test soil variant and one soil from another origin ([redacted]) to identify moisture and/or soil-specific effects. In these tests the route of BYI08330 degradation was similar, and it was found that degradation is more slowly in dried soil.

Table IIA 7.1.3-6: Synopsis of transformation of BYI08330 on sandy loam [redacted] (MEF-04/481)

Soil	[Azaspirodecenyl-3-^{14}C] BYI08330 (label #1)		[Azaspirodecenyl-5-^{14}C] BYI08330 (label #2)	
Soil type	Irradiated	Dark	Irradiated	Dark
k (1/d)	0.139	0.588	0.285	1.20
Experimental 1st order DT_{50} [d] of BYI08330	5.0	1.2	2.4	0.6
Experimental 1st order DT_{90} [d] of BYI08330	16.6	3.9	8.1	1.9
R^2	0.991	0.986	0.830	0.973
Environmental DT_{50} [d] in June at [redacted], Greece	41.6	N/A	20	N/A
Major transformation products *)	Ketohydroxy	Enol, Ketohydroxy	Enol, Ketohydroxy, Methoxy cyclohexanone	Enol, Ketohydroxy
Minor transformation products	Enol Benzoic acid Glyoxylic amide	Enol-Dimer Glyoxylic amide	Enol-Dimer Glyoxylic amide	Enol-Dimer Glyoxylic amide

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs

III CONCLUSIONS

The parent compound was well degraded on irradiated soil samples. However, the biotransformation in the dark controls was approx. four times faster and, considering real environmental conditions (e.g. in June at [redacted], Greece) even approx. 20 times faster compared to soil samples irradiated by natural sunlight. This kinetics results together with the findings that the pathway of degradation was similar

indicate that a distinct phototransformation product is not to be expected in soil after the use of spirotetramat under outdoor conditions. For the proposed overall pathway of degradation of spirotetramat in soil see Figure IIA 7.1-1.

Report:	**KIIA 7.1.3/02, [redacted], 2008 (MEF-08/233)**
Title:	**[Azaspirodecenyl-3-^{14}C]- and [Azaspirodecenyl-5-^{14}C]BYI08330: Phototransformation on a Sterile Soil**
Report No & Document No:	**MEF-08/233 (amended 2008-09-08) M-306375-02-1**
Guidelines:	Pesticide Assessment Guidelines, Subdivision N, Environmental Fate, US EPA, 161-3: Photodegradation Studies on Soil, 1982 Environmental Chemistry and Fate, Guidelines for Registration of Pesticides in Canada, 1987
GLP:	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates:	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of April to June 2008. Study completion date: 2008-08-26

EXECUTIVE SUMMARY

The phototransformation of [azaspirodecenyl-3-^{14}C]- and -[azaspirodecenyl-5-^{14}C]-BYI08330 (referred to as labels #1 or #2, respectively) was studied on a sterile sandy loam soil (pH 6.0, organic carbon 0.7%) for seven days at 20 ± 1 °C and at a moisture of approx. 75% of 1/3-bar water holding capacity.

BYI08330 was applied directly to the sterile soil at an initial concentration of about 2 mg/kg soil (DM), equivalent to a single maximum use rate of 288 g/ha (calculation based on homogeneous distribution for 1 cm soil depth). The treated samples were continuously exposed to artificial irradiation (xenon lamp with <290 nm cut-off filter). In addition, dark controls were set up. Test vessels were connected to traps for the collection of CO_2 and organic volatiles. Samples were taken and processed at 0, 0.25, 1, 2, 3, 4, and 7 days for the determination of the parent compound and transformation products. The soil samples were extracted three times with acidic acetonitrile/water, two times with acetonitrile/1 N aqueous hydrochloric acid (1/1, v/v), and once with acetonitrile. The BYI08330 residues were analyzed by reversed phase HPLC with radioactivity detection. Identification and confirmation of the parent compound and transformation products was done by HPLC-MS, HPLC-MS/MS and/or co-chromatography.

The mass balance was 103.0 ± 0.9% and 101.6 ± 1.8% of the applied radioactivity (AR) in the dark and irradiated label #1 soil samples, respectively. For label #2, the mass balances were 102.7 ± 2.6% and 101.6 ± 2.5% of the AR.

In general the non-extractable residues in the dark samples were less than 0.5% of the AR and less than 5% in the irradiated samples at test termination for both radiolabels. With respect to the low bound residues formed here compared to viable soil samples (see study KIIA 7.1.3/01), it can be concluded that bound residues of spirotetramat were formed exclusively by irreversible nature.

The experimental first order DT50 of BYI08330 (#1) in the dark and irradiated samples were 5.2 and 12.0 days, respectively. The experimental first order DT50 of BYI08330 (#2) in the dark and irradiated samples were 4.9 and 7.1 days, respectively.
Based on the experimental DT50 of 12.0 and 7.1 days of BYI08330 for the label #1 and #2 test systems,

respectively, the DT50 of BYI08330 under environmental conditions is calculated to be 60 and 35.5 solar summer days at [redacted], Arizona, USA.

Irradiating label #1 one major transformation product was detected, BYI08330-ketohydroxy at a maximum of 10.9% of the AR at day 4. One minor transformation product identified was BYI08330-enol, which was formed at a maximum of 6.3% of the AR at day 2. Irradiating label #2 two major and one minor transformation products were detected, BYI08330-ketohydroxy, BYI08330-methoxy cyclohexanone and BYI08330-enol at a maximum of 20.6% and 16.7% of the AR both at the end of the study and 5.0% of the AR at day 2, respectively. Contrary to the non-sterile soil study [KIIA 7.1.3/01] not any dimers were formed, which indicates that their formation is microbially controlled. In irradiated soil samples, at study termination, the CO_2 amounted to 0.8% and 0.9% of the AR, for label #1 and label #2 test systems, respectively. Organic volatile formation was negligible throughout the study (≤ 0.1% of the AR)

In the dark label #1 test systems, one major transformation product was detected, BYI08330-enol at a maximum of 59.2% of the AR by the end of the study. In the dark label #2 test systems also just one major transformation product was detected, BYI08330-enol at a maximum of 61.8% of the AR by the end of the study. As already observed in the irradiated samples, also in the dark controls not any dimers were formed. At study termination, the CO_2 in the dark samples were ≤0.1% of the AR for label #1 and label #2 test systems. Organic volatile formation was negligible throughout the study (≤0.1% of the AR).

This kinetics results in conjunction with the findings that the pathway of degradation was similar as already proposed in study KIIA 7.1.3/01 indicate that phototransformation in soil is not of importance for the degradation of Spirotetramat under outdoor conditions. A transformation pathway of Spirotetramat under the influence of simulated sunlight on soil is proposed, which is included in Figure IIA 7.1-1. However, a distinct phototransformation product is not to be expected after the use of Spirotetramat under outdoor conditions.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat; Code = BYI08330
Identity and purity of test item in the application solutions were checked

Label #1: Label position = [azaspirodecenyl-3-^{14}C]BYI08330 (sample ID: KATH 6558)
Specific activity 3.67 MBq/mg (100 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

Label #2: Label position = [azaspirodecenyl-5-^{14}C] (sample ID: KATH 6559)
Specific activity 4.03 MBq/mg (109 µCi/mg)
Radiochemical purity: >98 and 99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

2. Soil: The phototransformation of BYI08330 was studied in a sandy loam soil collected from the Bayer CropScience Southern Field Technology Station, field plot V, located in [redacted], Florida, USA. It was soil from the same location as used for the other studies before (see Table IIA 7.1.3-7). Once received at the test facility, the soil was stored in a refrigerator until study commencement.
The soil was sterilized at the beginning of the study on 2008-04-14. The test soil was sieved under moist conditions using a 2-mm mesh sieve and then sterilized. In a non-GLP pre-test, the following sterilization methods were tested:

a) Chemical ($HgCl_2$) sterilization: 100 g bulk test soil and 100 mL aqueous $HgCl_2$ solution (100 mg/L) were agitated for 48 h, centrifuged and the supernatant was decanted. The soil was dried for 2 days and the moisture was adjusted by adding sterile filter water (mesh 0.2 µm) on a sterile bench.

b) Physical sterilization (autoclave). 3 g of test soil were weighted into the test vessels and the moisture was adjusted by adding pure water. The vessels were covered by quartz and alumina foil and treated in an autoclave at 120°C for 20 min including heating and cooling phase of 1.5 h. After three days this procedure was repeated.

According to the pre-test, physical sterilization b) was found to be exhaustive and reliable, whereas sterilization by $HgCl_2$ was found to be less effective and incomplete. Moreover, the effect of $HgCl_2$ during photolysis is not known. Thus, physical sterilization was preferred for this type of study, although it may affect the physical and chemical properties of the soil. Note: a phototransformation study is not a "mobility" study and therefore changes of the physical and chemical properties of the soil may not affect the results.

Table IIA 7.1.3-7: Soil physicochemical properties (MEF-08/233)

Designation	Source	Soil Type (USDA)	pH ($CaCl_2$)	Organic Carbon [%]	Texture Analysis [% sand/silt/clay]
[redacted]	[redacted], Florida, USA	sandy loam	6.0	0.7	77 / 14 / 9

Cation exchange capacity (CEC) was 4.7 meq/100 g DM.
Measurement of initial & final soil biomass (mg microbial C/kg soil DM) indicated that the soil was not viable after the sterilization throughout the study.
The selected soil has been used also in study KIIA 7.1.3/01.

B. STUDY DESIGN

1. Experimental conditions: The tests were performed using individual static test systems held at aerobic conditions at 20 ± 1 °C for a maximum period of 7 days. They consisted of glass flasks (approx. 10.2 cm² irradiated area) filled with 3 g dry weight of sterile soil/replicate, closed by a quartz glass cover and attached with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds.
BYI08330 dissolved in 100 µL of acetonitrile/water (1:1, v:v) was applied directly to the surface of the soil using a variable Eppendorf pipette at an initial concentration of about 2 µg/g soil, equivalent to a single maximum use rate of 288 g./ha (calculation based on homogeneous distribution for 1 cm soil depth). The co-solvent was allowed to evaporate for 3-5 min. Moisture adjustment after application to 75% of 1/3 bar moisture was carried out for each individual flask by addition of deionized water, and the vessel initial weights were recorded in order to maintain the soil moisture.

Treated samples were either incubated in the dark as controls or continuously exposed to artificial irradiation (xenon lamp with <290 nm cut-off filter. The experimental light intensity (1054 W/m² and 1053 W/m² for labels #1 and #2) of continuous irradiation was in a way that 7 days of irradiation is equivalent to 35 solar days in June under extreme sunlight conditions at [redacted], AZ (USA) or to 53 days in June under extreme European conditions in Greece.

2. Sampling: Entire test flasks were taken at 0, 0.25, 1, 2, 3, 4, and 7 days for the determination of the parent compound and transformation products.

3. Description of analytical procedures: The extraction scheme was used to be consistent with earlier

experiment to ensure stability of the parent compound. The soil samples were extracted immediately after sampling. The extraction solvent A (5 mL, acetonitrile/water 1/1, v/v + 0.5% formic acid) was used to transfer the total amount of the soil sample into a 42-mL Teflon® centrifuge flask. The soil was extracted three times by 5 mL extraction solvent A, two times by 5 mL extraction solvent B (acetonitrile/1 N aqueous HCl 1/1, v/v), and finally by single extraction using 5 mL extraction solvent C (acetonitrile). The extraction was performed by a mechanical shaker at ambient temperature for 30 min each. Each extraction step was followed by centrifugation at 8000 rpm (DuPont Sorvall® RC-5B) for 15 min and by decanting the supernatant. The resulting extracts of extraction step 1-3 (extraction solvent A) were combined as organic extract. The resulting supernatants of extraction step 4-5 (extraction solvent B) were combined as acidic extract. The volume and the RA of the combined extracts A to C were determined and the samples were radioassayed by triplicate 100-µL aliquots. The volumes of the extracts A and B were vacuum-concentrated at ambient temperature (Speed Vac®) to about the half volume and again the samples were radioassayed by triplicate 100-µL aliquots. Extract C was not processed further, because the radioactivity was <3% of the AR.

Chromatographic analyses by the primary method (HPLC-RA detector) and confirmatory method (TLC) were performed within one day after preparation. Analysed extracts, the extracted soils and other samples were stored deep-frozen at approximately -30°C until further investigations.

The extracted soil was air dried in a laboratory hood and the NER were quantified by combustion, but not characterized.

If labelled and/or non-labelled reference standards were available, identification of transformation products was performed either by co-injecting the sample extracts with the non-radioactive standards and comparing the HPLC retention time of the cold standards (UV trace) to that of the sample extracts (^{14}C trace) and confirmation by TLC co-chromatography with the non-radioactive standards or by co-injecting the sample extract with a HPLC split of a known radiolabelled compound from another study and comparing the HPLC retention time.

C. Determination of Degradation Kinetics

The data for the test item were evaluated according to Focus Kinetics using the software KinGUI. The initial concentration at day 0 was included in the parameter optimisation procedure. Based on the chi^2 (X^2) error criterion and visual assessment the best fit kinetic model was chosen for the disappearance time evaluation.

For the determination of the degradation kinetics following procedure was followed:

- Values < LOD were set to 0.5 LOD for samples after or before a value > LOD, or for samples between values > LOD. The curve was cut off after the first non-detect.
- Values between LOD and LOQ were set to the measured values.

For the evaluation of the data the kinetic models shown in section 3.7.2 of the report were tested in order to find the most suitable approach based on the chi^2 error criterion and visual inspection.

II. RESULTS AND DISCUSSION

A. DATA

The data for the irradiated test systems and the dark controls are shown in Table IIA 7.1.3-8 to Table IIA 7.1.3-11.

B. MASS BALANCE

The mass balance was 103.0 ± 0.9% and 101.6 ± 1.8% of the applied radioactivity (AR) in the dark and irradiated label #1 soil samples, respectively. For label #2, the mass balances were 102.7 ± 2.6% and 101.6 ± 2.5% of the AR.

C. BOUND AND EXTRACTABLE RESIDUES

For irradiated test systems label #1 and #2, the extractable radioactivity was 102.4%/99.5% of the AR at day 0 and 95.8%/92.7% at day 7 (study end), respectively. NER was 0.0%/0.0% of the AR at day 0 and remained at a low level of 2.0%/4.2% of the AR at the end of the test, respectively. $^{14}CO_2$ formation increased up to 0.8%/0.9% by day 7, respectively. Organic volatile formation was negligible throughout the study (<1%).

For dark test systems label #1 and #2, the extractable radioactivity was 102.4%/99.5% of the AR at day 0 and 102.7%/102.2% at day 7, respectively. NER were 0.0%/0.0% of the AR at day 0 and remained at a very low level of 0.4%/0.3% of the AR at the end of the test, respectively.

In general the non-extractable residues in the dark samples were less than 0.5% of the AR and less than 5% in the irradiated samples at test termination for both radiolabels. With respect to the low bound residues formed here compared to viable soil samples (see study KIIA 7.1.3/01), it can be concluded that bound residues of spirotetramat were formed exclusively by microbial activity and thus indirectly indicating their irreversible nature.

D. VOLATILIZATION

In the irradiated and dark test systems treated by label #1 and #2 no $^{14}CO_2$ formation was observed. Organic volatile formation was negligible throughout the study (<1%).

E. TRANSFORMATION OF TEST ITEM

The parent compound was well degraded (for synopsis of results, i.e. DT_{50} and DT_{90} values of BYI08330 in dark and irradiated samples see Table IIA 7.1.3-12).

The degradation of BYI08330 in dark test systems was faster compared to irradiated test systems. Thus, the net experimental phototransformation rates (difference between dark and irradiated samples) can not be calculated, because they would result in negative values. The experimental DT_{50} of BYI08330 in the dark controls was 5.2 days (label #1) and 4.9 days (label #2). The experimental DT_{50} of BYI08330 in the irradiated samples was 12.0 days (label #1) and 7.1 days (label #2), showing excellent comparability of both systems.

Based on the experimental DT50 of 12.0 and 7.1 days of BYI08330 for the label #1 and #2 test systems, respectively, the DT50 of BYI08330 under environmental conditions is calculated to be 60.0 and 35.5 solar summer days at [redacted], Arizona, USA (see Table IIA 7.1.3-12).

In the irradiated label #1 test systems, BYI08330 decreased from an average of 99.1% of the AR at day 0 to 72.3% of the AR by the end of the study. One major transformation product was detected, BYI08330-ketohydroxy at a maximum of 10.9% of the AR at day 4. One minor transformation product identified was BYI08330-enol, which was formed at a maximum of 6.3% of the AR at day 2. Other

minor transformation products formed accounted for a total of 13.6% of the AR at day 2 with a single maximum amount of 3.9% of the AR. In the irradiated label #2 test systems, BYI08330 decreased from an average of 98.8% of the AR at day 0 to 39.1% of the AR by the end of the study. Two major and one minor transformation products were detected, BYI08330-ketohydroxy, BYI08330-methoxy cyclohexanone and BYI08330-enol at a maximum of 20.6% and 16.7% of the AR both at the end of the study and 5.0% of the AR at day 2, respectively.

In the dark label #1 test systems, BYI08330 decreased from an average of 99.1% of the AR at day 0 to 41.6% of the AR by the end of the study. One major transformation product was detected, BYI08330-enol at a maximum of 59.2% of the AR by the end of the study. A couple of minor transformation products formed accounted for a total of 2.7% of the AR at day 0. In the dark label #2 test systems, BYI08330 decreased from an average of 98.8% of the AR at day 0 to 38.5% of the AR by the end of the study. One major transformation product was detected, BYI08330-enol at a maximum of 61.8% of the AR by the end of the study. A couple of minor transformation products formed accounted for a total of 1.7% of the AR at day 3.

Contrary to the non-sterile soil study [KIIA 7.1.3/01] not any dimers were formed, which indicates that their formation is microbially controlled.

Tthe proposed overall pathway of degradation of Spirotetramat in soil shown in Figure IIA 7.1-1.

Table IIA 7.1.3-8: Phototransformation of BYI08330 in sterile sandy loam [redacted], mean ± SD of radiolabel #1 expressed as % of AR (MEF-08/233)

Compound	Irradiation Days After Treatment (DAT)													
	0		0.25		1		2		3		4		7	
BYI08330 (t.i.)	99.1	±0.2	99.3	±2.7	86.2	±2.7	72.6	±0.4	72.3	±2.3	70.5	±1.1	72.3	±0.2
BYI08330-ketohydroxy	0.0	±0.0	1.0	±1.0	5.2	±0.1	8.1	±1.1	10.7	±1.5	10.9	±0.1	9.0	±0.1
BYI08330-enol	0.0	±0.0	4.1	±1.3	5.2	±1.4	6.3	±1.0	4.7	±0.3	5.4	±0.7	5.3	±1.4
Minor metabolites	2.7	±0.1	0.0	±0.0	3.9	±0.7	13.6	±0.8	11.5	±0.2	10.5	±1.5	9.3	±0.1
Total extracted*	101.9	±0.1	104.3	±0.5	100.5	±0.7	100.7	±0.4	99.2	±0.7	97.4	±1.0	95.8	±1.4
$^{14}CO_2$	n.a.	±0.0	0.0	±0.0	0.0	±0.0	0.4	±0.2	0.4	±0.1	0.7	±0.1	0.8	±0.4
Volatile org.	n.a.	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
NER	0.0	±0.0	0.3	±0.1	0.7	±0.0	1.6	±0.1	1.7	±0.1	1.9	±0.2	2.0	0.1
Total RA recovery	101.9	±0.1	104.7	±0.4	101.3	±0.7	102.7	±0.3	101.3	±0.8	99.9	±1.4	98.6	±1.7

Radiolabel #1: cis-[azaspirodecenyl-3-^{14}C]BYI08330.
*: Including the final ACN extract that was not further considered for analyses due to its low RA content.

Table IIA 7.1.3-9: Transformation of BYI08330 in sterile sandy loam [redacted], mean ± SD of radiolabel #1 expressed as % of AR (MEF-08/233)

Compound	Dark Incubation Days After Treatment (DAT)													
	0		0.25		1		2		3		4		7	
BYI08330 (t.i.)	99.1	±0.2	96.0	±0.9	83.2	±0.5	70.5	±1.2	65.6	±1.8	55.0	±1.9	41.6	±1.5
BYI08330-ketohydroxy	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
BYI08330-enol	0.0	±0.0	6.1	±0.2	17.8	±0.1	31.3	±1.0	37.9	±0.6	47.4	±1.3	59.2	±2.4
Minor metabolites	2.7	±0.1	0.0	±0.0	0.9	±0.0	1.0	±0.0	1.1	±0.1	1.2	±0.2	1.5	±0.2
Total extracted *	101.9	±0.1	102.1	±0.7	101.9	±0.6	102.8	±0.1	104.5	±1.3	103.7	±0.7	102.3	±0.7
$^{14}CO_2$	n.a.	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
Volatile org.	n.a.	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
NER	0.0	±0.0	0.0	±0.0	0.1	±0.0	0.1	±0.0	0.1	±0.0	0.2	±0.0	0.4	±0.0
Total RA recovery	101.9	±0.1	102.1	±0.7	102.0	±0.6	102.9	±0.1	104.6	±1.3	103.9	±0.7	102.6	±0.7

Radiolabel #1 = cis-[azaspirodecenyl-3-^{14}C]BYI08330.
*: Including the final ACN extract that was not further considered for analyses due to its low RA content.

Table IIA 7.1.3-10: Phototransformation of BYI08330 in sterile sandy loam [redacted], mean ± SD of radiolabel #2 expressed as % of AR (MEF-08/233)

Compound	Irradiation Days After Treatment (DAT)													
	0		0.25		1		2		3		4		7	
BYI08330 (t.i.)	98.8	±0.4	96.1	±0.5	86.2	±2.6	82.6	±3.0	88.8	±1.3	74.3	±9.4	39.1	±4.0
BYI08330-ketohydroxy	0.0	±0.0	1.3	±0.0	5.6	±1.2	6.6	±1.2	4.7	±1.2	9.9	±2.8	20.6	±1.6
BYI08330-enol	0.0	±0.0	3.4	±0.3	4.9	±0.2	5.0	±0.8	2.9	±2.9	3.1	±0.8	5.6	±0.1
4- methoxy cyclohexanone	0.0	±0.0	0.0	±0.0	2.4	±0.5	2.3	±0.6	2.7	±0.6	5.3	±1.9	16.7	±0.5
Minor metabolites	0.0	±0.0	1.1	±0.1	3.5	±0.3	3.6	±0.7	5.7	±1.4	6.5	±1.4	10.8	±2.1
Total extracted *	98.8	±0.4	102.0	±0.3	102.6	±0.4	100.2	±0.2	104.9	±4.6	99.1	±2.5	92.7	±4.0
$^{14}CO_2$	n.a.	±0.0	0.0	±0.0	0.0	±0.0	0.1	±0.0	0.1	±0.1	0.1	±0.0	0.9	±0.5
Volatile org.	n.a.	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.1	±0.0
NER	0.0	±0.0	0.2	±0.0	1.0	±0.1	0.9	±0.1	1.1	±0.2	1.4	±0.3	4.2	±0.4
Total RA recovery	98.8	±0.4	102.2	±0.3	103.7	±0.2	101.1	±0.1	106.1	±5.0	100.6	±2.2	97.9	±3.1

Radiolabel #1 = cis-[azaspirodecenyl-3-^{14}C]BYI08330.
*: Including the final ACN extract that was not further considered for analyses due to its low RA content.

Table IIA 7.1.3-11: Transformation of BYI08330 in sterile sandy loam [redacted], mean ± SD of radiolabel #2 expressed as % of AR (MEF-08/233)

Compound	Dark Incubation Days After Treatment (DAT)													
	0		0.25		1		2		3		4		7	
BYI08330 (t.i.)	98.8	±0.4	96.5	±0.9	87.8	±0.7	73.0	±2.3	64.1	±1.5	55.5	±1.4	38.5	±6.2
BYI08330-ketohydroxy	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
BYI08330-enol	0.0	±0.0	6.5	±1.0	18.4	±0.3	30.2	±0.3	34.6	±2.7	43.5	±1.4	61.8	±3.1
4- methoxy cyclohexanone	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
Minor metabolites	0.0	±0.0	1.0	±0.0	0.0	±0.0	1.2	±0.2	1.7	±0.6	1.0	±0.0	1.5	±0.1
Total extracted *	98.8	±0.4	104.0	±0.0	106.2	±0.3	104.4	±1.5	100.4	±4.8	100.0	±0.0	101.8	±3.1
$^{14}CO_2$	n.a.	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
Volatile org.	n.a.	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.1	±0.1	0.0	±0.0
NER	0.0	±0.0	0.0	±0.0	0.1	±0.0	0.1	±0.0	0.1	±0.0	0.2	±0.0	0.3	±0.0
Total RA recovery	98.8	±0.4	104.1	±0.0	106.3	±0.3	104.5	±1.4	100.5	±4.8	100.3	±0.1	102.1	±3.1

Radiolabel #2 = cis-[azaspirodecenyl-5-^{14}C]BYI08330.
*: Including the final ACN extract that was not further considered for analyses due to its low RA content.

Table IIA 7.1.3-12: Synopsis of transformation of BYI08330 on sterile sandy loam [redacted] (MEF-08/233)

Sterile sandy loam [redacted]	[Azaspirodecenyl-3-^{14}C] BYI08330 (label #1)		[Azaspirodecenyl-5-^{14}C] BYI08330 (label #2)	
	Irradiated	Dark	Irradiated	Dark
Experimental SFO DT_{50} [d] of BYI08330	12.0	5.2	7.1	4.9
Experimental SFO DT_{90} [d] of BYI08330	39.9	17.1	23.7	16.2
Experimental DT_{50}/ DT_{90} Phototransformation [d]	Although sterile soil was used net experimental phototransformation kinetics could not be calculated, because negative rates would result (difference between dark and irradiated samples).			
Environmental DT_{50} [d] in June at [redacted], AZ (USA)	60.0		35.5	
Major transformation products *)	BYI08330-ketohydroxy	BYI08330-enol	BYI08330-ketohydroxy; 4-methoxy cyclohexanone	BYI08330-enol
Minor transformation products	BYI08330-enol		BYI08330-enol	

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs

III CONCLUSIONS

With respect to the low bound residues formed especially in sterile dark samples, it can be concluded that bound residues were formed exclusively by microbial activity and thus indirectly indicating their irreversible nature. The degradation of BYI08330 in dark test systems was faster compared to irradiated test systems. Thus, the net experimental phototransformation rates (difference between dark and irradiated samples) can not be calculated, because they would result in negative values. The experimental DT_{50} of BYI08330 in the dark controls was 5.2 days (label #1) and 4.9 days (label #2). The experimental DT_{50} of BYI08330 in the irradiated samples was 12.0 days (label #1) and 7.1 days (label #2), showing excellent comparability of both systems.

This kinetics results in conjunction with the findings that the pathway of degradation was similar as already proposed in Figure IIA 7.1-1 indicate that phototransformation in soil is not of importance for the degradation of Spirotetramat under outdoor conditions, and that a major phototransformation product is not to be expected. Furthermore, contrary to the non-sterile soil study not any dimers were formed.

Summary: Route of degradation of spirotetramat in soil

The biotransformation of [azaspirodecenyl-3-^{14}C]BYI08330 was studied in three EU soils and one US soil for 50 days (EU soils) or 360 days (US soil) under aerobic conditions in the dark at 20 ± 1 °C and 50% WHC_{max} (EU soils) or 75% of 1/3 bar moisture (US soil). The parent compound was quickly degraded: Already after 1-2 days more than 90% of the test item dissipated and declined. At study termination, evolved $^{14}CO_2$ (no volatile organics occurred) accounted up to 19.4% of AR at DAT-50 (EU soils), and accounted for 15.3% of AR for the US soil after 360 days. During the course of the study a number of degradates was observed. Five major degradates were present in all soils and were identified. Besides the two main soil metabolites BYI08330-enol (max. 24.3% of AR at DAT-3) and BYI08330-ketohydroxy (max. 16.3%, DAT-1), BYI08330-MA-amide (max. 6.4%, DAT-179) and two BYI08330-enol-dimers were found. In addition, two minor degradates were identified as BYI08330-desmethyl-enol and BYI08330-oxo-enol amounting to maximum 3.7% and 1.2% of AR, respectively. The route of oxidative BYI08330-enol dimerization leading to dimer 1 or dimer 2, and re-entry of the BYI08330-enol after their cleavage is considered as of minor importance for use according to the GAP, because the formation of dimers is regarded as an artificial process mainly caused by the hot spot application in this test.

Further, the biotransformation of spirotetramat was investigated in two soils using [azaspirodecenyl-3-^{14}C]BYI08330 for 127 days under outdoor climatic conditions realistic for the intended use. Thereby BYI08330 formulated as an OD 100 (pH 5) was applied at 94.6% of the highest recommended single use rate for field application (288 g/ha). The parent compound quickly and thoroughly degraded, and already one day after application, only 53.6 and 72.2% of the applied test item were detectable in both soils. During the course of the study a number of degradates was observed in all four soils. Only two major degradates were detected, the ketohydroxy (max. 25.3% AR, DAT-14) and the enol (max. 7.8% AR, DAT-7). Three further minor metabolites were identified as glyoxylic amide, benzoic acid and ketohydroxy-carboxy. The former two metabolites were originally identified within a laboratory aerobic soil photolysis study and were confirmed to occur in the current study under outdoor conditions. The results obtained confirmed and completed the pathway (see Figure IIA 7.1-1) already established in the guideline aerobic soil metabolism laboratory studies.
In the BYI08330 the soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero. However, under the acidic extraction conditions needed for spirotetramat, the major metabolite enol was found to be partly unstable. It degraded during extraction

to form BYI08330-ketohydroxy and others. Therefore, the degradation and metabolism of the BYI08330-enol in soil was investigated in a separate study (see below), and those results need to be included in the proposed overall metabolic pathway of spirotetramat in soil (see Figure IIA 7.1-1). This fact was also the reason to base the degradation kinetics of the major spirotetramat metabolites on the BYI08330-enol study, but not on the parent study.

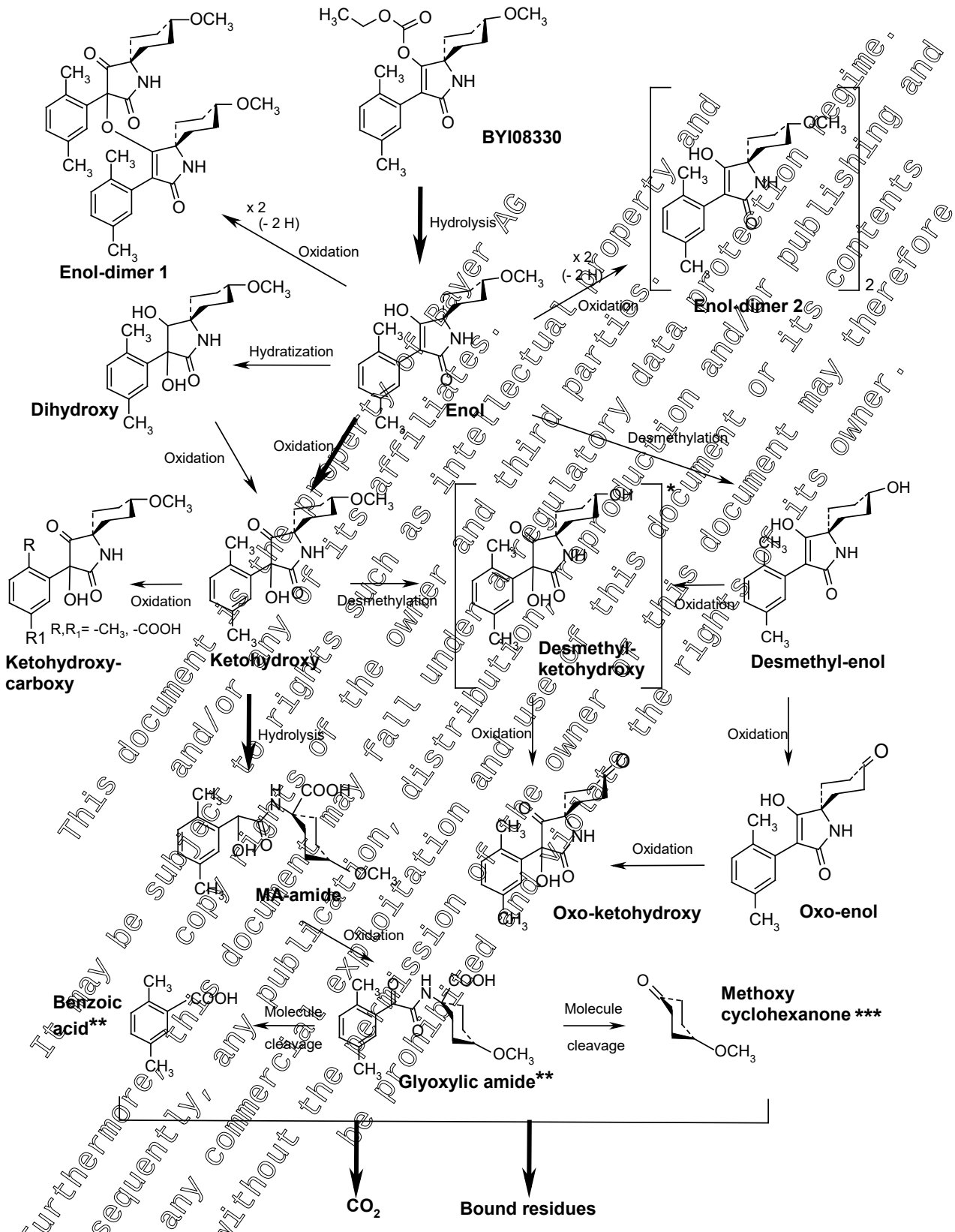
Thus, the biotransformation of [azaspirodecenyl-3-^{14}C] and [azaspiro-decenyl-5-^{14}C]BYI08330-enol was studied in three EU soils and one US soil for 119 days under aerobic conditions in the dark at 20 ±1 °C and at approx. 80% of 1/3 bar moisture (US soil) or 60% WHCmax (EU soils). The BYI08330-enol dissipated following pronounced biphasic kinetics with an extremely quick first phase. Within a second slower degradation phase, the test item declined to 2.7 to 6.1% of AR in the four soils at the end of the study at DAT-119. During the course of the study a large number of degradates was observed in all four soils. Label-specific degradates were not observed throughout the entire study, and the degradation pathway found in before-mentioned study on spirotetramat was confirmed. In addition, a metabolite previously not found, the BYI08330-oxo-ketohydroxy was identified. However, it was a very minor contaminant and was not quantifiable. In all four soils, BYI08330-ketohydroxy was analyzed at levels >10%. BYI08330-enol-dimer 1 amounted once to 5.0% (DAT-60) in one soil, and BYI08330-enol-dimer 2 was maximum 3.6% (DAT-14). BYI08330-MA-amide amounted once >5% (5.2% at DAT-4), and BYI08330-desmethyl-enol was max. 1.8% at DAT-4. All degradates were transient during the study and did not increase towards the end of the study (with the exception of BYI08330-enol-dimer 2 which exhibited scattering results).

Based on the degradate profiles obtained within an anaerobic soil metabolism study, a degradation pathway was proposed which is almost identical to the degradation pathway obtained in aerobic soil. It is concluded that BYI08330 applied to soil will be degraded rapidly in a subsequently flooded anaerobic soil situation, and will not form degradates different from those observed in soil under aerobic conditions, and/or known from abiotic hydrolysis experiments (see section later).

The parent compound was well degraded on irradiated soil samples of phototransformation study on soil surface. However, the biotransformation in the dark controls was approx. four times faster and, considering real environmental conditions (e.g. in June at [redacted], Greece) even approx. 20 times faster compared to soil samples irradiated by natural sunlight. This kinetics results together with the findings that the pathway of degradation was similar indicate that a distinct phototransformation product is not to be expected in soil after the use of spirotetramat under outdoor conditions.

A study using sterile soil surface confirmed the before-mentioned findings that phototransformation in soil is not of importance for the degradation of spirotetramat under outdoor conditions, and that a major phototransformation product is not to be expected. Furthermore, contrary to the non-sterile soil study not any dimers were found, and higher portions of bound residues were not formed. It can be concluded that bound residues of Spirotetramat were formed exclusively by microbial activity and thus indirectly indicating their irreversible nature.

Referring to the behavior in the environment it can be concluded that the active substance spirotetramat (BYI08330) predominantly degrades to the metabolite BYI08330-enol which is further oxidized to BYI08330-ketohydroxy. Subsequently BYI08330-ketohydroxy is hydrolytically opened to BYI8330-MA-amide, as it is included in the proposed overall metabolic scheme outlined in Figure IIA 7.1-1. All components are subject to further degradation to form non-extractable residues (NER) and CO_2.



*: postulated intermediate; ** and *** were identified in studies including light (dependent on radiolabel used)

Figure IIA 7.1-1: Proposed Metabolic Pathway for Spirotetramat (BYI08330) in Soil

IIA 7.2 Rate of degradation in soil(s) - laboratory studies

The laboratory studies presented in Section IIA 7.1 (route of degradation in soil) were also designed to derive information on the rate of degradation of spirotetramat and its significant metabolites under standardized laboratory conditions in soil. In this chapter the methods and results of the respective kinetics calculations were described in more detail.

In addition, an experimental degradation study was performed with BYI08330-methoxy cyclohexanone, a major metabolite found under irradiation conditions with simulated sunlight.

IIA 7.2.1 Aerobic degradation of the active substance in soils at 20°C

Report:	**KIIA 7.2.1/01, [redacted], 2005 (MEF-04/169)**
Title:	**Aerobic Degradation/Metabolism of BYI8330 in Four Different Soils**
Report No & Document No	**MEF-04/169 M-256849-02-2**
Guidelines	OECD: TG 307, Aerobic and Anaerobic Transformation in Soil, April 24, 2002; Commission Directive 95/36/EC amending Council Directive 91/414/EEC (Annexes I and II, Fate and Behavior in the Environment), July 14, 1995; US EPA Subdivision N, Section § 162-1; Japanese MAFF Guidelines,
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of March 2003 to March 2004. Study completion date, inclusive amendment no.1: 2005-07-12

EXECUTIVE SUMMARY

The biotransformation of cis-[azaspirodecenyl-3-^{14}C]BYI08330 was studied in three EU soils and one US soil for 50 days (EU soils) or 360 days (US soil) under aerobic conditions in the dark at 20 ± 1 °C and 50% WHC_{max} (EU soils) or 75% of 1/3 bar moisture (US soil).

The parent compound was quickly degraded: Already after 1-2 days more than 90% of the test item dissipated and declined. The degradation behavior of the test item followed first order kinetics with an extremely quick decline within the first 3 days, i.e. BYI08330 degraded with a DT50 of between 2.0 and 7.8 hours. The mean DT50 value for BYI8330 degradation in aerobic soils was calculated to 0.21 days. The calculated DT90 value was reached for all soils in this study (according to the SFO model on average 0.71 days).

When evaluating this spirotetramat study it has to be considered that the soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero. However, under the acidic extraction conditions needed for spirotetramat, the major metabolite BYI08330-enol was partly unstable, and degraded under formation of BYI08330-ketohydroxy and others. Therefore, the degradation of the BYI08330-enol in soils was necessarily investigated in a separate study (see next chapter), and those results have to be considered when evaluating the overall kinetics of degradation of spirotetramat residues in soil.

I. MATERIALS AND METHODS

A. MATERIALS

Materials used in this study are comprehensively described under Point IIA 7.1.1/01.

B. STUDY DESIGN

The study design used in this study is comprehensively described under Point IIA 7.1.1/01.

C. DETERMINATION OF DEGRADATION KINETICS

As a standard approach, mathematical model representation of the observed degradation of BY0I8330 in soil over time was based on single compartment first order kinetics (SFO), which is represented as:

$$M_p(t) = M_0 * e^{(-kt)}$$

$M_p(t)$ = concentration of BYI08330 at time t
M_0 = initial concentration of BYI08330
k = rate constant of degradation

The degradation rate constant k was estimated by automated curve fitting of an exponential function to the measured data, using the Simple First Order (SFO) routine of the software tool ®Model Manager, Version 1.1 (Cherwell Scientific, Oxford, UK) for Microsoft Windows NT 4.0. Based on degradation rate constant, dissipation times DT_{50}, DT_{75}, and DT_{90} (time of 50, 75 or 90% disappearance of the test item) were automatically derived according to:

$$DT50 = \frac{\ln 2}{k} \qquad DT75 = \frac{\ln 4}{k} \qquad DT90 = \frac{\ln 10}{k}$$

Model input datasets for the four soils were the mean abundances of residual BY0I8330 found in ambient plus "aggressive" extracts at each sampling date. All data points were weighed equally. For optimal goodness of fit, the initial value (M_0) was also allowed to be estimated by the model. A simple first order kinetic model approach provided acceptable quality of fit, reflected in an R^2 value of 0.974 for the US soil and even ≥ 0.989 for the EU soils.

II. KINETICS OF TEST ITEM DEGRADATION

The respective results for the four soils based on the data presented in Section IIA 7.1.1 are shown in Table IIA 7.2.1-1.

The degradation behavior of the test item followed first order kinetics, i.e. BYI08330 degraded with a DT50 of between 0.08 and 0.33 days. The mean DT50 value for BYI8330 degradation in aerobic soils was calculated to 0.21 days. The calculated DT90 value was reached for all soils in this study (according to the SFO model on average 0.71 days).

III. CONCLUSION

Spirotetramat is a very fast degrading compound in soil with DT50 values between 0.08 and 0.33 days.

Table IIA 7.2.1-1: Degradation of BYI08330 in four soils under aerobic conditions at 20 °C (MEF-04/169)

Soil type	[redacted]	[redacted]	[redacted]	[redacted]	Mean
Parameter	Simple 1st Order (SFO)				
M_0 (% AR)	94.5	91.3	90.9	93.2	92.5
k (1/days)	2.12	3.30	2.99	8.32	4.18
DT_{50} (days)	0.327	0.210	0.232	0.083	0.21
DT_{90} (days)	1.090	0.697	0.770	0.277	0.71
R^2	0.974	0.991	0.989	0.991	0.986

Report:	**KIIA 7.2.1/02, [redacted] and [redacted] 2006 (MEF-05/249)**
Title:	**Degradation of BYI08330 in Four Soils Under Aerobic Conditions: Kinetic Evaluation**
Report No & Document No	**MEF-05/249 M-277096-01-2**
Guidelines	The recommendations from the draft FOCUS guidelines (FOCUS, 2000) for kinetics modeling were considered in this study
GLP	N/A: modeling calculation
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the estimations in August 2006. Completion date, 2006-08-30

EXECUTIVE SUMMARY

The objective of this study is to characterize the degradation of BYI08330 in soil under aerobic conditions using kinetics modeling and derived degradation parameters for use in environmental exposure assessment and as trigger values for higher tier studies. Degradation of [azaspirodecenyl-3-^{14}C]BYI08330 was studied in three European soils and one US soil under laboratory aerobic conditions at 20 °C. Even though a number of degradation products were identified in the metabolism study, the kinetic evaluation was conducted only for BYI08330, since the soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero. However, under the acidic extraction conditions needed for BYI08330, the major metabolite BYI08330-enol was found to be partly unstable. It degrades under the formation of BYI08330-ketohydroxy and others. Therefore, the degradation/metabolism of the BYI08330-enol in soil was necessarily investigated in a separate study on which the degradation kinetics calculations of the major spirotetramat metabolites were based.

The found normalized geometric mean BYI08330 DT_{50} value of 0.13 days is a suitable input parameter for environmental fate models.

I. MATERIALS AND METHODS

A. MATERIALS

Materials used in this study are comprehensively described under Point IIA 7.1.1/01.

B. STUDY DESIGN

The study design used in this study is comprehensively described under Point IIA 7.1.1/01.

C. DETERMINATION OF DEGRADATION KINETICS

For modeling purposes a normalization factor f_θ was calculated to normalize the estimated laboratory DT_{50} values to moisture conditions corresponding to field capacity (FC) based on recommendations of the FOCUS working group on ground water scenarios (FOCUS, 2000). The moisture content at field capacity is assumed to correspond to the moisture content at a matrix potential of 0.1 bar (pF 2.0).
The relation between actual DT_{50} corresponding to the experimental conditions and the reference half life $DT_{50\text{-ref}}$ is

$$DT_{50-ref} = f_\theta \; DT_{50}$$

where, the normalization factor f_θ is calculated using the Walker equation (Walker, 1974) as follows:

$$f_\theta = \begin{cases} \left(\theta / \theta_{ref}\right)^B & \theta \le \theta_{ref} \\ 1 & \theta > \theta_{ref} \end{cases}$$

The symbol θ denotes the incubation soil moisture and θ_{ref} the reference soil moisture. The moisture contents are either given as volumetric or gravimetric water content. The parameter B was chosen according to FOCUS (2000) as B = 0.7. Values for the maximum water holding capacity and field capacity were estimated from soil texture according to FOCUS (2000). The field capacity value was used directly as θ_{ref}, which was used to calculate the actual water content θ.

Table IIA 7.2.1-2: Overview of water content and normalization factor (MEF-05/249)

Soil and Source	Texture	MWHC[a] % Vol.	MC at pF 2.5[b] % Vol.	θ % Vol.	θ_{ref}[c] % Vol.	f_θ
[redacted] (Germany)	sandy loam	27	15	13.5 (50% of MWHC)	19	0.79
[redacted] (Germany)	silt loam	32	21	16 (50% of MWHC)	26	0.71
[redacted] (Germany)	silt	31	21	15.5 (50% of MWHC)	27	0.68
[redacted] (Florida, USA)	sandy loam	27	15	11.25 (75% of MC, pF2.5)	19	0.69

[a] Maximum Water Holding Capacity – according to FOCUS (2000)
[b] Moisture Content at pF 2.5 – according to FOCUS (2000)
[c] Moisture Content at pF2.0 – according to FOCUS (2000)

A kinetics modeling tool, which was built within the frame-work of mathematical software, MATLAB (Ver. 7.0.4) (Matlab), was used in this study. The tool uses internal routines (Levenberg-Marquardt) of MATLAB to optimize the model parameters to fit a chosen kinetics model to the metabolism study data. The objective function used for the optimization of the parameters was to minimize the sum of squares between the calculated and observed time series data.
In this study, single first-order (SFO, see point 4.3.1 of report MEF-05/249) and Double First-Order in Parallel (DFOP, see point 4.3.3 of report MEF-05/249) models were used to characterize the degradation of BYI08330. Brief descriptions of these models are given here and more details are given in the FOCUS

report (FOCUS, 2006).

The goodness of fit was assessed by visual inspection and an error criterion based on a chi-square (χ^2) significance test. The visual inspection focuses on the residuals which should not be distributed systematically but randomly. However in the case of systematic but sufficiently small deviations a fit may still be qualified as visually acceptable. The χ^2 significance test evaluates the likeliness that a given model is a correct description of the values observed. In addition to these, coefficient of determination (r^2) was calculated and reported by the kinetics modeling tool.

II. KINETICS OF TEST ITEM DEGRADATION

For the evaluation of trigger values for higher tier experiments the FOCUS working group on kinetics (FOCUS, 2006) recommends to use the DT_{50} and DT_{90} values derived from the best fit kinetics. As the results of modeling shown in Table IIA 7.2.1-3 indicate for BYI08330 this was the DFOP evaluation of the laboratory data.

Table IIA 7.2.1-3: Summary of results for use as trigger values: DFOP model fits (MEF-05/249)

Parameters	Soil AXXa	AIII	[redacted]	[redacted]	Geom. mean
Optimized Parameters:					
DT50 (d)	0.24	0.26	0.09	0.30	0.20
DT90 (d)	0.89	0.97	0.34	1.26	0.78
Goodness of Fit:					
χ2 Scaled Error (%)	1.37	0.81	4.00	6.12	

For the use in environmental fate models, first-order DT_{50} values were calculated from the optimized first-order rate constants and were normalized to the soil moisture at field capacity according to FOCUS rules:

Table IIA 7.2.1-4: Summary of results of SFO model fit for use in exposure models (MEF-05/249)

Parameters	Soil AXXa	AIII	[redacted]	[redacted]	Geom. mean
Optimized Parameters:					
DT50 (d)	0.24	0.23	0.08	0.33	
DT90 (d)	0.69	0.77	0.28	1.09	1.07
DT50-normalized (d)	0.17	0.16	0.05	0.23	0.13
Goodness of Fit:					
χ2 Scaled Error (%)	8.52	8.81	9.50	21.79	

The DFOP kinetic model fitted the experimental data best in terms of visual assessment and the χ^2 scaled errors. The normalized DFOP DT50 of 0.15 days and DT90 of 0.78 days are suitable endpoints for use as trigger values.

SFO is the preferred model for deriving parameters to be used in environmental fate models. The visual assessment of the SFO plots indicated an acceptable fit of the data to the SFO model. For three of the four studied soils the χ^2 scaled errors was below 10% indicating good agreement between the modeled and measured data. Despite the fact, that the χ^2 scaled error in case of the [redacted] soil was estimated to

be 8% higher than the indicator value suggested by the FOCUS group (FOCUS, 2006), the SFO model was found to adequately represent all four studied soils. The normalized SFO DT50 of 0.13 days, which in fact is almost identical to the DFOP half-life, is suitable for use in environmental fate models.

III. Conclusion

Spirotetramat is a very fast degrading compound in soil.

IIA 7.2.2 Aerobic degradation of the active substance in soils at 10°C

The Arrhenius equation is a validated relationship which can be used to describe temperature effects on transformation (SANCO Doc. 9188/VI/97 rev. 8). A Q10-value of 2.20 could reasonably be used to extrapolate DT50 data derived at 20 °C to expected values at 10 °C (FOCUS, SANCO Doc. 7617/VI/96). Spirotetramat is rapidly degraded in soil with DT50 values between 0.08 and 0.33 days. Multiplying these DT_{50}-values by a factor of 2.2 will result in DT50 values between 0.18 and 0.73 days, clearly far below the trigger value for field dissipation trials of 60 days.

IIA 7.2.3 Aerobic degradation of relevant metabolites in soils at 20°C

Metabolite BYI08330-Enol

Chemical name (CAS): cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one; CAS #: 203312-38-3

Report:	**KIIA 7.2.3/01, [redacted], 2006 (MEF-05/157)**
Title:	**[Azaspirodecenyl-3-^{14}C]- and [Azaspirodecenyl-5-^{14}C]-Labeled BYI08330-*cis*-Enol: Comparative Aerobic Soil Metabolism/Degradation in Four Soils**
Report No & Document No	**MEF-05/157, M-269304-01-2**
Guidelines:	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.
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of January 2004 to June 2005. Study completion date: 2006-01-17

EXECUTIVE SUMMARY

The biotransformation of cis-[azaspirodecenyl-3-^{14}C]BYI08330-enol and cis-[azaspiro-decenyl-5-^{14}C]BYI08330-enol was studied in three EU soils and one US soil for 119 days under aerobic conditions in the dark at 20 ±1°C and at approx. 80% of 1/3 bar moisture (US soil) or 60% WHCmax (EU soils). The application rate of BYI08330-enol to the soils was calculated based on the highest recommended single use rate of spirotetramat for field application (288 g/ha) and an assumed worst case amount of its first soil metabolite BYI08330-enol. Following the respective required conversion factors a rate of 0.13 and 0.309 mg test item per kg DM of soil was calculated for the US soil and the EU soils. The soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test

item at time zero (i.e. slightly alkaline extraction conditions were needed for the test item).
The test item dissipated following pronounced biphasic kinetics. In three soils more than 82% of the test item dissipated within six hours (DAT-0.25), i.e. indicating an extremely quick first phase. In the fourth soil (silt loam soil [redacted]) this happened in the same manner but slightly later at DAT-1. Within a second slower degradation phase, the test item declined to 2.7 to 6.1% of AR in the four soils at the end of the study at DAT-119. The metabolites of BYI08330-enol formed were transient during the study and did not increase towards the end of the study.
The respective kinetic modeling of test item by using MatLab® (application KinGUI) indicated that the best fit DT50 (days) of test item resulted by using the bi-exponential model DFOP (double first order in parallel). This model yielded BYI08330-enol DT_{50} values ranging between 0.02 and 0.09 days for the four soils (a mean DT50 of 0.08 days; chi^2 statistics mean value of 7.7). Thus it can be concluded that BYI08330-enol is a fast degrading metabolite of spirotetramat in soil.

I. MATERIALS AND METHODS

A. MATERIALS

Materials used in this study are comprehensively described under Point IIA 7.1.1, report /03.

B. STUDY DESIGN

The study design used in this study is comprehensively described under Point IIA 7.1.1, report /03.

C. DETERMINATION OF DEGRADATION KINETICS

DT_{50} and DT_{90} values were determined for the degradation of the test item BYI08330-enol and its metabolites BYI08330-ketohydroxy and BYI08330-MA-amide. Determination of the kinetics values followed the recommendations of FOCUS rules. A detailed report on the calculation of kinetics values for modeling aspects was prepared separately (see later in this chapter).
The data for the test item were evaluated using the software MatLAB® (application KinGUI) version 7.0.4.365. DAT-0 values in the Tables and Appendices actually corresponded to values determined approximately 1 to 2 minutes after DAT-0 but were kept as DAT-0 values for the modeling calculations. For the transformation products ketohydroxy and MA-amide the dissipation was evaluated starting from DAT-0. Based on the chi^2 scaled-error criterion the best fit kinetic model was chosen for the disappearance time evaluation. For the determination of the degradation kinetics the LOD was set to 0.1% AR. Values below the LOD were reported as < 0.1% AR. For the data evaluation the simple first order model (SFO), the first order multi compartment model (FOMC) and the bi-exponential model (double first order in parallel, DFOP) were tested in order to identify the most suitable approach (best fit). Based on the chi^2 scaled-error criterion and visual inspection the following model was the most appropriate:

Bi-exponential model (double first order in parallel, DFOP):

$$M_P(t) = M_1 \exp^{(-k_1 t)} + M_2 \exp^{(-k_2 t)}$$

$M_P(t)$	= Total amount of chemical present at time t
M_1	= Amount of chemical applied to compartment 1 at time t = 0
M_2	= Amount of chemical applied to compartment 2 at time t = 0
k_1	= Rate constant in compartment 1 [d^{-1}]
k_2	= Rate constant in compartment 2 [d^{-1}]

For the DFOP model, DT_{50}, and DT_{90} were derived by the computer software from cross-over points of

the dissipation curves with the respective 50% or 90% horizontal lines. Model input datasets for the four soils were the mean amounts of BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide found in aqueous, ambient and "aggressive" extracts at each sampling date (see Section IIA 7.1.1). All data points were weighed equally. For optimal goodness of fit, the initial value was also allowed to be estimated by the model.

II. KINETICS OF TEST ITEM DEGRADATION

The degradation kinetics of BYI08330-enol in aerobic soil indicated that the test item degrades following biphasic kinetics, with the initial phase lasting less than a day, with more than 80% degradation. In the second phase that followed, further degradation of up to 6% of the applied test item was observed at the end of the study period of 119 days.

The kinetic evaluation was started by assuming a simple-first-order dissipation for the test item (see for kinetics parameters in Table IIA 7.2.3-1). The visual inspection of the SFO fits exhibited an overestimation of the test item dissipation. This was confirmed by high chi^2 scaled-error values, significantly >15% (range 27.8 – 68.3%) and a low range of R^2 values (see Table IIA 7.2.3-1). In a second step, a biphasic model was checked for the test item. The first-order multi-compartment model (FOMC) according to Gustafson-Holden was chosen. The statistical results and the visual inspection of the fits demonstrated that the FOMC fits represented a relatively appropriate kinetic model to describe the dissipation of the test item (chi^2 scaled-error values in the range of 7.3 – 13.0, R^2 values ≥ 0.9675; see Table IIA 7.2.3-1).

In a third step, as another biphasic model the bi-exponential model DFOP (double first order in parallel) was checked for the test item and was found to lead to an improved fit. The respective chi^2 scaled-error values ranged from 6.9 – 8.7% (R^2 values ≥ 0.9675; see Table IIA 7.2.3-1), and moreover the visual inspection of the fits demonstrated that the DFOP fits represented the most appropriate kinetic model for the BYI08330-enol dissipation in the four soils. Using this model, DT_{50} values of BYI08330-enol in the four soils tested range from 0.02 to 0.2 days, with a geometric mean of 0.08 days. The DT_{90} values in the four soils range from 22.9 to 106.9 days, with a geometric mean of 64.4 days (see Table IIA 7.2.3-1).

Table IIA 7.2.3-1: Summary of the kinetics evaluation of the degradation of [^{14}C]BYI08330-enol in aerobic soils (MEF-05/157)

Soil Type	[redacted]	[redacted]	[redacted]	[redacted]
Soil type	Sandy loam	Sandy loam	Silt loam	Silt
Parameter	1st step: Simple first order regression (SFO)			
Chi^2	41.9	56.2	27.8	68.3
R^2	0.9667	0.3901	0.9710	0.4272
Parameter	2nd step: First order multi compartment regression (FOMC)			
Chi^2	8.0	13.0	7.3	12.3
R^2	0.9938	0.9675	0.9956	0.9811
Parameter	3rd step: Double first order regression in parallel (DFOP)			
DT_{50} (days)	0.09	0.02	0.2	0.02
DT_{90} (days)	53.7	106.9	22.9	74.0
Chi^2	6.9	8.7	7.2	8.1
R^2	0.9959	0.9870	0.9963	0.9927
Mean DT_{50} (days)	0.08			
Mean DT_{90} (days)	64.4			

The respective results of estimating the degradation kinetics of BYI08330-ketohydroxy and BYI08330-MA-amide in aerobic soil („trigger evaluation") are shown Table IIA 7.2.3.2.

Table IIA 7.2.3-2: Summary of the kinetics evaluation of the degradation of BYI08330-ketohydroxy and BYI08330-MA-amide in soils (MEF-05/157)

Soil Type	[redacted]	[redacted]	[redacted]	[redacted]
Soil type	Sandy loam	Sandy loam	Silt loam	Silt
BYI08330-ketohydroxy				
Parameter	Simple first order regression (SFO)			
DT_{50} (days)	20.0	4.8	5.8	2.0
DT_{90} (days)	66.4	15.8	19.2	6.7
Chi^2	13.5	12.4	9.0	10.9
R^2	0.9442	0.9872	0.9885	0.9947
Mean DT_{50} (days)	8.2			
Mean DT_{90} (days)	27.0			
BYI08330-MA-Amide				
Parameter	Simple first order regression (SFO)			
DT_{50} (days)	6.8	1.3	3.1	0.6
DT_{90} (days)	22.5	4.3	10.2	2.1
Chi^2	30.5	31.3	12.7	20.3
R^2	0.8602	0.8966	0.9855	0.9736
Mean DT_{50} (days)	3.0			
Mean DT_{90} (days)	9.8			

Major metabolite BYI08330-ketohydroxy (maximum 24.0% AR in the present study; maximum 16.3% in the spirotetramat metabolism study KIIA 7.1.1/01) appeared very quick in all four soils and peaked already at around DAT-1 in three of the four soils). In the biologically most active [redacted] soil its peak value was reached already at "DAT-0". Using the kinetic SFO modeling, DT_{50} values of 20.0, 4.8, 5.8 and 2.0 days (mean 8.2 days) were calculated for the BYI08330-ketohydroxy in soils [redacted], [redacted], [redacted] and [redacted], respectively. As respective DT_{90} values 66.4, 15.8, 19.2 and 6.7 days (mean 27.0 days) were calculated for the four soils. The respective chi^2 scaled-error values (9.0 – 13.5%) and R^2 values ($\geq$ 0.9442) and moreover the visual inspection of the fits demonstrated that the SFO fits represented an appropriate kinetic model for the BYI08330-ketohydroxy dissipation in the four soils.

BYI08330-MA-amide, which amounted maximum 6.4% AR in the spirotetramat metabolism study (KIIA 7.1.1/01) and reached maximum 5.2% in the present study peaked later during the course of the study (at around DAT-4 in the two [redacted] soils, at around DAT-7 in soil [redacted], and already at DAT-1 in most active soil [redacted]). Using the same SFO kinetic modeling, DT_{50} values of 6.8, 1.3, 3.1 and 0.6 days (mean 3.0 days) were calculated in soils [redacted], [redacted] and [redacted], respectively. As respective DT_{90} values 22.5, 4.3, 10.2 and 2.1 days (mean 9.8 days) were calculated for the four soils. The respective chi^2 scaled-error values (12.7 – 31.3%) and R^2 values ($\geq$ 0.8602) were considered acceptable regarding the low individual % AR-values measured for this metabolite. As a support, the visual inspection of the fits demonstrated that the SFO fits represented an appropriate kinetic model for the BYI08330-MA-amide dissipation in the four soils.

III. CONCLUSIONS

The current laboratory study demonstrated that BYI08330-enol quickly degrades in aerobic soil under the formation of numerous degradates and readily mineralized to CO_2. The test item degraded following biphasic kinetics, with the initial phase lasting less than a day, with more than 80 % degradation. The DT_{50} and DT_{90} values are expected to be in the range of < 1 day and 64.4 days, respectively. Metabolites generated from BYI08330-enol are further degraded and are expected not to accumulate in the environment. Considering these results terrestrial field dissipation studies were not necessary for this dossier (see IIA 7.3) since residues of spirotetramat in field soils can be reliably estimated from the data on dissipation in laboratory soils.

In order to calculate a normalized laboratory DT50 for spirotetramat and its major soil metabolites, needed e.g. for estimating Predicted Environmental Concentrations (PECs) the following detailed report on the calculation of kinetic values for modeling aspects was prepared separately. It was based on a conceptual metabolic pathway, also considering the knowledge of other relevant studies described in section IIA 7.1.1.

Report:	**KIIA 7.2.3/02, [redacted], 2006 (MEF-06/199)**
Title:	**Kinetic Evaluation of Laboratory Soil Degradation Studies of BYI08330-Enol, BYI08330-Ketohydroxy, BYI08330-MA-Amide**
Report No & Document No	**MEF-06/199 (amended 2007-08-23) M-277178-03-1**
Guidelines	The recommendations from the draft FOCUS guidelines (FOCUS, 2000) for kinetics modeling were considered in this study
GLP	N/A: modeling calculation
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted] [redacted], GER, conducted the estimations in July 2006. Completion date: 2006-08-30, 2[nd] version of report dated 2007-05-31.

EXECUTIVE SUMMARY

By this modeling investigation the before-mentioned laboratory study on biotransformation of [^{14}C]BYI08330-enol performed with three EU soils and one US soil (KIIA 7.2.3/01) was evaluated to derive kinetic parameters and obtain half-lives for the BYI08330 soil degradates (BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide) as endpoints for use as persistence trigger values and as suitable input parameters for environmental fate models. The initial soil concentration was freely fitted together with all degradation rates and formation fractions, based on equally weighted soil residues.

Since BYI08330-enol shows an extremely biphasic behavior, a mechanistic explanation was sought. The single first order reversible binding (=SFORB) model suited the analyzed dataset best, whereby the compound is rapidly degraded in the equilibrium domain but also rapidly transfers into a stronger bound soil compartment, where it is not susceptible to degradation and only slowly transferred back into the equilibrium domain. The visual assessments and the calculated scaled errors indicated acceptable fits of the five-compartment model with SFORB kinetics to the experimental data. The degradation rates of BYI08330-enol, -ketohydroxy and -MA-amide pass the t-test at acceptable probabilities. The parameters obtained from the SFORB kinetics model were transformed into the equivalent parameters used by the PEARL kinetic sorption model in order to be usable in common environmental fate models.

First-order DT_{50} values were calculated from the optimized first-order rate constants of the five-compartment model and were normalized to the soil moisture at field capacity according to FOCUS rules (FOCUS, 2002). The normalized DT_{50} values range from 0.02 to 0.13 days for BYI08330-enol, from 1.2 to 12.1 days for BYI08330-ketohydroxy and from 0.2 to 3.9 days for BYI08330-MA-amide. A maximum of 24% of the degradation of BYI08330-enol leads to the formation of BYI08330-ketohydroxy, and a maximum of 5.2% to BYI08330-MA-amide.

The resulting geometric mean normalized $DT_{50\text{-ref}}$ values of 0.03 days for BYI08330-enol, 3.8 days for BYI08330-ketohydroxy and 1.0 days for BYI08330-MA-amide are suitable input parameters for environmental fate models. It must be noted, that the extremely short half life time of 0.03 days for BYI08330-enol may only be used for modelling purposes and in conjunction with the SFORB model or a kinetic sorption model as implemented in PEARL.

As the estimated biphasic SFORB half-lives of BYI08330-enol are not valid outside the domain of kinetic sorption models, alternative conservative half-live values were recalculated from the FOMC DT_{90} as suggested by FOCUS (2006) for the parameterization of e.g. 1[st] tier PEC_{Soil} and PEC_{SW} model approaches. The resulting geometric mean normalized $DT_{50\text{-ref}}$ value of 1.16 days (recalculated by FOMC DT_{90}/3.32 days) for BYI08330-enol is a suitable worst case input parameter for environmental fate models which do not allow the consideration of aged sorption.

For the evaluation of trigger values for higher tier experiments the FOCUS working group on kinetics

(FOCUS, 2006) recommends to use the DT_{50} and DT_{90} values derived from the best fit kinetics. As for BYI08330 this was the DFOP evaluation of the laboratory data, which gave the results shown in Table IIA 7.2.3-4.

I. MATERIALS AND METHODS

A. MATERIALS

Materials used in this study are comprehensively described under Point IIA 7.1.1, report /03.

B. STUDY DESIGN

The study design used in this study is comprehensively described under Point IIA 7.1.1, report /03.

C. DETERMINATION OF DEGRADATION KINETICS

Since BYI08330-enol shows an extremely biphasic behavior, a mechanistic explanation was sought. The single first order reversible binding (=SFORB) model fitted the analyzed dataset best, whereby the compound is rapidly degraded in the equilibrium domain but also rapidly transfers into a stronger bound soil compartment, where it is not susceptible to degradation and only slowly transferred back into the equilibrium domain. The visual assessments and the calculated scaled errors indicated acceptable fits of a five-compartment model with SFORB kinetics to the experimental data. The degradation rates of BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide pass the t-test at acceptable probabilities.

The parameters obtained from the SFORB kinetics model were transformed into the equivalent parameters used by the PEARL kinetic sorption model in order to be usable in common environmental fate models.

First-order DT_{50} values were calculated from the optimized first-order rate constants of the five-compartment model and were normalized to the soil moisture at field capacity according to FOCUS rules (FOCUS, 2002).

II. KINETICS OF TEST ITEM DEGRADATION

The normalized DT_{50} values ranged from 0.02 to 0.13 days for BYI08330-enol, from 1.2 to 12.1 days for BYI08330-ketohydroxy and from 0.2 to 3.9 days for BYI08330-MA-amide. A maximum of 24% of the degradation of BYI08330-enol leads to the formation of BYI08330-ketohydroxy and a maximum of 5.2% to BYI08330-MA-amide. The geometric mean normalized $DT_{50\text{-ref}}$ values of 0.03 days for BYI08330-enol, 3.8 days for BYI08330-ketohydroxy and 1.0 day for BYI08330-MA-amide are suitable input parameters for environmental fate models. It must be noted, that the extremely short half life time of 0.03 days for BYI08330-enol may only be used for modeling purposes and in conjunction with the SFORB model or a kinetic sorption model as implemented in PEARL.

As the estimated biphasic SFORB half-lives of BYI08330-enol are not valid outside the domain of kinetic sorption models, alternative half-life values are required for the parameterization of e.g. 1st tier PEC_{Soil}, PEC_{SW} model approaches. According to the recommendations of the FOCUS working group on kinetics (FOCUS, 2006) the recalculated DT_{50} value from the DT_{90} value of the biphasic FOMC model can be used for modeling purposes as a worst case assumption where the concentration of the test substances decreases to 10% of the initial concentration during the experiment. As the recalculated half-lives ($DT_{50} = DT_{90}/3.32$) are longer than the original half-lives, the use in environmental fate models will result in an overestimation of the soil residues and hence represent a worst case. The visual

assessments of the FOMC fits were acceptable and the calculated scaled errors ε ranged from 5.6% to 19% for BYI08330-enol. The geometric mean normalized $DT_{50\text{-ref}}$ value of 1.16 days (FOMC DT_{90}/3.32) for BYI08330-enol is a suitable worst case input parameter for environmental fate models which do not allow the consideration of aged sorption (e.g. 1st tier PEC_{Soil}, PEC_{SW}).

The detailed results of the kinetic evaluations (SFO, SFORB, FOMC, and DFOP) for each soil, considering only the final simultaneous fits of parent and metabolite, are given in Table 10 to Table 13 in the report MEF-06/199.

Table IIA 7.2.3-3: DT_{50} values of BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide, based on laboratory data normalized to standard moisture conditions (MEF-06/199)

	DT_{50} (days) at pF2 and 20°C				
	BYI08330-enol			BYI08330-ketohydroxy	BYI08330-MA-amide
Soil	$DT_{50\text{-ref}}$	f_{me}	K_d	$DT_{50\text{-ref}}$	$DT_{50\text{-ref}}$
[redacted]	0.04	193.2	0.013	12.1	3.9
[redacted]	0.02	693.1	0.012	3.8	1.0
[redacted]	0.13	28.9	0.037	4.1	1.5
[redacted]	0.02	593.7	0.012	1.2	0.2
Geom. mean	0.03			3.8	1.0

For the evaluation of trigger values for higher tier experiments the FOCUS working group on kinetics (FOCUS, 2006) recommends to use the DT_{50} and DT_{90} values derived from the best fit kinetics. Like in case of BYI08330 this was the DFOP evaluation of the laboratory data, which gave the following results:

Table IIA 7.2.3-4: Trigger $DT_{50/90}$ values of BYI08330-enol evaluated with the DFOP - model (MEF-06/199)

BYI08330-enol	[redacted]	AXXa	AIII	[redacted]	Geom. mean
DT_{50} (d)	0.06	0.02	0.18	0.02	0.05
DT_{90} (d)	28.93	40.9	16.8	10.9	21.6
χ²	5.5	5.6	2.4	5.2	
R^2	0.99	0.99	0.99	0.99	
t-probability	<0.001	<0.001	<0.001	<0.001	

III. CONCLUSIONS

Spirotetramat is a very fast degrading compound in soil. The metabolites generated from BYI08330-enol, the predominant first metabolite of BYI08330, are further degraded quickly and are expected not to accumulate in the environment.

Metabolite BYI08330-ketohydroxy

Chemical name (CAS): cis-3-(2,5-Dimethylphenyl)-3-hydroxy-8-methoxy-1-azaspiro[4.5]decan-

2,4-dione

The fate of the metabolite BYI08330-ketohydroxy was described earlier in this chapter, already (see KIIA 7.2.3/01 and 02).

Major metabolite BYI08330-ketohydroxy (maximum 24.0% AR in the enol study; maximum 16.3% in the spirotetramat metabolism study (KIIA 7.1.1/01) appeared very quick in all four soils and peaked already at around DAT-1 in three of the four soils). In the biologically most active [redacted] soil its peak value was reached already at “DAT-0”. Using the kinetic SFO modeling, DT_{50} values of 20.0, 4.8, 5.8 and 2.0 days (mean 8.2 days) were calculated for the BYI08330-ketohydroxy in soils [redacted], [redacted], [redacted] and [redacted], respectively. As respective DT_{90} values 66.4, 15.8, 19.2 and 6.7 days (mean 27.0 days) were calculated for the four soils. The respective chi^2 scaled-error values (9.0 – 13.5%) and R^2 values ($\geq$ 0.9442) and moreover the visual inspection of the fits demonstrated that the SFO fits represented an appropriate kinetic model for the BYI08330-ketohydroxy dissipation in the four soils.

The respective results of estimating the degradation kinetics of BYI08330-ketohydroxy in aerobic soil („trigger evaluation“) were summarized in Table IIA 7.2.3-2. The geom. mean normalized laboratory degradation half-live of BYI08330-ketohydroxy, as needed for the calculation of predicted environmental concentrations (PECs), was estimated to be 3.8 days (see Table IIA 7.2.3-3).

Metabolite BYI08330-MA-amide

Chemical name (IUPAC): (1s,4s)-1-{[(2,5-dimethylphenyl)(hydroxy)acetyl]amino}-4-methoxy-cyclohexanecarboxylic acid

The fate of the metabolite BYI08330-MA-amide was described earlier in this chapter, already (see KIIA 7.2.3/01 and 02).

MA-amide, which amounted maximum 6.4% AR in the spirotetramat metabolism study (KIIA 7.1.1/01) and reached maximum 5.2% in the enol study peaked later during the course of the study (at around DAT-4 in the two [redacted] soils, at around DAT-7 in soil [redacted], and already at DAT-1 in most active soil [redacted]). Using the same SFO kinetic modeling, DT_{50} values of 6.8, 1.3, 3.1 and 0.6 days (mean 3.0 days) were calculated in soils [redacted], [redacted], [redacted] and [redacted], respectively. As respective DT_{90} values 22.5, 4.3, 10.2 and 2.1 days (mean 9.8 days) were calculated for the four soils. The respective chi^2 scaled-error values (12.7 – 31.3%) and R^2 values ($\geq$ 0.8602) were considered acceptable regarding the low individual % AR-values measured for this metabolite. As a support, the visual inspection of the fits demonstrated that the SFO fits represented an appropriate kinetic model for the MA-amide dissipation in the four soils.

The respective results of estimating the degradation kinetics of BYI08330-MA-amide in aerobic soil („trigger evaluation“) were summarized in Table IIA 7.2.3-2. The geom. mean normalized laboratory degradation half-live of BYI08330-MA-amide, as needed for estimating e.g. PEC_{Soil}, was estimated to be 1.0 days (see Table IIA 7.2.3-3).

Metabolite BYI08330-methoxy cyclohexanone

Chemical name (CAS): Cyclohexanone, 4-methoxy (CAS No.: 013482-23-0)

Report:	**KIIA 7.2.3/03, [redacted] & [redacted], 2006 (MEF-05/485)**
Title:	**[Carbonyl-^{14}C] 4-Methoxycyclohexanone: Aerobic Soil Degradation in Three Soils**
Report No & Document No	**MEF-05/485 M-269022-02-2**
Guidelines:	EC-Directive 91/414/EEC Annex I Part 7 and Annex II Part 9, OECD Guideline 307: Aerobic and Anaerobic Transformation in Soil (2002)
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of July 2005 to October 2005. Study completion date: 2006-02-28, Amendment No.1 of 2006-07-18

EXECUTIVE SUMMARY

The biotransformation of radiolabeled 4-methoxycyclohexanone was studied in two European soils ([redacted]a: loam / [redacted]: silt loam) and in one US soil ([redacted]: loamy sand), under aerobic laboratory conditions for 14 days ([redacted], [redacted]) and 3 days ([redacted]) at 20 ± 1 °C in the dark. Soil moisture was maintained constant throughout the test, targeting 55% of maximum water holding capacity.

4-Methoxycyclohexanone, a soil photolysis transformation product of spirotetramat, was applied at the nominal rate of 0.133 mg/kg soil, equivalent to a virtual field rate of 0.050 kg/ha (conversion based on homogenous distribution within 2.5 cm topsoil layer, bulk density 1.5 g/cm^3).
The static test systems each containing 100 g of soil dry matter equivalents were attached with solid phase traps for the collection of CO_2 and volatile organics. The test systems of the EU soils were incubated and processed first (the samples were analyzed at 0, 1, 3, 7 and 14 days of incubation). The experience made in these studies led to a shortening of the sampling intervals and an adjustment of the extraction procedure for the later investigated [redacted] soil (sampling intervals were 0, 3, 7 hours and 1, 2, 3 days after treatment). The soil was extracted at room temperature by six cycles of repeated shaking and decanting. The combined extractable residues were analyzed by reversed-phase HPLC-flow-through 14C-radioactivity detection. Exemplarily, result verification was done by normal phase TLC-14C-phosphor-imaging, and identification of 4-methoxy cyclohexanone was verified by co-chromatography.

The material balances of the tested soil systems ranged from 71.3% to 97.8% of AR for [redacted], from 70.4% to 99.0% of AR for [redacted] and from 95.4% to 99.7% of AR for [redacted], respectively. The observed loss at days 1 and 3 clearly was caused by liberation of ^{14}C-carbon dioxide bound in the soil during the extraction procedure using acidic solvent. In order to prevent losses at the following sampling intervals, the first extraction step was performed with test systems closed with a soda lime trap. Accordingly the balances upon analysis were found above 95% of AR.

It is concluded from this study that 4-methoxy cyclohexanone is fast and steadily degrading in soil, mainly to carbon dioxide and partly to several minor transformation products (single peak < 5% of AR) and NER. At study termination the amount of $^{14}CO_2$ ranged from 66.3% to 75.8% of AR. The calculated half-life of 4-methoxy cyclohexanone in the [redacted] soil and the estimated half lives for the EU soils were each lower than 1 day under aerobic laboratory conditions. Thus, there is no potential for accumulation of 4-methoxycyclohexanone residues in viable soils. The observed higher level of 4-

methoxy cyclohexanone residues in the phototransformation laboratory study on soil surface (see KIIA 7.1.3/01) might have been caused by a decreasing viability of test soil due to the irradiation conditions.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BYI08330-methoxy cyclohexanone (CAS name: cyclohexanone, 4-methoxy): Identity and purity of test item in the application solution were checked

Label position:	[Carbonyl-^{14}C]
Sample ID:	BECH 1831 (reference synthesis: KML 3292-3
Specific activity:	4.71 MBq/mg (= 127.31 µCi/mg))
Radiochemical purity:	97.0% (acc. radio-HPLC)
Chemical purity:	96.9% (GC-FID)

2. Soil: The biotransformation of BYI08330-methoxy cyclohexanone was studied in two European soils ([redacted]a: loam, pH 7.0, org. C 1.0% / [redacted]: silt loam, pH 6.8, org. C 2.4%) and in one US soil ([redacted]: loamy sand, pH 6.7, org. C 0.7%), under aerobic laboratory conditions for 14 days ([redacted], [redacted]) and 3 days ([redacted]) at 20 ± 1 °C in the dark. Soil moisture was maintained constant throughout the test, targeting 55% of maximum water holding capacity. All soils were taken freshly from the A horizon (ca. 0-20 cm depth) of their respective sampling area. Soil collection and handling prior to the experimental work were in accordance to ISO 10381-6:1993(E). Stones and plant material were removed, and soil moisture was partially reduced by spreading the soil at ambient temperature to allow for sieving to a particle size of 2 mm. Finally, the soil batches were each mixed thoroughly for optimal batch homogeneity. Initial and final microbial biomass of soils was determined. In case of soil [redacted] the microbial activity was measured only at the start of the study incubation period, since no relevant decline in microbial soil activity was expected within the short experimental phase of three days.

B. STUDY DESIGN

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 20 ±1 °C. The test system consisted of Erlenmeyer flasks (300 mL) attached with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of lipophilic organic volatiles. For preparation of the test systems, 100 g dry matter equivalents of the sieved soils were weighed into the Erlenmeyer flasks. Moisture adjustments to the target test moistures were carried out for each individual flask by addition of de-ionized water, and the vessel initial weights were recorded. All vessels were then fitted with the solid phase traps for volatiles. To allow for soil acclimatization to the intended study incubation conditions, the test systems were placed in the temperature controlled incubation chamber 5 days before application and in case of soil "[redacted]" 10 days before application. For all three soils and all sampling intervals, duplicates were set up and investigated.

BYI08330-methoxy cyclohexanone, a soil photolysis transformation product of spirotetramat, was applied at the nominal rate of 0.133 mg/kg soil, equivalent to a virtual field rate of 0.050 kg/ha (conversion based on homogenous distribution within 2.5 cm topsoil layer, bulk density 1.5 g/cm^3).

2. Sampling: The test systems of the EU soils were incubated and processed first; the experiences made lead to a shortening of the sampling intervals and an adjustment of the extraction procedure for the latter investigated [redacted] soil. Duplicate samples of [redacted] and [redacted] were analyzed at 0, 1, 3, 7 and 14 days of incubation, in case of [redacted] the sampling intervals were 0, 3, 7 hours and 1, 2, 3 days

after treatment.

3. Description of analytical procedures: The soil was extracted at room temperature by six cycles of repeated shaking and decanting, two cycles with acetonitrile/1 M aqueous HCl (1/1, v/v), three cycles with acetonitrile/water/formic acid (100/100/0.5, v/v/v) and one cycle with pure acetonitrile. The combined extractable residues were radio-assayed by LSC and analyzed by reversed-phase high performance liquid chromatography (HPLC-flow-through ^{14}C-radioactivity detection), with exemplary result verification in normal phase thin layer chromatography (TLC-^{14}C-phosphor-imaging). Identification of BYI08330-methoxy cyclohexanone was verified by co-chromatography with an HPLC-NMR characterized ^{14}C-labeled reference item in both chromatography systems.

In order to determine the non-extractable soil residues (NER), the extracted soils were air-dried at room temperature and homogenized by grinding to powder in a mill prior to combustion analysis of aliquots.

4. Determination of degradation kinetics: The data for the test item were evaluated with the software MatLab®, version 7.0.4.365 (The Mathworks). The initial concentration at day 0 was included in the parameter optimization procedure. Based on the chi² error criterion and visual assessment the best fit kinetic model was chosen for the disappearance time evaluation.

For the determination of the degradation kinetics following procedure was followed:

- Values < LOD were set to 0.5 LOD for samples after or before a value > LOD, or for samples between values > LOD. The curve was cut off after the first non-detect.
- Values between LOD and LOQ were set to the measured values.

For the evaluation of the data the following kinetic models were tested in order to find the most suitable approach based on the chi²-error criterion and visual inspection:

Simple first order model (SFO):

$$M_P(t) = M_0 \exp^{(-kt)}$$

$M_P(t)$ = Total amount of chemical present at time t
M_0 = Total amount of chemical present at time t = 0
k = Rate constant

First order multi compartment model (FOMC):

$$M_P(t) = M_0 \left(\frac{t}{\frac{\alpha}{\beta}} + 1\right)^{-\alpha}$$

$M_P(t)$ = Total amount of chemical present at time t
M_0 = Total amount of chemical present at time t = 0
a = Shape parameter determined by CV of k values
b = Location parameter

II. RESULTS AND DISCUSSION

A. DATA

The respective results for the three soils are shown in Table IIA 7.2.3-5 to Table IIA 7.2.3-7.

B. MASS BALANCE

The material balances of the tested soil systems ranged from 71.3 to 97.8% of AR for [redacted], from 70.4 to 99.0% of AR for [redacted] and from 95.4 to 99.7% of AR for [redacted], respectively. The

reason for the losses in the soil systems of [redacted] and [redacted] at days 1 and 3 clearly was caused by liberation of ^{14}C-carbon dioxide bound in the soil during the extraction procedure using acidic solvent. In order to prevent losses, at the following sampling intervals the first extraction step was performed with test systems closed with a soda lime trap. Accordingly the balances upon analysis were found above 95% of AR.

C. BOUND AND EXTRACTABLE RESIDUES

A steady decrease in extractable radioactivity with incubation time was observed for all tested soils. At study termination approximately twice 3% and 12% of AR was still detected in solvent extracts of soils [redacted], [redacted] and [redacted], respectively.

Formation of ^{14}C containing non-extractable soil residues (NER) was observed for the tested soils, reaching maximum rates of about 25% of AR at day 1 in the European soils and 23% of AR at day 2 in the US soil. A slight decrease in NER until study termination demonstrated the steady mineralization of the bound residues.

D. VOLATILIZATION

It can be concluded that 4-methoxycyclohexanone was mainly to $^{14}CO_2$ in soil. At study termination the amount of $^{14}CO_2$ ranged from 66.3 to 75.8% of AR. The chemical identity of $^{14}CO_2$ trapped in the soda lime of the traps was checked for each tested soil by [^{14}C]$BaCO_3$ precipitation. No volatile organics were evolved from the test systems.

E. TRANSFORMATION OF TEST ITEM

BYI08330-methoxy cyclohexanone was shown to be subject to aerobic soil biodegradation. Degradation occurred at a very fast rate under the conditions of this laboratory experiment. This also was supported by the $^{14}CO_2$ formation rates. Except the high amounts of formed $^{14}CO_2$ several minor transformation products (single peak <5% of AR) and NER were found, only.

The calculated half life of BYI08330-methoxy cyclohexanone in the [redacted] soil and the estimated half lives for the EU soils were each lower than 1 day under aerobic laboratory conditions. Thus, there is no potential for accumulation of BYI08330-methoxy cyclohexanone residues in viable soils. The observed higher level of BYI08330-methoxy cyclohexanone residues in the phototransformation laboratory study on soil surface (see KIIA 7.1.3/01) might have been caused by a decreasing viability of test soil due to the irradiation conditions.

Table IIA 7.2.3-5: Biotransformation of BYI08330-methoxy cyclohexanone in loamy sand [redacted] - values expressed as % of AR (MEF-05/485)

	Sampling times, i.e. days after treatment (DAT)					
	0	0.13	0.29	1	2	3
4-methoxy cyclohexanone	99.5 ± 1.5	94.9 ± 0.8	82.1 ± 0.8	22.7 ± 0.6	3.8 ± 0.2	5.6 ± 4.3
ROI 2				4.5 ± 0.2	2.6 ± 0.1	2.5 ± 0.1
ROI 4				3.2 ± 0.7	3.6 ± 0.7	3.4 ± 1.3
Unidentified RA				1.4 ± 0.0		0.8 ± 0.8
Total extracted RA	99.5 ± 1.5	94.9 ± 0.8	82.1 ± 0.8	31.9 ± 0.9	10.0 ± 0.3	12.2 ± 4.7
$^{14}CO_2$	n.a.	2.9 ± 0.5	10.4 ± 0.2	46.6 ± 0.4	62.0 ± 2.1	66.3 ± 0.4
Volatile organics	n.a.	n.d.	n.d.	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
Non extractable residues	0.2 ± 0.0	1.6 ± 0.4	4.8 ± 0.1	18.3 ± 0.6	22.6 ± 0.1	18.9 ± 4.2
Total RA recovery	99.7 ± 1.5	99.3 ± 0.1	97.2 ± 1.0	96.8 ± 0.1	94.7 ± 2.3	97.4 ± 0.3

Blank boxes: values < LOD
n.a.: not analyzed;
n.d.: not detected

Table IIA 7.2.3-6: Biotransformation of BYI08330-methoxy cyclohexanone in loam [redacted] - values expressed as % of AR (MEF-05/485)

	Sampling times, i.e. days after treatment (DAT)				
	0	1	3	7	14
4-methoxy cyclohexanone	98.6 ± 1.3	0.5 ± 0.5			
ROI 2		2.9 ± 0.5			
ROI 3		1.3 ± 0.2	1.5 ± 0.1	1.5 ± 0.8	0.8 ± 0.8
ROI 4		3.6 ± 0.5	3.6 ± 0.2	2.6 ± 0.7	2.1 ± 0.6
Unidentified RA					
Total extracted RA	98.6 ± 1.3	8.3 ± 0.6	5.0 ± 0.0	4.1 ± 0.0	2.8 ± 0.5
$^{14}CO_2$	n.a.	37.4 ± 0.9	49.7 ± 7.1	72.8 ± 1.2	75.8 ± 0.2
Volatile organics	n.a.	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Non extractable residues	0.4 ± 0.1	24.3 ± 0.3	22.6 ± 0.1	20.9 ± 0.0	19.6 ± 0.0
Total RA recovery	99.0 ± 1.4	70.2 ± 0.6	77.5 ± 7.1	97.8 ± 1.1	98.2 ± 0.6

Blank boxes: values < LOD; n.a.: not analyzed; n.d.: not detected

Table IIA 7.2.3-7: Biotransformation of BYI08330-methoxy cyclohexanone in silt loam [redacted] - values expressed as % of AR (MEF-05/485)

	Sampling times, i.e. days after treatment (DAT)				
	0	1	3	7	14
4-methoxy cyclohexanone	94.2 ± 3.0	0.5 ± 0.5			
ROI 1	2.9 ± 0.2				
ROI 2		2.0 ± 0.0			
ROI 3		1.0 ± 0.0	1.4 ± 0.0	1.3 ± 0.6	0.7 ± 0.7
ROI 4		2.7 ± 0.1	3.1 ± 0.1	2.5 ± 0.6	2.1 ± 0.7
Unidentified RA					
Total extracted RA	97.1 ± 2.8	6.2 ± 0.7	4.5 ± 0.1	3.9 ± 0.0	2.8 ± 0.3
$^{14}CO_2$	n.a.	39.7 ± 4.6	55.1 ± 8.8	70.1 ± 1.9	74.1 ± 0.7
Volatile organics	n.a.	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
Non extractable residues	0.7 ± 0.1	24.9 ± 0.1	22.6 ± 0.1	22.3 ± 1.4	20.2 ± 0.1
Total RA recovery	97.8 ± 2.7	70.9 ± 5.2	82.3 ± 8.7	96.4 ± 0.6	97.1 ± 0.5

Blank boxes: values < LOD; n.a.: not analyzed, n.d.: not detected

Table IIA 7.2.3-8: Kinetics results of transformation of BYI08330-methoxy cyclohexanone in soil (MEF-05/485)

Soil	DT_{50} [days]	DT_{90} [days]	Best Fit Kinetic Model (X^2 error)
[redacted]	< 1	--	Not evaluated[1]
[redacted]	1	--	Not evaluated[2]
[redacted]	0.6	1.8	SFO (11.2 %)

III CONCLUSIONS

Based on the results obtained within the present laboratory investigation in three aerobic soils it is concluded that radiolabelled BYI08330-methoxy cyclohexanone is fast and steadily degrading in soil, mainly to $^{14}CO_2$ and partly to several minor transformation products and NER. Since the half-life of BYI08330-methoxy cyclohexanone in aerobic soil is expected to be lower than 1 day, there is no potential for accumulation of BYI08330-methoxy cyclohexanone residues in viable soils. The observed higher level of BYI08330-methoxy cyclohexanone in the laboratory study on phototransformation of BYI08330 on soil surface (see KIIA 7.1.3/01) might have been caused by a decreasing viability of test soil during the strong irradiation in such a laboratory test system.

[1] For the European soils no statistical DT_{50}/DT_{90} values were calculated due to the very short dissipation and therefore the lack of data points after the treatment.

IIA 7.2.4 Anaerobic degradation of the active substance in soil

Report:	**KIIA 7.2.4/01, [redacted], 2006 (MEF-05/515)**
Title:	**BYI08330: Anaerobic Soil Metabolism**
Report No & Document No	**MEF-05/515 M-270739-01-2**
Guidelines:	Official Journal of the EC, Commission Directive, 95/36/EC, amending Council Directive 91/414/EEC: Annexes I+II, Fate and Behavior in the Environment; OECD Guideline for Testing of Chemicals, Guideline 307, Aerobic and Anaerobic Transformation in Soil, Adopted Document; US EPA Subdivision N, Section 162-2; CAN PMRA DACO 8.2.3.4.4; Japanese MAFF New Test Guidelines for Supporting Registration of Chemical Pesticides, 12 Nohsan 8147
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and dates	Bayer CropScience AG, RD, Metabolism and Environmental Fate, D-[redacted], Germany, conducted the study during the period April 2003 to March 2006. Study completion date: 2006-05-03

EXECUTIVE SUMMARY

The aerobic/anaerobic biotransformation of BYI08330 (spirotetramat) in soil under dark laboratory conditions has been investigated in one sandy loam soil using [azaspirodecenyl-3-^{14}C]BYI08330 (label covering the most stable and representative part of the molecule). Samples were incubated for approx one half-life (i.e. 4.8 hours) under aerobic conditions in the dark at 20 °C and about 50 % of maximum water holding capacity. Following the short aerobic phase, the samples were flooded with oxygen-depleted de-ionized water (3 cm layer above soil level), set under nitrogen atmosphere, and maintained in the dark under anaerobic conditions for 180 days at 20 °C.
Applying first order multi compartment (FOMC) kinetics, a DT_{50} value for BYI08330 of 0.06 days and a DT_{90} value of 1.33 days were calculated for the entire system (Chi^2 error value 5.3 %). Other models tested (single first order and double first order parallel) exhibited clearly higher Chi^2 error values of 17.9 and 9.2 %, respectively.

It is concluded that BYI08330 applied to soil will be degraded rapidly in a subsequently flooded anaerobic soil situation, and will not form degradates different from those observed in soil under aerobic conditions, and/or known from abiotic hydrolysis experiments. Table IIA 7.1.2-3 gives a synopsis of results.

I. MATERIALS AND METHODS

A. MATERIALS

Materials used in this study are comprehensively described under Point IIA 7.1.2/01.

B. STUDY DESIGN

The study design used in this study is comprehensively described under Point IIA 7.1.2/01.

II DETERMINATION OF DEGRADATION KINETICS

Applying first order multi compartment (FOMC) kinetics, a DT_{50} value for BYI08330 of 0.06 days and a DT_{90} value of 1.33 days were calculated for the entire system (Chi^2 error value 5.3%). Other models tested (single first order and double first order parallel) exhibited clearly higher Chi^2 error values of 17.9 and 9.2%, respectively. The synopsis in Table IIA 7.1.2-3 contains the kinetics results, also.

III. CONCLUSION

It is concluded that BYI08330 applied to soil will be degraded rapidly in a subsequently flooded anaerobic soil situation, and will not form degradates different from those observed in soil under aerobic conditions, and/or known from abiotic hydrolysis experiments.

IIA 7.2.5 Anaerobic degradation of relevant metabolites in soil

This point is covered by point IIA 7.2.4. There (see study KIIA 7.2.4/01), the parent compound was aged for one half-life in soil. Thus, the relevant metabolites had built up prior to changing the incubation conditions to anaerobic.

IIA 7.3 Field studies

Due to the results of the laboratory soil degradation studies demonstrating the rapid dissipation of spirotetramat and the major metabolites in soil, field studies were not required in the EU. Hence, they were not performed in the EU. According to the tiered evaluation procedures relevant for EU registration the residues of spirotetramat in field soils can be reliably estimated from the data on dissipation in laboratory soils (see IIA 7.2).

However, terrestrial field dissipation studies were performed on four different sites in the USA as part of registration requirements to US-EPA.

IIA 7.3.1 Soil dissipation testing in a range of representative soils

Soil analytical method for terrestrial field dissipation studies

Report:	**KIIA 7.3.1/01, [redacted], D.J., 2006 (RAFNX012)**
Title:	**In House Laboratory Validation Of An Analytical Method For The Determination of BYI08330 And Its Metabolites BYI08330–enol, BYI08330-ketohydroxy And BYI08330-MA-amide In Soil And Sediment by LC/MS/MS**
Report No & Document No	**RAFNX012** **M-277365-01-1**
Guidelines:	U.S. EPA Ecological Effects Test Guidelines OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods and US EPA Residue Chemistry Test Guidelines OPPTS 860.1340 Method Validation
GLP	GLP Compliant meeting the requirements of 40 CFR 160.
Testing Laboratories and dates	Bayer CropScience LP, Environmental Research, [redacted], [redacted], USA; Study completion date: August 29, 2006.

EXECUTIVE SUMMARY

The purpose of this study is to perform an in house laboratory validation (IHLV) of an analytical method (FN-002-S05-02) for the determination of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide in soil and sediment by LC/MS/MS.
Soil and sediment samples were analyzed by liquid chromatography using a triple quadruple mass spectrometry detection system (LC/MS/MS). Isotopically-labeled internal standards were used in the calibration standards and added to the samples prior to analysis to correct for any instrument drift or matrix enhancement or suppression.
The method was validated using one sediment and two soil samples. Residues of BYI8330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide were extracted from soil using an acidic extraction solution in the presence of cysteine hydrochloride and utilizing microwave extraction. The samples were fortified with an isotopic internal standard and an aliquot of the final extract analyzed by LC/MS/MS.
The limit of quatitation (LOQ) was demonstrated to fall at or below the target of 5ng/g for BYI08330 and its metabolites based on five times the standard deviation of the 5 ng/g spiked samples for both soil and sediment. The calculated method detection limits (MDL) are summarized below:

Table IIA 7.3.1-1: Calculated method detection limits, MDL (RAFNX012)

Matrix	Analyte	Calculated MDL (µg/kg)
Soil	BYI08330	0.5
	BYI08330-enol	0.5
	BYI08330-MA-amide	2.1
	BYI08330-ketohydroxy	0.9
Sediment	BYI08330	0.5
	BYI08330-enol	0.6
	BYI08330-MA-amide	2.7
	BYI08330-ketohydroxy	1.0

The mean recovery and relative standard deviation (RSD) found for BYI08330 and its metabolites in soil and sediment based on determinations from two soil samples and one sediment sample, each fortified seven times at the target LOQ, 5ng/g and three times at 5x LOQ, 25ng/g (ppb).

The mean recoveries were all within the limits of 70 to 120% and the precision values as measured by the relative standard deviation are all less than 20%.The calibration was linear over the range from 0.1 to 10ng/mL. Using that calibration range, samples fortified at 5x LOQ were analyzed without dilution. On completion of this study the method was issued and assigned a method number of FN-002-S05-02. This method is suitable for the determination of residues of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide in soil and sediment by LC/MS/MS.

I. MATERIALS AND METHODS

1. Test Items:

Code Name:	**BYI08330**
CAS Number:	[203313-25-1]
Molecular Formula:	C21 H27 N O5
Molecular Weight:	373.44
ID No.:	K-1338
Reference No.:	1105200304
Purity:	99.2%
Expiration Date:	May 02, 2008
Storage Conditions:	Frozen
Source:	[redacted], [redacted]

Code Name:	**$^{13}C_3$-BYI08330 (BYI08330-azaspirodecenyl-2,3,4-$^{13}C_3$)**
CAS Name:	cis-3-(2,5-Dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl-2,3,4-13C3 ethyl carbonate
Molecular Formula:	C_{21} H_{27} N O_5
Molecular Weight:	376.4570
ID No.:	K-1381
E.R. Reference No.:	KML 3386-1-2
Reference No.:	0622200501
Purity:	99.6%
Expiration Date:	June 21, 2015
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	**BYI08330-MA-amide (FHN14065 or AE 1786350)**
CAS Name:	2 cis-1-[[(2,5-Dimethylphenyl)hydroxyacetyl]amino]-4-methoxycyclohexanecarboxylic acid
Molecular Formula:	C_{18} H_{25} N O_5
Molecular Weight:	335.39
ID No.:	K-1387
Reference No.:	0706200414
Purity:	96.1%
Expiration Date:	March 31, 2009
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	**FHN 14065-acetyl-^{13}C ($^{13}C_3$ BYI08330-MA-amide)**
CAS Name:	1-[[(2,5-Dimethylphenyl)hydroxyacetyl-13C2]amino]-4-methoxycyclohexanecarboxylic-13C acid
Molecular Formula:	C_{18} H_{25} N O_5
Molecular Weight:	338.36
ID No.:	K-1384
E.R. Reference No.:	KML-1-4
Reference No.:	0728200501
Chemical Purity:	98.8%
Expiration Date:	July 25, 2015
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	**BYI08330–ketohydroxy**
CAS Name:	cis-3-(2,5-Dimethylphenyl)-3-hydroxy-8-methoxy-1-azaspiro[4.5]decane-2,4-dione
Molecular Formula:	C_{18} H_{23} N O_4
Molecular Weight:	317.3795
ID No.:	K-1339
Reference No.:	1105200305
Chemical Purity:	95.3%
Expiration Date:	10/19/09
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	**$^{13}C_3$-BYI08330-keto-hydroxy**
CAS Name:	3-(2,5-Dimethylphenyl)-3-hydroxy-8-methoxy-1-azaspiro[4.5]decane-2,4-dione-2,3,4-13C3
Molecular Formula:	C_{18} H_{23} N O_4
Molecular Weight:	320.34
ID No.:	K-1383
E.R. Reference No.:	KML 3387-1-8
Reference No.:	0622200503
Purity:	100%
Expiration Date:	June 21, 2015
Date of Analysis:	June 21, 2005
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	**BYI08330–enol**
CAS Name:	cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one
Molecular Formula:	C_{18} H_{23} N O_3
Molecular Weight:	301.3801
ID No.:	K-1337
Reference No.:	1105200303
Purity:	99.4%

Expiration Date:	February 8, 2008
Date of Analysis:	February 8, 2005
Storage Conditions:	Frozen
Source:	[redacted], Germany
Code Name:	**$^{13}C_3$BYI08330-enol**
CAS Name:	cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one-2,3,4-$^{13}C_3$
Molecular Formula:	C_{18} H_{23} N O_3
Molecular Weight:	304.35
ID No.:	K-1382
E.R. Reference No.:	KML 3384-1-1
Reference No.:	0622200502
Purity:	98.3%
Expiration Date:	June 21, 2015
Date of Analysis:	June 21, 2005
Storage Conditions:	Frozen
Source:	[redacted], Germany

2. Test system: Soil samples from New York and Washington terrestrial field dissipation study sites (KIIA 7.3.1/02 and KIIA 7.3.1/05) and sediment samples collected from University of [redacted] were used to validate the BYI08330 soil and sediment analysis method.

3. Principle: Residues of BYI08330 and its metabolites BYI 08330-enol, BYI 08330-ketohydroxy and BYI08330-MA-amide are extracted from soil using an acidic extraction solution in the presence of cysteine hydrochloride and utilizing microwave extraction. The extraction solvent consists of a mixture of water (containing 8 g/L cysteine hydrochloride), acetonitrile, ethyl acetate and formic acid. An aliquot of the final extract is analyzed by LC/MS/MS. Quantification of residues is based on the use of isotopically labeled internal standards and comparison of peak areas with those of known standards.

The final quantitative detection of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide is accomplished by LC/MS/MS detection in Multiple Reaction Monitoring (MRM) mode using instrumentation and conditions summarized in the following Table IIA 7.3.1-2.

Table IIA 7.3.1-2: Instrumentation and conditions (RAFNX012)

Instrument used	Perkin Elmer Sciex API 4000 LC/MS/MS System with Shimadzu LC-10AD VP HPLC pumps (2), Gilson 215 autosampler and a Valco Divert Valve
HPLC Column	e.g. Luna 3u C8(2) 100A, size 50 mm x 2 mm, Part No. 00B4248-B0, Phenomenex, 63741 Aschaffenburg, GER
Injection Volume	80 µL
HPLC Method	Solvent A: 0.5% acetic acid in HPLC grade water; Solvent B: Acetonitrile Flow rate (column): 0.2 mL/min; Flow rate (into MS): 0.2 mL/min Gradient:

Time (min)	0	0.1	2.9	3.0	5.0	7.0	8.1	11.0	11.1	13.0
% A	85.0	85.0	85.0	70.0	55.0	55.0	35.0	35.0	85.0	85.0
% B	15.0	15.0	15.0	30.0	45.0	45.0	65.0	65.0	15.0	15.0

MS/MS System	e.g. API 4000 with turbo-ionspray interface mass selective detector (MS/MS) (Perkin Elmer Sciex Instruments, Weiterstadt, GER)

~ Retention Time (min)	Analyte	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	Parameter	Start	Stop
6.1	BYI 08330-MA-amide	336	290	200	DP	71	71
					CE	22	22
					CXP	15	15
6.1	BYI 08330-MA-amide $^{13}C_3$	339	292	200	DP	71	71
					CE	22	22
					CXP	10	10
6.4	BYI 08330-enol	302	216	200	DP	86	86
					CE	30	30
					CXP	15	15
6.4	BYI 08330-enol -$^{13}C_3$	305	219	200	DP	86	86
					CE	30	30
					CXP	15	15
6.8	BYI08330-ketohydroxy	318	268	200	DP	26	26
					CE	29	29
					CXP	14	14
6.8	BYI08330-ketohydroxy -$^{13}C_3$	321	271	200	DP	26	26
					CE	29	29
					CXP	14	14
8.9	BYI08330	374	302	200	DP	31	31
					CE	23	23
					CXP	16	16
8.9	BYI08330-$^{13}C_3$	377	305	200	DP	31	31
					CE	23	23
					CXP	16	16

Common Parameter (Period 1, Experiment 1)	Parameter	Value
	CAD:	4.0
	CUR:	10.0
	GS1:	40.0
	GS2:	20.0
	IS:	4200.0 volts
	TEM	500° C
	Ihe	on
	EP	10

IS: Ion Spray Voltage	EP: Entrance Potential	CXP: Collision Cell Exit Potential
DP: Declustering Potential	CE: Collision Energy	CAD: Collistion Gas (Collision Activated Dissociation)
CUR: Curtain Gas	GS1: Ion Source Gas 1	GS2: Ion Source Gas 2
TEM: Temperature	Ihe: Interface Heater	
TIS+:	Turbo Ion Source in positive ion mode, i.e. production of positive ions	

II. RESULTS AND DISCUSSION

1. Accuracy and Precision: The precision and accuracy experiments were carried out at the targeted LOQ of 5 ppb and at 5x LOQ of 25 ppb for each analyte. Seven replicates each of the two soil samples and one sediment sample were analyzed after fortification at 5ppb. Three additional replicates of each soil and sediment samples were analyzed after fortification at 25 ng/g for each analyte. The mean recovery values were between the target values of 70% to 120%, and relative standard deviation (RSDs) were below the target value of 20%.

2. Method detection limit and limit of quantitation: Separate method detection limits (MDL) and limits of quantitation were determined for soil and sediment samples. An estimate of the potential method limit of quantitation or method LOQ was determined in this study by examining the variability in the recovery as measured by the standard deviation of the amount found at the level of the target LOQ of 5 ng/g. For each set of soil samples seven samples were fortified at the target LOQ giving a total of 14 analyses. One of the results was rejected as no internal standard peaks were detected in the chromatogram. Therefore, the MDL and LOQ values were calculated using a total of 13 results. A total of seven sediment samples were fortified at the target LOQ.
The LOQ for each analyte was calculated as five times the standard deviation of the concentrations recovered based on the thirteen replicate analyses plus the average apparent residue in the untreated control samples and the estimated potential method detection limit for each analyte was calculated using the equation shown below:

$$\text{MDL(calculated)} = (\text{standard deviation} \times t_{0.99}) + \text{average apparent residue in the untreated control}$$

where $t_{0.99}$ = one-tailed t-statistic at the 99% confidence level for n-1 replicates.

As 13 replicate analyses were performed during the soil validation and the calculated LOQ's and MDL's for BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide are shown in Table IIA 7.3.1-3 below.

Table IIA 7.3.1-3: Results of validation of soil and sediment analytical method (FN-002-S05-02): Calculated MDL and LOQ (RAFNX012)

Matrix	Analyte	Calculated MDL (ng/g)	Calculated LOQ (ng/g)
Soil	BYI08330	0.5	0.9
	BYI08330-enol	0.5	0.9
	BYI08330-MA-amide	2.1	3.9
	BYI08330-ketohydroxy	0.9	1.7
Sediment	BYI08330	0.5	0.8
	BYI08330-enol	0.6	1.0
	BYI08330-MA-amide	2.7	4.4
	BYI08330-ketohydroxy	1.0	1.5

III. CONCLUSIONS

The Bayer CropScience analytical method, FN-002-S05-02 is suitable for the determination of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide in soil and sediment by LC/MS/MS. This method was successfully validated for the determination of

residues of BYI08330 and its metabolites in soil and sediment. The method was evaluated by determining the average recoveries and relative standard deviation at the LOQ of 5 ng/g and at 5x LOQ (25 ng/g). The average recoveries for all analytes were between 70 and 120 percent with a relative standard deviation of less than 20%. The method was shown acceptable of quantifying BYI08330 and its metabolites at the targeted LOQ of 5 ng/g and is suitable for analyzing soil samples from terrestrial field dissipation studies.

Soil dissipation studies

Report:	**KIIA 7.3.1/02; [redacted], M., 2006 (MEFNY004)**
Title:	**Terrestrial Field Dissipation of BYI 08330 in New York Soil, 2004**
Report No & Document No.	**MEFNY004** **M-277191-01-1**
Guidelines:	EPA Guideline Ref. No.: 164-1 Terrestrial Field Dissipation; PMRA Data Code No.: 8.3.2.1
GLP	GLP Compliant meeting the requirements of 40 CFR 160. Exception: weather data, soil moisture data, kinetic modeling, control soil collected before study initiation and standards used before re-certification results were finalized.
Testing Laboratories and dates	Bayer CropScience LP., Environmental Research, [redacted], [redacted], USA; AGVISE Laboratories, [redacted], North Dakota, USA; A.C.D.S. [redacted] New York, USA Field Phase of the study conducted between June 2004 to December 2005 Sample analysis completion date: June 09, 2006 Study completion date: September 05, 2006

Report:	**KIIA 7.3.1/03; [redacted], M., 2006 (MEFNX055)**
Title:	**Terrestrial Field Dissipation of BYI 08330 in Florida Soil, 2004**
Report No & Document No.	**MEFNX055** **M-277184-01-1**
Guidelines:	EPA Guideline Ref. No.: 164-1 Terrestrial Field Dissipation; PMRA Data Code No.: 8.3.2.1
GLP	GLP Compliant meeting the requirements of 40 CFR 160. Exception: weather data, soil moisture data, kinetic modeling, control soil collected before study initiation and standards used before re-certification results were finalized.
Testing Laboratories and dates	Bayer CropScience LP., Environmental Research, [redacted], [redacted], USA; AGVISE Laboratories, [redacted], North Dakota, USA; Florida [redacted] Field Phase of the study conducted between April 2004 to October 2005 Sample analysis completion date: June 22, 2006 Study completion date: September 05, 2006.

Report:	**KIIA 7.3.1/04; [redacted], M., 2006 (MEFNY002)**
Title:	**Terrestrial Field Dissipation of BYI 08330 in California Soil, 2004**
Report No & Document No.	**MEFNY002** **M-277186-01-1**
Guidelines:	EPA Guideline Ref. No.: 164-1 Terrestrial Field Dissipation; PMRA Data Code No.: 8.3.2.1
GLP	GLP Compliant meeting the requirements of 40 CFR 160. Exception: weather data, soil moisture data, kinetic modeling, control soil collected before study initiation and standards used before re-certification results were finalized.
Testing Laboratories and dates	Bayer CropScience LP., Environmental Research, [redacted], [redacted], USA; AGVISE Laboratories, [redacted], North Dakota, USA; Bayer CropScience, [redacted], California, USA. Field Phase of the study conducted between May 2004 to November 2005 Sample analysis completion date: July 13, 2006 Study completion date: September 05, 2006.

Report:	**KIIA 7.3.1/05; [redacted], M., 2006 (MEFNY003)**
Title:	**Terrestrial Field Dissipation of BYI 08330 in Washington Soil, 2004**
Report No & Document No.	**MEFNY003** **M-277189-01-1**
Guidelines:	EPA Guideline Ref. No.: 164-1 Terrestrial Field Dissipation; PMRA Data Code No.: 8.3.2.1
GLP	GLP Compliant meeting the requirements of 40 CFR 160. Exception: weather data, soil moisture data, kinetic modeling, control soil collected before study initiation and standards used before re-certification results were finalized.
Testing Laboratories and dates	Bayer CropScience LP., Environmental Research, [redacted], [redacted], USA; AGVISE Laboratories, [redacted], North Dakota, USA; Qualls Agricultural Laboratory, Inc., Ephrata, Washington. Field Phase of the study conducted between June 2004 to November 2005 Sample analysis completion date: June 30, 2006 Study completion date: September 05, 2006.

EXECUTIVE SUMMARY

Terrestrial field dissipation of BYI08330 and its major metabolites were conducted in four sites in the US. The objective was to evaluate the dissipation and mobility of BYI08330 under actual field use conditions. Environmental fate laboratory studies (see Sections IIA 7.1) showed that parent BYI08330 and the transformation products BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide should be considered to determine the magnitude and distribution of residues of BYI08330 in soil.

The target rate of application for all the four sites was 438 g as/ha, which corresponds to the maximum seasonal application rate proposed in the US. BYI08330, formulated as BYI08330 100 OD, was applied on three replicate plots at each site. While the New York site had only bare ground plots, the Florida, California and Washington sites has bare ground as well as cropped plots. In addition a control plot was maintained at each site, away from the treated plots. Applications to the treated plots were verified by

using Whatman filter paper and soil pan application monitors. An average of 80% to 97% of active ingredient equivalent was recovered from soil pans on the bare ground plots, and 83% to 98% on the cropped plots. On the filter paper application monitors (saturation pads) an average of 85% to 132% of active ingredient equivalent was recovered on bare ground plots and 82% to 119% on the cropped plots.

The field dissipation studies were conducted for 540 days in all the four locations, with nominal soil sampling times of 0, 1, 3, 7, 14, 28, 60, 120, 179, 289, 365 and 540 days after application to a depth of 0-122 cm in 15 cm increments. The actual sampling times varied with each site. The soil samples were extracted using a microwave extractor at a maximum of 800 watts (70° C) with a mixture of acetonitrile, cysteine hydrochloride and ethyl acetate. Residues of BYI08330, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide were analyzed by HPLC/MS/MS (summarized at the beginning of this section). The method detection limit (MDL) for each analyte in soil was 0.5, 0.5, 0.9 and 2.1 µg /kg, respectively. The target limit of quantitation (LOQ) was 5 µg /kg for each analyte.

The average zero-time concentration of BYI08330 in the 0-15 cm soil segment was 37.9 to 100.0 µg a.i./kg in bare ground plots and 59.1 to 89.4 µg a.i./kg in cropped plots. BYI08330 degraded rapidly in both bare ground and cropped plots below detection limits within 14 days after application at all the four sites. The major transformation products were BYI08330-enol and BYI08330-ketohydroxy. Residues of BYI08330 or its transformation products above the limit of quantitation (5 µg/kg) were not detected below the surface soil layer (0-15 cm) at New York (bare ground), California (bare ground and cropped) and Washington (bare ground and cropped) sites. At the Florida site (bare ground and cropped plots) BYI08330 residues above the limit of quantitation were not detected below the 15 - 30 cm soil layer. It should be noted that the Florida site represents a worst case condition with heavy rainfall and very light soil (95% sand in the surface layer) with very low organic matter (0.5 %).

A kinetics modeling tool was used to characterize the dissipation of BYI08330 residues in the field. The first-order dissipation half-life (DT_{50}) in the bare ground plots ranged from 0.4 to 1.0 days. The DT_{50} values (0.3 to 1.0 days) in the cropped plots were not significantly different from the values from the bare ground plots. The DT_{50} of the combined residues (BYI08330, BYI08330-enol, and BYI08330-ketohydroxy), expressed as BYI08330 equivalents ranged from 6.3 to 23.4 days in bare ground plots and 5.0 to 10.2 days in cropped plots.

As seen in the laboratory environmental fate studies, residues of BYI08330 dissipated rapidly under field conditions without possibility of carry-over to the next season. The residues were generally confined to the upper 30 cm of the soil even under worst case conditions and therefore leaching and groundwater contamination is not likely with BYI08330. Considering the results from laboratory soil metabolism studies and terrestrial field dissipation studies the major route(s) of dissipation for BYI08330 are degradation to BYI08330-enol and BYI08330-ketohydroxy, subsequent biodegradation to non-extractable soil residues and mineralization to CO_2.

I. MATERIALS AND METHODS

1. Test Material: BYI08330 (Spirotetramat)

Formulation:	formulated as oil dispersible (OD) BYI08330 OD 100
Appearance:	Brown suspension
Density:	0.981 g/ml
Lot/Batch #:	08030/0110(0073)
Purity:	102 g/L of active ingredient, BYI08330

The stability of test compound had been approved for storage in room temperature until February 23, 2005 at room temperature.

2. Test sites and Programme of Studies: The terrestrial field dissipation studies were conducted in four different sites in the US, as representative growing regions of the major crops for which BYI08330 is labelled for. The study programmes were designed to determine the residue levels of BYI08330 and its major metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide and to provide estimates of the dissipation time (DT_{50} and DT_{90}) of BYI08330 under field conditions. A summary of the terrestrial field dissipation programme is provided in Table IIA 7.3.1-4 and the management history at each site is provided in Table IIA 7.3.1-5. Table IIA 7.3.1-6 summarizes the physical characteristics of the treatment fields and the physicochemical properties of the surface soil at each site.

3. Experimental treatments: BYI08330 formulated as BYI08330 100 OD (102 g a.s./L) was applied at the maximum recommended rate of application of 438 g a.s./ha to bare ground plots at all the four sites in the US. In addition the Florida, California and Washington sites had additional treatment plots with crops. The treatment area in each of the sites was divided into three subplots of equal size – bare ground and cropped plots, where applicable. In addition a control plot was established sufficiently away from the treated plots, which was managed similar to the treated plots except for the application of BYI08330 spray mix.

BYI08330 was applied to bare ground at the proposed label rate of 439 g a.s./ha as a liquid spray, which is typical for the product. The OD formulation was stored at ambient temperature from receipt until use. A boom sprayer was equipped with flat fan nozzles spaced 20 inches (50.8 cm) apart and set at 15 to 28 inches (38.1 to 71 cm) above the ground. The tractor speed and nozzle output were calibrated prior to application to yield 20 to 25 gallons per acre (187 to 234 L/ha).

In the cropped plots, the seeds were sown several days before application and the product was applied either before the crop emerged or shortly after emergence. Therefore, no significant crop interception can be expected in the cropped plots because majority of the sprayed substance was expected to reach the soil.

4. Application verification: Application to the treatment plots were verified using saturation pads and soil pans. The saturation pads consisted of either two Whatman 24 cm diameter filter papers or two Gelman solvent saturation pads (13.7 cm × 22 cm) placed on cardboard squares or aluminium trays. The pads were then placed at one subsection per subplot before the application. Immediately after application each pair of pads were collected and placed in labelled bottles. The samples were stored frozen until analysis. The soil pans consisted of a layer of pre-weighed, sieved and air dried control soil in 9.5 × 13 inch (24.13 × 33.02 cm) rectangular or 12 inch diameter (30.5 cm) or 7 inch diameter (17.8 cm) circular aluminium trays. One soil pan was placed in each subplot prior to application. Immediately after application soil from the pans were transferred to a labelled bottle. The soil samples were stored frozen until analysis. The saturation pads and soil pans received the same application as that of the treatment plots.

Table IIA 7.3.1-4: Programme (2004-2005) of US field dissipation studies with BYI08330

Site	Cropping	Treatment plot sizes (m × m)	Application Rate (g a.s./ha)	Start and End Dates for Field Phase (days)	Report and Author
New York	Bare ground	25.9 × 4.6	438.0	June 14, 2004 - December 8, 2005	KIIA 7.3.1/02; [redacted], M., 2006
Florida	Bare ground Cropped (Bush bean)	36.6 × 3.0 36.6 × 3.0	438.0	April 27, 2004 – October 19, 2005	KIIA 7.3.1/03; [redacted], M., 2006
California	Bare ground Cropped (Germain's Seed)	90.0 × 6.0 90.0 × 6.0	438.0	May 26, 2004 – November 15, 2005	KIIA 7.3.1/04; [redacted], M., 2006
Washington	Bare ground Cropped (Yellow Sweet Spanish onions)	45.7 × 18.3 45.7 × 18.3	438.0	June 8, 2004 – November 28, 2005	KIIA 7.3.1/05; [redacted], M., 2006

Table IIA 7.3.1-5: Management history of the test sites in the previous three years

Site	Crops grown	Pesticides used	Fertilizers used	Report and Author
New York	Alfalfa and corn	Fusilade DX 2EC; Balan 60DF; Buctril 2EC; Pursuit 2AS; Baythroid 2EC; Post Plus 1EC; Kerb 50 WP; Sencor 75 DF; Baythroid 2L; Velpar 2L	Information not available	KIIA 7.3.1/02; [redacted], M., 2006
Florida	Sweet corn	Warrior (Lambda cyhalothrin)	10-10-10 and 34-0-0	KIIA 7.3.1/03; [redacted], M., 2006
California	None	Scala (pyrimethanil), Roundup (glyphosate)	Information not available	KIIA 7.3.1/04; [redacted], M., 2006
Washington	Wheat and potato	Assure II; Gramoxone Extra; Roundup Ultra; Fusilade; Goal; Touchdown; Buctril; Poast	Information not available	KIIA 7.3.1/05; [redacted], M., 2006

Table IIA 7.3.1-6: Site physical and soil characteristics

Characteristic	New York	Florida	California	Washington
Report and Author	KIIA 7.3.1/02; [redacted], M., 2006	KIIA 7.3.1/03; [redacted], M., 2006	KIIA 7.3.1/04; [redacted], M., 2006	KIIA 7.3.1/05; [redacted], M., 2006
Location:				
Latitude	[redacted]	[redacted]	[redacted]	[redacted]
Longitude	[redacted]	[redacted]	[redacted] W	[redacted]
Ecoregion	8.1	8.5	11.1	10.1
Slope gradient (%)	0.0	≤ 1%	0%	0 – 1%
Depth to ground water level	> 6 ft (1.8 m)	36 to 48" (0.9 – 1.2 m)	≥ 30 ft (9.1 m)	22.9 m ± 3 m
Soil				
Series name	Oakville series	St. Johns – Malabar-Wabasso complex	Hanford Series	Timmerman series
Taxonomic class	Mixed, mesic Typic Udipsamments	Sandy to loamy, siliceous, hyperthermic Typic Alaquods (St. Johns), hyperthermic Alfic Alaquods (Wabasso), and hyperthermic Grossarenic Endoaqualfs (Malabar)	Coarse-loamy, mixed, superactive, nonacid, thermic Typic Xerorthents	Mixed, Haplcambids, Aridisols
Surface soil texture (USDA)	Loamy sand	Sand	Sandy loam	Sandy loam
Average from all treatment plots				
Sand (%)	85	95	69	75
Silt (%)	6	3	23	20
Clay (%)	9	2	8	5
pH				
1:1 soil:water	6.3	7.1	7.0	8.1
0.01 M $CaCl_2$	5.5	N/A	N/A	N/A
Organic carbon (%)	1.3	0.4	0.3	0.4
Disturbed bulk density (g/cm^3)	1.4	1.4	1.46	1.45

5. Sampling and sample processing: Each treatment subplot (bare ground our cropped) divided into sampling subsections. Five cores from a randomly selected subsection were sampled from each subplot at 4 days prior to application. Subsequent samplings at the four sites were taken on the following days:

1. New York: 0, 1, 3, 7, 14, 28, 60, 121, 179, 289, 365, and 542 days after application.
2. Florida: 0, 1, 3, 7, 14, 28, 59, 126, 185, 266, 367, and 540 days after application.
3. California: 0, 1, 2, 7, 14, 28, 62, 120, 187, 275, 357 and 538 days after application.
4. Washington: 0, 1, 3, 7, 14, 28, 58, 120, 175, 269, 363, and 538 days after application.

Each soil core consisted of two segments, a 0-15 cm and a 15-122 cm. All samples were frozen and shipped by freezer truck to the analytical laboratory. Samples were stored under freezer conditions at the analytical laboratories. The 15-122 cm core was segmented at every 15 cm to the depth of the core. Segments from the same depth from each subplot were composited into one sample per depth. The composited samples from each sampling interval were homogenized from the deepest to the shallowest soil depth. Each composited soil sample was milled with dry ice by a hammer mill into a clean plastic

bag directly from the grinder. The milled sample was mixed using a bucket mixer equipped with fixed inner paddles.

6. Irrigation: Supplemental irrigation was applied to the field to maintain approximately 122% of the historical precipitation. Air temperature and precipitation data were recorded from a weather station located at each of the test site. In addition, supplemental weather data such as radiation, relative humidity, and wind speed were either collected from on-site weather station or from a National Oceanic and Atmospheric Administration (NOAA) weather station located close to the test site. Table IIA 7.3.1-7 summarizes the source of primary and supplemental weather data at each site. In addition, soil moisture was monitored by ML2x Theta Probes (Dynamax Inc.) at a depth of 2.0 inches (5.08 cm) and 12 inches (30.5 cm) in the bare ground plot.

Table IIA 7.3.1-7: Summary of source of weather data at the terrestrial field dissipation test sites

Site	Source of Primary Weather data (Rainfall and air temperature)	Source of supplemental weather data	Target water input (natural precipitation + irrigation)
New York	On-site	[redacted], NY (NOAA), 5 miles (8 km) from test site	122% of historical precipitation
Florida	On-site	[redacted], FL (NOAA); 20 miles (32 km) from test site	134% of 29-year historical precipitation
California	On-site	[redacted] International Airport; 13 miles (21 km) from test site	144% of 30-year evapotranspiration; 16 times the annual precipitation
Washington	On-site	[redacted], WA (NOAA); 22 miles (35 km) from test site	135% of 30-year evapotranspiration; 9 times the annual precipitation

6. Description of analytical procedure: The analytical procedure used in the analysis of the soil samples from the field dissipation studies is described in the beginning of this section. Residues of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide are extracted from soil using an acidic extraction solution in the presence of cysteine hydrochloride and utilizing microwave extraction. The extraction solvent consists of a mixture of water (containing 8 g/L cysteine hydrochloride), acetonitrile, ethyl acetate and formic acid. An aliquot of the final extract is analyzed by LC/MS/MS. Quantification of residues is based on the use of isotopically labeled internal standards and comparison of peak areas with those of known standards. The method detection limit (MDL) for each analyte in soil was 0.5, 0.5, 0.9 and 2.1 µg /kg, respectively for BYI08330, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide. The target limit of quantitation (LOQ) was 5 µg /kg for each analyte.

7. Determination of dissipation rate of BYI08330 residues: The dissipation of BYI08330, BYI08330-enol and BYI08330-ketohydroxy under terrestrial field conditions was characterized using kinetics modeling. A kinetics modeling tool that was built within the frame-work of mathematical software, MATLAB (Ver. 7.0.4) was used for fitting the kinetics models to the experimental data. The goodness of fit was assessed by visual inspection and an error criterion based on a chi-square (χ^2) significance test. In addition to these, coefficient of determination (r^2) was also used as a secondary measure of goodness of fit.

Soil concentrations above the MDL were considered without change for kinetics modeling. The residue concentrations from all the soil layers that were above the MDL were added and used in the kinetics

modelling and value that were lower than MDL were set to zero. For kinetics modeling, concentrations of the metabolites were converted to parent equivalents, using appropriate molecular mass of the compounds. Field dissipation rates for BYI08330 and combined residues BYI08330 were calculated.

II. RESULTS AND DISCUSSION

A. APPLICATION VERIFICATION

The nominal application rate for the treatments corresponded to the maximum seasonal application rate of 438 g/ha. Application verification solvent saturation pads showed an average parent equivalent recovery ranging from 85% to 132% of the nominal application rate from the bare ground plots and 82% to 119% of the nominal application rate from cropped plots. And the soil pans showed an average parent equivalent recovery of 80 to 96% of the nominal application rate from the bare ground plot and 83 to 98% pf the nominal application rate from the cropped plots. The following table summarizes the application verification results from each site.

Table IIA 7.3.1-8: Summary of recoveries from application verification saturation pads and soil pans

Site	Recovery from saturation pads (%)	Recovery from soil pans (%)
New York	94	96
Florida		
Bare ground	85	95
Cropped	82	98
California		
Bare ground	132	97
Cropped	119	89
Washington		
Bare ground	98	80
Cropped	106	83

B. MASS ACCOUNTING

The total recovery of BY08330 in this field study is limited to the recovery of the analytes of concern which are parent, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide. Table IIA 7.3.1-9 through Table IIA 7.3.1-14 show the measured soil concentrations of BYI08330 residues at the bare ground plots at the four field dissipation study sites. BYI08330 rapidly dissipated in soil in less than 14 days. From the results of dissipation studies, it can be demonstrated that biodegradation is the primary route of dissipation; however the mass balance is limited to the recovery of parent, BYI 08330-enol, BYI08330-ketohydroxy and BYI 08330 MA-amide.

Among the measured bulk densities of the soils in the test sites it should also be noted that the measured bulk density (1.0 g/cm^3) for the Florida soil appears to be an outlier. According to the USDA soil properties database, STATSGO, the bulk densities of the soils in New York, Florida, California and Washington test sites are 1.42, 1.4, 1.55 and 1.3 g/cm^3, respectively. Considering the bulk densities from STATSGO database the theoretical soil concentrations in the 0 to 15 cm layer range from 188 to 225 µg/kg in the treated plots. The initial (Day 0) combined residues of parent BYI08330 and the parent-equivalents of BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide in the 0 to 15 cm layer accounted for 48% to 68% and 53% to 72% of theoretical soil concentration in bare ground and cropped plots respectively. The aerobic soil metabolism study of BYI08330 (Section IIA 7.1.1) shows that biodegradation to non-extractable residue is a significant route of dissipation, which accounted for

up to 35.2% of total applied radioactivity after 3 days of incubation. Also, up to 19.4% of applied radioactivity was mineralized to CO_2 after only 50 days. The observed difference between the theoretical and actual initial soil concentration can therefore be attributed to rapid biodegradation to non-extractable residues or mineralization to CO_2, both of which were not within the scope of the soil analysis.

BYI08330 is not volatile as indicated by a vapour pressure of $1.5x10^{-10}$ Pa at 25 °C (1.12×10^{-10} mmHg). Therefore, volatilization is not a significant dissipation route.

Leaching was evaluated by this terrestrial field dissipation study using 122 cm soil cores to adequately monitor potential movement of the test analytes through the soil profile. It was shown BYI08330 and its transformation products did not move below 15 cm in New York, California and Washington sites. In the Florida site residues of BYI08330-enol and BYI08330-ketohydroxy were detected above the LOQ at 15 – 30 cm layer between 1 day and 7 days after application. After that the residues completed degradation to less than LOQ and MDL. Therefore, leaching is not a dissipation route.

Table IIA 7.3.1-9 through Table IIA 7.3.1-15 shows that BYI08330 nearly completely degrades to BYI08330-enol and BYI08330-ketohydroxy within 14 days after treatment. Analyzing the measured residues of BYI08330 from 1 to 14 days after application, greater than 78% to 100% of the measured initial concentration of BYI08330 can be accounted for by converting the transformation products. This clearly shows that degradation of BYI08330 to its metabolites is a major route of dissipation observed at the test sites.

It was also observed that there was no significant difference between bare ground and cropped plots.

Table IIA 7.3.1-9: Average soil concentrations of BYI08330 residues from treated bare

ground plots in New York

Compound	Depth (cm)	Days After Treatment (DAT)												
		-4	0	1	3	7	14	28	60	121	179	289	365	542
		Average Concentration Found in Three Subplots (µg/kg; dry weight)												
BYI08330	0-15	n.d.	74.1	17.7	<LOQ (2.2)	<LOQ (1.3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60												n.d.	n.d.
	60-75													
	75-90													
	90-105													
	105-120													
	Total	n.d.	74.1	17.7	<LOQ (2.2)	<LOQ (1.3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BYI08330-Enol	0-15	n.d.	8.7	46.0	40.3	32.2	39.5	16.8	14.3	8.3	5.8	11.6	4.8	4.2
	15-30			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60												n.d.	n.d.
	60-75													
	75-90													
	90-105													
	105-120													
	Total	n.d.	8.7	46.0	40.3	32.2	39.5	16.8	14.3	8.3	5.8	11.6	4.8	4.2
BYI08330-Keto-hydroxide	0-15	n.d.	12.2	26.0	24.5	22.6	16.9	12.0	<LOQ (3.0)	<LOQ (1.5)	<LOQ (1.3)	<LOQ (1.8)	<LOQ (1.2)	n.d.
	15-30			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60												n.d.	n.d.
	60-75													
	75-90													
	90-105													
	105-120													
	Total	n.d.	12.2	26.0	24.5	22.6	16.9	12.0	<LOQ (3.0)	<LOQ (1.5)	<LOQ (1.3)	<LOQ (1.8)	<LOQ (1.2)	n.d.
BYI08330-MA-amide	0-15	n.d.	n.d.	n.d.	<LOQ (2.7)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60												n.d.	n.d.
	60-75												n.d.	n.d.
	75-90													
	90-105													
	105-120													
	Total	n.d.	n.d.	n.d.	<LOQ (2.7)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Method Detection Limit (MDL) = BYI08330 0.5 ng/g
BYI08330-enol 0.5 ng/g
BYI08330-MA-amide 2.1 ng/g
BYI08330-ketohydroxy 0.9 ng/g

Limit of Quantitation (LOQ) = 5 ng/g

n.d. = not detected, below method detection limit

Note: Concentrations in each soil layer are added for convenience in representing dissipation in the soil column. The sum is not intended to express a concentration by depth, but rather approximate a concentration if all residues measured were confined to a single soil layer.

Table IIA 7.3.1-10: Average soil concentrations of BYI08330 residues from treated bare

ground plots in Florida

Compound	Depth (cm)	Days After Treatment (DAT)									
		-4	0	1	3	7	14	28	59	126	185
		Average Concentration Found in Three Subplots (ng/kg; dry weight)									
BYI08330	0-15	n.d.	37.9	23.5	9.13	<LOQ (1.3)	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		25.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		<LOQ (2.7)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.	n.d.	n.d.	n.d.				
	60-75										
	75-90										
	90-105										
	105-120										
	Total	n.d.	37.9	51.7	9.13	1.3	n.d.	n.d.	n.d.	n.d.	n.d.
BYI08330-Enol	0-15	n.d.	28.5	51.7	9.13	1.3	1.27	5.2	5.1	3.2	n.d.
	15-30	n.d.		51.7	n.d.	3.2	<LOQ (2.0)	n.d.	<LOQ (0.8)	n.d.	n.d.
	30-45	n.d.		<LOQ (1.8)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.	n.d.	n.d.	n.d.				
	60-75										
	75-90										
	90-105										
	105-120										
	Total	n.d.	28.5	30.3	15.4	19.8	14.5	5.2	5.9	3.2	n.d.
BYI08330-ketohydroxy	0-15	n.d.	32.6	30.3	15.4	19.8	14.5.0	<LOQ (4.1)	<LOQ (2.3)	n.d.	n.d.
	15-30	n.d.		30.3	n.d.	7.2	<LOQ (2.7)	<LOQ (3.8)	<LOQ (1.4)	n.d.	n.d.
	30-45	n.d.		<LOQ (1.4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.	n.d.	n.d.	n.d.				
	60-75										
	75-90										
	90-105										
	105-120										
	Total	n.d.	32.6	46.3	38.3	26.9	17.7	7.9	3.7	n.d.	n.d.
BYI08330-MA-amide	0-15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.	n.d.	n.d.	n.d.				
	60-75										
	75-90										
	90-105										
	105-120										
	Total	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Method Detection Limit (MDL) = BYI08330 0.5 ng/g
BYI08330-enol 0.5 ng/g
BYI08330-MA-amide 2.1 ng/g
BYI08330-ketohydroxy 0.9 ng/g
Limit of Quantitation (LOQ) = 5 ng/g
n.d. = not detected, below method detection limit
Note: Concentrations in each soil layer are added for convenience in representing dissipation in the soil column. The sum is not intended to express a concentration by depth, but rather approximate a concentration if all residues measured were confined to a single soil layer.

Table IIA 7.3.1-11: Average soil concentrations of BYI08330 residues from treated cropped plots in Florida

Compound	Depth (cm)	Days After Treatment (DAT)									
		-4	0	1	3	7	14	28	59	126	185
		Average Concentration Found in Three Subplots (µg/kg; dry weight)									
BYI08330	0-15	n.d.	59.1	35.2	18.2	<LOQ (0.8)	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		35.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		<LOQ (2.7)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.	n.d.	n.d.	n.d.	n.d.			n.d.
	60-75						n.d.				
	75-90						n.d.				
	90-105						n.d.				
	105-120						n.d.				
	Total	n.d.	59.1	70.7	18.2	0.8	n.d.	n.d.	n.d.	n.d.	n.d.
BYI08330-Enol	0-15	n.d.	33.3	22.6	16.1	8.6	7.1	6.7	<LOQ (3.5)	<LOQ (3.1)	<LOQ (1.9)
	15-30	n.d.		12.9	n.d.	8.9	<LOQ (2.8)	n.d.	<LOQ (1.1)	<LOQ (1.2)	<LOQ (1.0)
	30-45	n.d.		n.d.	n.d.	<LOQ (0.8)	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.	n.d.	n.d.	n.d.	n.d.			n.d.
	60-75						n.d.				
	75-90						n.d.				
	90-105						n.d.				
	105-120						n.d.				
	Total	n.d.	33.3	35.5	16.1	17.5	9.4	6.7	4.6	4.3	2.9
BYI08330-Keto-hydroxide	0-15	n.d.	41.8	30.5	46.4	19.3	13.3	5.5	<LOQ (1.8)	<LOQ (0.7)	n.d.
	15-30	n.d.		17.1	n.d.	17.7	<LOQ (4.5)	n.d.	<LOQ (1.7)	<LOQ (0.7)	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.	n.d.	n.d.	n.d.	n.d.			n.d.
	60-75						n.d.				
	75-90						n.d.				
	90-105						n.d.				
	105-120						n.d.				
	Total	n.d.	41.8	47.6	46.4	37.0	17.8	5.5	3.5	1.4	n.d.
BYI08330-MA-amide	0-15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.	n.d.	n.d.	n.d.	n.d.			n.d.
	60-75						n.d.				
	75-90						n.d.				
	90-105						n.d.				
	105-120						n.d.				
	Total	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Method Detection Limit (MDL) = BYI08330 0.5 ng/g
BYI08330-enol 0.5 ng/g
BYI08330-MA-amide 2.1 ng/g
BYI08330-ketohydroxy 0.9 ng/g

Limit of Quantitation (LOQ) = 5 ng/g

n.d. = not detected, below method detection limit

Note: Concentrations in each soil layer are added for convenience in representing dissipation in the soil column. The sum is not intended to express a concentration by depth, but rather approximate a concentration if all residues measured were confined to a single soil layer.

Table IIA 7.3.1-12: Average soil concentrations of BYI08330 residues from treated bare ground plots in California

Compound	Depth (cm)	Days After Treatment (DAT)								
		-1	0	1	2	7	14	28	62	120
		Average Concentration Found in Three Subplots (ng/kg; dry weight)								
BYI08330	0-15	n.d.	100.0	68.0	23.4	<LOQ (0.6)	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.		n.d.	n.d.	n.d.	n.d.	n.d.
	Total	n.d.	100.0	68.0	23.4	<LOQ (0.6)	n.d.	n.d.	n.d.	n.d.
BYI08330-Enol	0-15	n.d.	<LOQ (2.6)	7.0	13.6	13.1	9.4	5.1	<LOQ (2.9)	<LOQ (0.8)
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.		n.d.	n.d.	n.d.	n.d.	n.d.
	Total	n.d.	<LOQ (2.6)	7.0	13.6	13.1	9.4	5.1	<LOQ (2.9)	<LOQ (0.8)
BYI08330-Ketohydroxy	0-15	n.d.	9.8	46.9	52.6	38.9	22.2	11.8	<LOQ (3.7)	<LOQ (1.2)
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60			n.d.		n.d.	n.d.	n.d.	n.d.	n.d.
	Total	n.d.	9.8	46.9	52.6	38.9	22.2	11.8	<LOQ (3.7)	<LOQ (1.2)
BYI08330-MA-amide	0-15	n.d.	n.d.	n.d.	<LOQ (2.3)	<LOQ (3.6)	n.d.	<LOQ (2.7)	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60					n.d.	n.d.	n.d.	n.d.	n.d.
	Total	n.d.	n.d.	n.d.	<LOQ (2.3)	<LOQ (3.6)	n.d.	<LOQ (2.7)	n.d.	n.d.

Method Detection Limit (MDL) = BYI08330 0.5 ng/g
BYI08330-enol 0.5 ng/g
BYI08330-MA-amide 2.1 ng/g
BYI08330-ketohydroxy 0.9 ng/g

Limit of Quantitation (LOQ) = 5 ng/g
n.d. = not detected, below method detection limit
Note: Concentrations in each soil layer are added for convenience in representing dissipation in the soil column. The sum is not intended to express a concentration by depth, but rather approximate a concentration if all residues measured were confined to a single soil layer.

Table IIA 7.3.1-13: Average soil concentrations of BYI08330 residues from treated cropped plots in California

Compound	Depth (cm)	Days After Treatment (DAT)								
		-1	0	1	2	7	14	28	62	120
		Average Concentration Found in Three Subplots (µg/kg; dry weight)								
BYI08330	0-15	n.d.	89.4	55.9	19.0	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		<LOQ (1.8)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		<LOQ (0.6)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.			n.d.	n.d.	n.d.		n.d.	n.d.
	Total	n.d.	89.4	58.3	19.0	n.d.	n.d.	n.d.	n.d.	n.d.
BYI08330-Enol	0-15	n.d.	<LOQ (3.0)	<LOQ (4.5)	6.7	9.8	9.7	<LOQ (4.6)	<LOQ (2.3)	<LOQ (0.9)
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
	Total	n.d.	<LOQ (3.0)	<LOQ (4.5)	6.7	9.8	9.7	<LOQ (4.6)	<LOQ (2.3)	<LOQ (0.9)
BYI08330-ketohydroxy	0-15	n.d.	5.9	37.7	43.6	30.4	27.9	13.6	<LOQ (2.7)	n.d.
	15-30	n.d.		<LOQ (1.1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.			n.d.	n.d.	n.d.		n.d.	n.d.
	Total	n.d.	5.9	38.8	43.6	30.4	27.9	13.6	<LOQ (2.7)	n.d.
BYI08330-MA-amide	0-15	n.d.	n.d.	n.d.	n.d.	<LOQ (4.0)	<LOQ (4.5)	<LOQ (2.7)	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.			n.d.	n.d.	n.d.		n.d.	n.d.
	Total	n.d.	n.d.	n.d.	n.d.	<LOQ (4.0)	<LOQ (4.5)	<LOQ (2.7)	n.d.	n.d.

Method Detection Limit (MDL) = BYI08330 0.5 ng/g
BYI08330-enol 0.5 ng/g
BYI08330-MA-amide 2.1 ng/g
BYI08330-ketohydroxy 0.9 ng/g

Limit of Quantitation (LOQ) = 5 ng/g

n.d. = not detected, below method detection limit

Note: Concentrations in each soil layer are added for convenience in representing dissipation in the soil column. The sum is not intended to express a concentration by depth, but rather approximate a concentration if all residues measured were confined to a single soil layer.

Table IIA 7.3.1-14: Average soil concentrations of BYI08330 residues from treated bare ground plots in Washington

Compound	Depth (cm)	Days After Treatment (DAT)									
		-4	0	1	3	7	14	28	58	120	175
		Average Concentration Found in Three Subplots (µg/kg; dry weight)									
BYI08330	0-15	n.d.	85.4	28.5	<LOQ (2.1)	<LOQ (0.5)	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	60-75										n.d.
	75-90										n.d.
	90-105										n.d.
	105-120										n.d.
	Total	n.d.	85.4	28.5	<LOQ (2.1)	<LOQ (0.5)	n.d.	n.d.	n.d.	n.d.	n.d.
BYI08330-Enol	0-15	n.d.	19.3	8.2	6.6	<LOQ (4.0)	<LOQ (2.4)	<LOQ (1.7)	<LOQ (0.7)	n.d.	<LOQ (1.2)
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	60-75										n.d.
	75-90										n.d.
	90-105										n.d.
	105-120										n.d.
	Total	n.d.	19.3	8.2	6.6	<LOQ (4.0)	<LOQ (2.4)	<LOQ (1.7)	<LOQ (0.7)	n.d.	<LOQ (1.2)
BYI08330-Keto-hydroxide	0-15	n.d.	36.6	79.5	71.8	53.4	26.6	12.4	5.3	<LOQ (3.2)	<LOQ (2.8)
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	60-75										n.d.
	75-90										n.d.
	90-105										n.d.
	105-120										n.d.
	Total	n.d.	36.6	79.5	71.8	53.4	26.6	12.4	5.3	<LOQ (3.2)	<LOQ (2.8)
BYI08330-MA-amide	0-15	n.d.	n.d.	n.d.	<LOQ (3.5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	60-75										n.d.
	75-90										n.d.
	90-105										n.d.
	105-120										n.d.
	Total	n.d.	n.d.	n.d.	<LOQ (3.5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Method Detection Limit (MDL) = BYI08330 0.5 ng/g
BYI08330-enol 0.5 ng/g
BYI08330-MA-amide 2.1 ng/g
BYI08330-ketohydroxy 0.9 ng/g

Limit of Quantitation (LOQ) = 5 ng/g

n.d. = not detected, below method detection limit

Note: Concentrations in each soil layer are added for convenience in representing dissipation in the soil column. The sum is not intended to express a concentration by depth, but rather approximate a concentration if all residues measured were confined to a single soil layer.

Table IIA 7.3.1-15: Average soil concentrations of BYI08330 residues from treated cropped plots in Washington

Compound	Depth (cm)	Days After Treatment (DAT)									
		-4	0	1	3	7	14	28	58	120	175
		Average Concentration Found in Three Subplots (µg/kg; dry weight)									
BYI08330	0-15	n.d.	69.7	17.3	<LOQ (0.5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	60-75										n.d.
	75-90										n.d.
	90-105										n.d.
	105-120										n.d.
	Total	n.d.	69.7	17.3	<LOQ (0.5)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BYI08330-Enol	0-15	n.d.	24.1	8.7	<LOQ (4.7)	<LOQ (3.6)	<LOQ (2.4)	<LOQ (1.9)	<LOQ (0.9)	<LOQ (0.7)	<LOQ (0.9)
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	60-75										n.d.
	75-90										n.d.
	90-105										n.d.
	105-120										n.d.
	Total	n.d.	24.1	8.7	<LOQ (4.7)	<LOQ (3.6)	<LOQ (2.4)	<LOQ (1.9)	<LOQ (0.9)	<LOQ (0.7)	<LOQ (0.9)
BYI08330-Keto-hydroxide	0-15	n.d.	39.0	84.6	83.4	40.5	14.2	<LOQ (3.2)	n.d.	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	60-75										n.d.
	75-90										n.d.
	90-105										n.d.
	105-120										n.d.
	Total	n.d.	39.0	84.6	83.4	40.5	14.2	<LOQ (3.2)	n.d.	n.d.	n.d.
BYI08330-MA-amide	0-15	n.d.	n.d.	n.d.	<LOQ (3.0)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	15-30	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	30-45	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	45-60	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	60-75										n.d.
	75-90										n.d.
	90-105										n.d.
	105-120										n.d.
	Total	n.d.	n.d.	n.d.	<LOQ (3.0)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Method Detection Limit (MDL) = BYI08330 0.5 ng/g
BYI08330-enol 0.5 ng/g
BYI08330-MA-amide 2.1 ng/g
BYI08330-ketohydroxy 0.9 ng/g

Limit of Quantitation (LOQ) = 5 ng/g

n.d. = not detected, below method detection limit

Note: Concentrations in each soil layer are added for convenience in representing dissipation in the soil column. The sum is not intended to express a concentration by depth, but rather approximate a concentration if all residues measured were confined to a single soil layer.

C. DISSIPATION OF THE PARENT

Applied BYI08330 dissipated rapidly to BYI08330-enol and BYI08330-ketohydroxy. In all the sites, the soil concentration of parent BYI08330 was less than the method detection limit within 7 days after application. Also, parent BYI08330 did not move below the surface layer (0 – 15 cm).

In all the four sites, BYI08330 dissipation followed biphasic decline – a rapid initial decline, followed by a relatively slower decline. However, a simple first order decline model sufficiently explained the dissipation of BYI08330 (χ^2 scaled error less than 15% and r^2 ranging from 0.94 to 0.99). Table IIA 7.3.1-16 summarizes the simple first-order degradation and half-life (DT_{50}) values. The time required for dissipation of 90% of the initial concentration of BYI08330 ranged from 1.1 to 3.5 days. There was no significant difference between the dissipation rates of BYI08830 calculated for cropped and bare ground plots.

Table IIA 7.3.1-16: Summary of dissipation rates of BYI08330 in field dissipation studies

Compound	Estimated Initial Conc. C_0 (µg/kg)	First-Order Rate Constant k (d^{-1})	Half-life DT_{50} (d)	DT_{90} (d)	χ^2 (%)	r^2
New York						
Bare ground	74.1	1.4175	0.49	1.62	5.1	0.94
Florida						
Cropped	149.6	0.7062	1.0	3.2	0.4	0.96
Bare ground	111.7	0.7842	0.9	2.9	2.2	0.96
California						
Cropped	101.8	0.6856	1.0	3.4	12.6	0.97
Bare ground	116.9	0.6646	1.0	3.5	12.1	0.98
Washington						
Cropped	145.5	2.1289	0.3	1.1	0.4	0.99
Bare ground	152.4	1.6671	0.4	1.4	1.6	0.99

E. DISSIPATION OF MAJOR METABOLITES

In bare ground as well as cropped plots the major transformation products were BYI08330-enol and BYI08330-ketohydroxy. Residues of BYI08330 did not move below the surface layer (0 – 15 cm) in all the sites, except in Florida where residues of BYI08330-enol and BYI08330-ketohydroxy were detected above the LOQ at 15 – 30 cm layer between 1 day and 7 days after application. After that the residues completed degradation to less than LOQ and MDL. It should be noted that the Florida site represents a worst case condition with heavy rainfall and very light soil (95% sand in the surface layer) with very low organic matter (0.5 %).

The measured soil concentrations of BYI08330-enol one day after application accounted for 76% to 98% of the initial concentration BYI08330. This clearly shows that biodegradation of BYI08330 is the predominant dissipation route for BYI08330. Similarly, the measured residues of BYI08330-ketohydroxy clearly show that biodegradation is major route of dissipation for of BYI08330-enol also.

In all the four sites, BYI08330-MA-amide was not detected above the LOQ in any of the soil layers. The magnitude and temporal pattern of the metabolites observed in cropped plots were not significantly different from that of the bare ground plots.

The dissipation of the combined residues (of BYI08330, BYI08330-enol, and BYI08330-ketohydroxy) was analyzed using kinetics modelling in order to derive dissipation rates. It should be noted that the BYI08330-MA-amide was not included in the calculation of total BYI08330 residues because it was not detected above LOQ at any point during the four studies. The combined BYI08330 residues rapidly degraded in soil following a biphasic decline. However, a simple first-order kinetics model adequately described the dissipation residues. Table IIA 7.3.1-17 summarizes the simple first-order degradation and half-life (DT_{50}) values of the combined residues of BYI08330. The first-order half-life (DT_{50}) of the combined residues range from 5.0 to 23.4 days and the DT_{90} values ranged from 16.7 to 77.8 days.

Table IIA 7.3.1-17: Summary of dissipation rates of combined residues of BYI08330 (BYI08330, BYI08330-enol and BYI08330-ketohydroxy) in field dissipation studies

Sites	Estimated Initial Conc. C_0 (µg/kg)	First-Order Rate Constant k (d^{-1})	Half-life DT50 (d)	DT90 (d)	χ^2 (%)	r^2
New York						
Bare ground	96.7	0.0296	23.4	77.8	16.4	0.93
Florida						
Cropped	162.9	0.1212	5.7	19.0	18.7	0.90
Bare ground	123.4	0.0915	7.6	25.2	22.3	0.86
California						
Cropped	100.7	0.0679	10.2	33.9	13.7	0.91
Bare ground	124.3	0.0825	8.4	27.9	11.3	0.95
Washington						
Cropped	147.5	0.1381	5.0	16.7	5.0	0.98
Bare ground	146.7	0.1096	6.3	21.0	8.5	0.98

F. CARRY OVER OF RESIDUE

BYI08330 degraded to less than the MDL levels (0.5 µg/kg) within 14 days after application. The soil concentration the metabolites of BYI08330 were below the LOQ within 28 to 365 days after application. Based on these results, the carry over potential of BYI08330 residues from one year to another is very low.

III. CONCLUSIONS

BYI08330 dissipated rapidly in soil under field conditions. The dissipation rates of BYI08330 calculated for four sites in the US, resulted in half-life (DT_{50}) values from 0.9 to 1.0 days and the periods required for 90% dissipation (DT_{90}) ranged 1.1 to 3.5 days with no apparent obvious correlation with soil properties or the management (bare ground Vs. cropped). The DT50 values of the combined residues of BYI08330 (i.e. BYI08330, BYI08330-enol, and BYI08330-ketohydroxy) ranged from 5.0 to 23.4 days

and the DT90 values ranged from 16.7 to 77.8 days.

Residues of BYI08330 did not move below the surface layer (0 to– 15 cm) in all the sites, except in Florida where residues of BYI08330-enol and BYI08330-ketohydroxy were detected above the LOQ at 15 to 30 cm layer between 1 day and 7 days after application. After that the residues completed degradation to less than LOQ and MDL. It should be noted that the Florida site represents a worst case condition with heavy rainfall and very light soil (95% sand in the surface layer) with very low organic matter (0.5 %). Therefore, leaching and groundwater contamination is not likely with BYI08330.

BYI08330 degraded to less than the MDL levels (0.5 µg/kg) within 14 days after application. The soil concentration the metabolites of BYI08330 were below the LOQ within 28 to 365 days after application. Based on these results, the carry over potential of soil residues from one year to another is very low.

Considering the results from laboratory soil metabolism studies and terrestrial field dissipation studies the major route(s) of dissipation for BYI08330 are degradation to BYI08330-enol and BYI08330-ketohydroxy, subsequent biodegradation to non-extractable soil residues and mineralization to CO_2.

Storage stability of BYI08330 residues in soil

Report:	**KIIA 7.3.1/06; [redacted], D.J., 2006 (RAFNX018)**
Title:	**Stability of BYI08330 And Its Metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide in Soil During Frozen Storage, USA, 2005 (Reported through a maximum of 334 days storage)**
Report No & Document No.	**RAFNX018 M-277371-01-1**
Guidelines:	EPA Residue Chemistry Test Guidelines: OPPTS 860.1380 Storage Stability; EU Directive: 7032/VI/95 Rev.5 A Appendix H.
GLP	This study was conducted in accordance with the Final Rule of the EPA FIFRA Good Laboratory Practice Standards (40 CFR 160; Federal Register 17 August 1989).
Testing Laboratories and dates	Bayer CropScience LP., Environmental Research, [redacted], [redacted], USA; Study reporting date: August 29, 2006.

EXECUTIVE SUMMARY

This study was initiated to establish the stability of BYI08330, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide in soil during frozen storage in order to provide stability data to support the data generated in the BYI08330 terrestrial field dissipation studies as soil samples had been stored in frozen condition for 30 days or longer.

Untreated soils were obtained from terrestrial soil dissipation studies performed in New York, Florida, California and Washington. A separate storage stability trial was performed on each of these four soils. Pre-weighed samples of soil were fortified with BYI08330, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide and then placed in frozen storage. Samples were withdrawn, at intervals, from frozen storage and analyzed for the appropriate analyte. In addition, pre-weighed samples of soil were fortified with ^{14}C-BYI08330-enol in order to better account for mass balance during storage. Samples were withdrawn, at intervals, from frozen storage and analyzed for ^{14}C-BYI08330-enol.

Aged samples were analyzed for BYI08330, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide using analytical method FN-002-S05-02 (summarized earlier in this Section). Residues of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide were extracted from soil using an acidic extraction solution in the presence of cysteine hydrochloride and utilizing microwave extraction. The samples were fortified with an isotopic internal standard and an aliquot of the final extract analyzed by LC/MS/MS.

Samples that were fortified with ^{14}C-BYI08330-enol were extracted using the same procedure and the extraction solution was radio assayed by liquid scintillation counting and by HPLC coupled to a radio detector. The un-extracted radioactivity in the extracted soil samples fortified with ^{14}C-BYI08330-enol was determined by oxidative combustion

BYI08330 and its metabolites BYI08330-ketohydroxy and BYI08330-MA-amide showed no evidence of any degradation in the four soils during a maximum storage interval of 334 days in frozen storage and there was little variation in the results for the four soils. BYI08330-enol recoveries declined during storage, with the majority of the loss occurring during the first 30 days of storage. The primary causes of these low recoveries were degradation of BYI08330-enol to BYI08330-ketohydroxy and binding of the analyte to soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Items:

Code Name:	BYI08330
CAS Number:	[203313-25-1]
Molecular Formula:	C21 H27 N O5
Molecular Weight:	373.44
ID No.:	K-1338
Reference No.:	1105200304
Purity:	99.2%
Expiration Date:	May 02, 2008
Storage Conditions:	Frozen
Source:	[redacted], [redacted]
Code Name:	$^{13}C_3$-BYI08330 (BYI08330-azaspirodecenyl-2,3,4-$^{13}C_3$)
CAS Name:	cis-3-(2,5-Dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl-2,3,4-13C3 ethyl carbonate
Molecular Formula:	$^{13}C_3$ C_{18} H_{27} N O_5
Molecular Weight:	376.4570
ID No.:	K-1381
E.R. Reference No.:	KML 3386-1-2
Reference No.:	0622200501
Purity:	99.6%
Expiration Date:	June 21, 2015
Storage Conditions:	Frozen
Source:	[redacted], Germany
Code Name:	BYI08330-MA-amide (FHN14065 or AE 1786350)
CAS Name:	2 cis-1-[[(2,5-Dimethylphenyl)hydroxyacetyl]amino]-4-methoxycyclohexanecarboxylic acid

Molecular Formula:	C_{18} H_{25} N O_5
Molecular Weight:	335.39
ID No.:	K-1387
Reference No.:	0706200414
Purity:	96.1%
Expiration Date:	March 31, 2009
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	FHN 14065-acetyl-^{13}C ($^{13}C_3$BAY BYI08330 Mandelic Acid Amide)
CAS Name:	1-[[(2,5-Dimethylphenyl)hydroxyacetyl-13C2]amino]-4-methoxycyclohexanecarboxylic-13C acid
Molecular Formula:	C_{18} H_{25} N O_5
Molecular Weight:	338.36
ID No.:	K-1384
E.R. Reference No.:	KML-1-4
Reference No.:	0728200501
Chemical Purity:	98.8%
Expiration Date:	July 25, 2015
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	BYI08330-ketohydroxy
CAS Name:	cis-3-(2,5-Dimethylphenyl)-3-hydroxy-8-methoxy-1-azaspiro[4.5]decane-2,4-dione
Molecular Formula:	C_{18} H_{23} N O_4
Molecular Weight:	317.3795
ID No.:	K-1339
Reference No.:	1105200305
Chemical Purity:	95.3%
Expiration Date:	10/19/09
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	$^{13}C_3$-BYI 08330-keto-hydroxy
CAS Name:	3-(2,5-Dimethylphenyl)-3-hydroxy-8-methoxy-1-azaspiro[4.5]decane-2,4-dione-2,3,4-13C3
Molecular Formula:	C_{18} H_{23} N O_4
Molecular Weight:	320.34
ID No.:	K-1383
E.R. Reference No.:	KML 3387-1-8
Reference No.:	0622200503
Purity:	100%
Expiration Date:	June 21, 2015
Date of Analysis:	June 21, 2005
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	BYI08330–enol
CAS Name:	cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one
Molecular Formula:	C_{18} H_{23} N O_3
Molecular Weight:	301.3801
ID No.:	K-1337
Reference No.:	1105200303
Purity:	99.4%
Expiration Date:	February 8, 2008
Date of Analysis:	February 8, 2005
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	$^{13}C_3$BYI08330-enol
CAS Name:	cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one-2,3,4-$^{13}C_3$
Molecular Formula:	C_{18} H_{23} N O_3
Molecular Weight:	304.35
ID No.:	K-1382
E.R. Reference No.:	KML 3384-1-1
Reference No.:	0622200502
Purity:	98.3%
Expiration Date:	June 21, 2005
Date of Analysis:	June 21, 2005
Storage Conditions:	Frozen
Source:	[redacted], Germany

Code Name:	BYI 8330 enol-azaspirodecenyl-3-^{14}C
CAS Name:	3-(2,5-dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-one-azaspirodecenyl-3-^{14}C
Molecular Formula:	C18 H23 N O3
Molecular Weight:	301.4
ID No.:	C-1067
Reference No.:	2005BRP022-0012
Purity:	97.6%
Concentration:	56.7 µCi/mL
Expiration Date:	November 10, 2008
Storage Conditions:	Frozen
Source:	[redacted], [redacted], KS

B. STUDY DESIGN

1. Fortification and storage: Soil samples were obtained from the four terrestrial dissipation study sites, where BYI08330 dissipation studies were conducted. Representative sub-samples (25 g each) of each of the four bulk matrices were weighed into wide mouthed, screw capped glass jars. Soil samples for aging were fortified separately with BYI08330, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide to a level of 25 ppb by glass pipette. The glass jars were then capped and stored frozen prior to analysis.

Each analytical set included the following samples:

- 1 Unfortified control sample
- 2 Samples freshly fortified with BYI08330, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide (fresh fortifications were made together)
- 2 Aged frozen samples fortified with BYI08330
- 2 Aged frozen samples fortified with BYI08330-enol
- 2 Aged frozen samples fortified with BYI08330-ketohydroxy
- 2 Aged frozen samples fortified with BYI08330-MA-amide

Between fortification and analysis, the aged samples were kept in the maintained at an average temperature of -22.7 °C (temperature range -26.8 to -1.3°C).

As a supplement to these frozen storage stability tests, a shorter ^{14}C-BYI08330-enol storage stability test was performed in order to determine the level of any un-extracted residues present in the soil and to determine the presence of any additional analytes formed by the degradation of BYI08330-enol.

2. Sampling: Starting from initial sampling (Day 0), one complete set of samples was analyzed immediately after fortification and at three further time points: 29, 70 and 334 days after fortification for New York soil; 30, 70 and 329 days after fortification for Florida soil; 28, 68 and 325 days after fortification for California soil; and 24, 71 and 329 days after fortification for Washington soil. The ^{14}C-BYI08330-enol fortified samples were analyzed 0, 3 and 20 days after fortification.

3. Description of analytical procedures: The soil analytical method summarized earlier in this Section was used to analyze the fortified soil samples. Residues of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide were extracted from soil using an acidic extraction solution in the presence of cysteine hydrochloride and utilizing microwave extraction. The samples were fortified with an isotopic internal standard and an aliquot of the final extract analyzed by LC/MS/MS.

The ^{14}C-BYI08330-enol fortified samples were extracted from soil using an acidic extraction solution in the presence of cysteine hydrochloride and utilizing microwave extraction. On completion of the extraction the sample extract was diluted to 100 mL with acetonitrile. Three 1.0 mL aliquots were removed from each of the solutions and radio assayed by liquid scintillation counting.

Un-extracted residues were determined for the 3 and 20 day aged samples, and the 20 day fresh sample, by combusting aliquots of the ^{14}C-BYI08330-enol dosed soils left over following extraction. The soil samples were allowed to air dry overnight in a fume hood. The next morning the samples were re-weighed. The dry weight was used as the basis for all subsequent calculations. The samples were weighed and mixed for analysis. A total of three sample aliquots for each of the two replicate samples were combusted to determine the total un-extracted residue.

II. RESULTS AND DISCUSSION

A summary of the analytical results from storage stability tests are presented in Table IIA 7.3.1-18. The results are expressed as a percent of fortified amount, corrected for the mean fresh recovery as each time point.

Table IIA 7.3.1-18: Summary of results - stability of BYI08330, BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide residues in soil during frozen storage

(RAFNX018)

Site	Days after Fortification	Percent Recovery (corrected for fresh recovery)			
		BYI08330	BYI08330-enol	BYI08330-ketohydroxy	BYI08330-MA-amide
New York	0	94	66	94	93
	29	87	43	92	112
	70	88	51	101	122
	334	83	37	101	107
Washington	0	101	93	91	95
	24	101	47	89	128
	71	97	32	84	116
	329	104	43	83	100
Florida	0	105	100	84	103
	30	99	67	81	118
	70	109	57	80	120
	329	100	24	81	106
California	0	102	72	74	95
	28	101	45	74	127
	68	97	41	76	116
	325	101	55	75	108

BYI08330 and its metabolites BYI08330-ketohydroxy and BYI08330-MA-amide showed no evidence of any degradation in the four soils during a maximum storage interval of 334 days in frozen storage. In addition, there is little variation in the results for the four soils. However, reduced BYI08330-enol recoveries were observed for each of the aged soils. Inspection of the results showed that significant BYI08330-enol loss occurred during the first month of storage, after which BYI08330 loss continued at a slower rate.

The results for the aged samples fortified with BYI08330-enol were further examined and it was observed that while no BYI08330 or BYI08330-MA-amide residues were observed, BYI08330-ketohydroxy was present in each of the samples. These results were not unexpected as a degradation study[6] concluded that BYI08330-enol partially degrades in the acidic extraction conditions required to obtain acceptable BYI08330 recoveries.

In order to determine the total residue extracted from the aged samples fortified with BYI08330-enol, the BYI08330-ketohydroxy residues detected in the aged samples fortified with BYI08330-enol were converted to BYI08330-enol equivalents and the total % recovery corrected with the concurrent BYI08330-enol recovery. The results obtained are summarized in Table IIA 7.3.1-19.

Table IIA 7.3.1-19: Summary of mass of BYI08330-enol and BYI08330-ketohydroxy recovered from soil samples fortified with BYI08330 and stored under frozen conditions (RAFNX018)

Soil	Fortification Level (µg/kg)	Storage Interval (Days)	Average BYI08330-enol residues (µg/kg)	Average BYI08330-ketohydroxy residues as BYI08330-enol equivalent (µg/kg)	Total residues extracted – BYI08330-enol equivalent (µg/kg)	Percent Recovery (Corrected for fresh recovery)
New York	25	0	17.7	0.6	18.3	68
		29	12.2	1.6	13.9	49
		70	14.0	1.4	15.4	56
		334	7.6	4.9	12.6	61
Washington		0	20.8	0.4	21.2	95
		24	11.7	3.4	15.1	61
		71	8.5	3.2	11.6	44
		329	9.1	2.9	12.0	57
Florida		0	23.2	0.3	23.5	102
		30	17.5	2.0	19.5	75
		70	15.5	2.4	17.9	66
		329	5.2	8.7	13.9	64
California		0	15.7	0.9	16.6	76
		28	11.9	1.7	13.6	51
		68	10.8	1.6	12.4	47
		325	12.5	1.5	13.9	62

The above table shows that while combining the BYI08330-enol and BYI08330-ketohydroxy residues results in an increased BYI08330-enol recovery, the recoveries obtained are still lower than expected. The results also show that the majority of the BYI08330-enol loss occurred during the first month of storage. In a radiolabeled soil metabolism study of BYI08330-enol (Section IIA 7.1.1), degradation to non-extractable residues accounted for 27% to 57% of applied radioactivity within 1 day of incubation, and reached stable levels within 1 to 4 days after incubation.

The total activity present in the aged extracted and un-extracted residues of the ^{14}C-BYI08330-enol fortified samples at 3 and 20 days after fortification were compared to the activity initially added at day 0. This yielded the percent recovery from the stored samples and results are summarized in Table IIA 7.3.1-19

Table IIA 7.3.1-20: Summary of extracted and unextracted residues from soil samples fortified with ^{14}C-BYI08330-enol and stored under frozen conditions (RAFNX018)

Soil	Days after fortification	Total DPM in stored sample			DPM added to sample	Percent Recovery		
		Extracted	Bound	Total		Extracted	Bound	Total
New York	3	565417	58401	623818	661133	86	9	94
	20	564317	98781	663098	661133	85	15	100
Washington	3	589883	43525	633409	661133	89	7	96
	20	621317	52222	673538	661133	94	8	102
Florida	3	595400	22180	617580	661133	90	3	93
	20	582933	22852	605786	661133	88	3	92
California	3	576400	40910	617310	661133	87	6	93
	20	607033	55900	662933	661133	92	8	100

DPM: Disintegrations per minute

The above results show that the mass balance of the radioactivity in the extract and post extracted solids ranged from 92 to 102%. After 20 days storage, between 3 to 15% of the radioactivity remained bound to the soil.
The extracted residues for both the fresh and aged samples were evaporated to reduced volume and analyzed by a HPLC coupled to a radio-detector. Inspection of the results showed that while the fresh samples contained only BYI08330-enol, the aged samples contained both BYI08330-enol and BYI08330-ketohydroxy. No additional peaks were observed in the aged samples.

The results of the radiolabeled study show mass balance of radioactivity and thereby no loss radioactivity by generation of new transformation products or volatility.

III. CONCLUSIONS

BYI08330 and its metabolites BYI08330-ketohydroxy and BYI08330-MA-amide showed no evidence of any degradation in the four soils during a maximum storage interval of 334 days in frozen storage and there was little variation in the results for the four soils.

BYI08330-enol recoveries declined during storage, with the majority of the loss occurring during the first 30 days of storage. The primary causes of these low recoveries were degradation of BYI08330-enol to BYI08330-ketohydroxy and binding of the analyte to soil.

IIA 7.3.2 Soil residue testing

This point is covered by point IIA 7.3.

IIA 7.3.3 Soil accumulation testing on relevant soils

This point is covered by point IIA 7.3.

Summary: Rate of degradation of spirotetramat residues in soil

The biotransformation of [azaspirodecenyl-3-^{14}C]BYI08330 was studied in three EU soils and one US soil for 50 days (EU soils) or 360 days (US soil) under aerobic laboratory conditions in the dark at 20 ± 1 °C and 50% WHC_{max} (EU soils) or 75% of 1/3 bar moisture (US soil). Spirotetramat was found to be a very fast degrading compound in soil. The normalized laboratory DT50 for spirotetramat at 20 °C was calculated to be 0.14 days (geometric average), and the worst case 90th percentile DT50 was 0.21 days. Furthermore, the biotransformation of spirotetramat was investigated in two soils using ^{14}C-BYI08330 for 127 days under outdoor climatic conditions realistic for the intended use. Thereby ^{14}C-BYI08330 formulated as an OD 100 (pH 5) was applied at 94.6% of the highest recommended single use rate for field application (288 g/ha). The parent compound was quickly and thoroughly degraded, and a mean DT50 of approx. 2 days was estimated.

In the degradation/metabolism study on BYI08330 the soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero. However, under the acidic extraction conditions needed for spirotetramat, the major metabolite BYI08330-enol was found to be partly unstable. It degraded during extraction under the formation of BYI08330-ketohydroxy and others. Therefore, the degradation/metabolism of BYI08330-enol in soil was investigated in a separate study,

and those results need to be included in the proposed overall metabolic pathway of spirotetramat in soil. This fact was also the reason to base the degradation kinetics of the major spirotetramat metabolites on the BYI08330-enol study, and not on the parent study

The biotransformation of [azaspirodecenyl-3-^{14}C] and [azaspiro-decenyl-5-^{14}C]BYI08330-enol was studied in three EU soils and one US soil for 119 days under aerobic conditions in the dark at 20 ± 1 °C and at approx. 80% of 1/3 bar moisture (US soil) or 60% WHCmax (EU soils). The BYI08330-enol dissipated following pronounced biphasic kinetics, with an extremely quick first phase. Within a second slower degradation phase, the test item declined to 2.7 to 6.1% of AR in the four soils at the end of the study. This portion is regarded as a strong bound fraction of BYI08330-enol in soil (see chapter IIA 7.4.2 and IIA 7.4.4). The respective kinetic modeling of BYI08330-enol by using MatLab® (application KinGUI) showed that the best fit DT_{50} (days) could be achieved by using the bi-exponential model DFOP (double first order in parallel). This model yielded a mean BYI08330-enol DT_{50} value of 0.08 days (chi^2 statistics mean value of 7.7). Thus it can be concluded that BYI08330-enol is a fast degrading major metabolite of spirotetramat in soil.

A more detailed kinetic modeling based on the results of BYI08330-enol studies yielded normalized laboratory DT_{50} for BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide of 0.03, 3.8 and 1.0 days (see Table IIA 7.2.3-3).

Based on the results obtained within a further laboratory soil degradation study using three aerobic soils it was shown that the metabolite BYI08330-methoxy cyclohexanone is fast and steadily degrading in soil (DT_{50} < 1 day) and that there is no potential for accumulation of BYI08330-methoxy cyclohexanone residues in viable soils. The observed higher level of BYI08330-methoxy cyclohexanone in the laboratory study on phototransformation of BYI08330 on soil surface might have been caused by a decreasing viability of test soil during the strong irradiation in such a laboratory test system.

From an anaerobic soil metabolism study it is concluded that BYI08330 applied to soil will be degraded rapidly in a subsequently flooded anaerobic soil situation, and will not form degradates different from those observed in soil under aerobic conditions, and/or known from abiotic hydrolysis experiments.

Compared to the before mentioned fast biotransformation in dark soils (DT50 < 1 day) phototransformation of BYI08330 on soil surface is not regarded as a relevant degradation process under environmental sunlight irradiation conditions. The degradation of BYI08330 in dark sterile soil was faster compared to the irradiated soil samples. The experimental DT50 of BYI08330 in the dark controls was approx. 5.0 days, and that in the irradiated samples was approx 9.6 days. Thus, the net experimental phototransformation rates (difference between dark and irradiated samples) cannot be calculated, because they would result in negative values. Based on the experimental DT50 of 12.0 and 7.1 days of BYI08330 for the label #1 and #2 test systems, respectively, the DT50 of BYI08330 under environmental conditions is calculated to be 60.0 and 35.5 solar summer days at [redacted], Arizona, USA. A study using sterile soil surface confirmed the before-mentioned findings that a major photo-transformation product is not to be expected. Furthermore, contrary to the non-sterile soil study not any dimers were found, and higher portions of bound residues were not formed. It can be concluded that bound residues of Spirotetramat were formed exclusively by microbial activity and thus indirectly indicating their irreversible nature.

From all the laboratory studies and a radiolabelled outdoor study it can be concluded that spirotetramat is a very fast degrading compound in soil, and all metabolites generated from BYI08330-enol, the predominant first metabolite, are further degraded quickly and are expected not to accumulate in the environment. The soil dissipation testing in a range of representative soils and locations in the USA

confirmed that findings.

In order to determine the residues during US terrestrial field dissipation trials an analytical method (FN-002-S05-02) for the determination of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide in soil and sediment by LC/MS/MS was developed and successfully validated for the determination of residues in soil and sediment. The method was evaluated by determining the average recoveries and relative standard deviation at the LOQ of 5 ng/g and at 5x LOQ (25 ng/g).
BYI08330 dissipated rapidly in soil under field conditions. The dissipation rates of BYI08330 calculated for four sites in the US, resulted in half-life (DT_{50}) values from 0.9 to 1.0 days and the periods required for 90% dissipation (DT_{90}) ranged 1.1 to 3.5 days with no apparent obvious correlation with soil properties or the management (bare ground Vs. cropped). The DT50 values of the combined residues of BYI08330 (i.e. BYI08330, BYI08330-enol, and BYI08330-ketohydroxy) ranged from 5.0 to 23.4 days and the DT90 values ranged from 16.7 to 77.8 days.

Residues of BYI08330 did not move below the surface layer (0 to 15 cm) in all the sites, except in Florida where residues of BYI08330-enol and BYI08330-ketohydroxy were detected above the LOQ at 15 to 30 cm layer between 1 day and 7 days after application. After that the residues completed degradation to less than LOQ and MDL. It should be noted that the Florida site represents a worst case condition with heavy rainfall and very light soil (95% sand in the surface layer) with very low organic matter (0.5 %). Therefore, leaching and groundwater contamination is not likely with BYI08330.

BYI08330 degraded to less than the MDL levels (0.5 µg/kg) within 14 days after application. The soil concentration the metabolites of BYI08330 were below the LOQ within 28 to 365 days after application. Based on these results, the carry over potential of soil residues from one year to another is very low.

Considering the results from laboratory soil metabolism studies and terrestrial field dissipation studies the major route(s) of dissipation for BYI08330 are degradation to BYI08330-enol and BYI08330-ketohydroxy, subsequent biodegradation to non-extractable soil residues and mineralization to CO_2.

BYI08330 and its metabolites BYI08330-ketohydroxy and BYI08330-MA-amide showed no evidence of any degradation in the four soils during a maximum storage interval of 334 days in frozen storage and there was little variation in the results for the four soils. BYI08330-enol recoveries declined during storage, with the majority of the loss occurring during the first 30 days of storage. The primary causes of these low recoveries were degradation of BYI08330-enol to BYI08330-ketohydroxy and binding of the analyte to soil.

IIA 7.4 Mobility studies

IIA 7.4.1 Adsorption and desorption of the active substance

Report:	**KIIA 7.4.1/01, [redacted], 2005 (MEF-04/373)**
Title:	**BYI08330: Adsorption/Desorption in Five Soils**
Report No & Document No	**MEF-04/373** **M-266755-01-2**
Guidelines:	OECD Guideline No. 106; US EPA Subdivision N, Section 163-1; Canada PMRA DACO Number 8.2.4.2; Japanese MAFF New Test Guidelines for Supporting Registration of Chemical Pesticides
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and dates	Bayer CropScience AG – D – Metabolism / Environmental Fate, [redacted], Germany; Experimental work: 2004-04-27 – 2004-10-18; Study completion date: 2005-10-21

EXECUTIVE SUMMARY

Freundlich adsorption and desorption constants K_F and K_{OC} of spirotetramat (BYI08330) have been determined in batch equilibrium experiments with five different soils using radiolabeled test substance ([azaspirodecenyl-3-^{14}C]BYI08330). Since significant degradation of spirotetramat was observed in a pre-test, the main test was performed with sterilized soil (pre-equilibration of soils in 0.01 M $CaCl_2$ solution containing 50 mg/L mercuric(II)chloride for two days) in the concentration range 0.01 mg a.s./L – 1.0 mg a.s./L, using a soil/solution ratio of 1:10 and an equilibrium time of 3 hours. K_{OC} values for the different soils were in the range of 159 to 435 mL/g with a mean K_{OC} of 281 mL/g (1/n = 0.941). Based on this value, spirotetramat can be classified as low mobile in soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat: Code = BYI08330
Label position: [azaspirodecenyl-3-^{14}C]BYI08330
Sample ID: BECH 1537 (pre-test), BECH 1597 (main experiment)
Specific activity: 3.67 MBq/mg (99.1 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Identity and purity of test item in the application solution was checked.

2. Soils: In sum five soils were used in the batch equilibrium experiments. The pH values of the soil batches were measured in 0.01 M aqueous $CaCl_2$. Three soils originated from Germany, and one from USA and Canada, each. The soils were air-dried and homogenized by sieving (≤ 2 mm). The detailed parameters of soils are shown in following Table 7.4.1-1.

Table 7.4.1-1 Physico-chemical characteristics of test soils used for adsorption/desorption study (MEF-04/373)

	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Origin	[redacted], Germany	[redacted], Germany	[redacted], Germany	[redacted], Florida, USA	[redacted], Canada
Texture class (USDA) Sand: Silt: Clay:	Loamy Sand 83.0 % 10.0 % 7.0 %	Sandy Loam 52.5 % 30.9 % 16.7 %	Silt Loam 19.0 % 66.0 % 15.0 %	Sandy Loam 77.3 % 12.7 % 10.0 %	Loam 27.3 % 48.3 % 24.4 %
pH (0.01 M $CaCl_2$)	6.1	6.8	5.9	5.4	4.7
pH (in supernatant of adsorption test)	6.6	6.2	6.9	6.6	6.3
Organic carbon [a)]	2.38 %	0.87 %	2.33 %	0.93 %	2.33 %
Cation exchange capacity (CEC)	11.0 meq/100 g	4.8 meq/100 g	12.9 meq/100 g	6.0 meq/100 g	19.9 meq/100g

a) % organic carbon = % organic matter / 1.724

B. STUDY DESIGN

1. Experimental conditions: Adsorption and desorption constants K_{OC} of spirotetramat were determined for 5 soils with batch equilibrium experiments using [azaspirodecenyl-3-^{14}C]BYI08330 in 0.01 M aqueous $CaCl_2$ solution at 5 different concentrations. In pre-tests, the stability of the test substance, an adequate soil/solution ratio as well as appropriate adsorption and desorption equilibration times were determined.

In pre-test I it was found that BYI08330 rapidly degrades to BYI08330-enol in the presence of soil. Hence, to prevent microbial degradation of the test item within the timescale of the test, the test soils were chemically sterilized. Furthermore, by reduction of the equlibration time to three hours, test item stability could be maintained. Under these conditions, no degradation product was observed during adsorption and desorption in the supernatant by HPLC. Samples without soil were used as control and did not show adsorption to the vessel wall or degradation.

For the definitive adsorption test each 2 g (dry weight) of soil was weighed into centrifuge tubes and aqueous 0.01 M $CaCl_2$ solution (containing 50 mg/mL $HgCl_2$) was added to reach a final solution volume of 18 mL. Control samples without soil were prepared in the same way. After pre-equilibration for at least two days, 2 mL of the application solution was added. The adsorption / desorption measurements were performed with five concentrations of BYI08330 in the range of 0.01 mg/L to 1.0 mg/L, covering two orders of magnitude. The tubes were closed and the suspensions were agitated using an overhead shaker at constant temperature (20 ± 1°C) and in the dark. Samples were then centrifuged and the radioactive content of the supernatants were determined by LSC. Supernatants in the pre-test were analyzed by HPLC to check the stability of the test item over the time course of the experiment.

For the desorption experiment the supernatant was completely removed after centrifugation (3000 g, 10 min) and a corresponding volume of aqueous 0.01 M $CaCl_2$ solution was added. After agitation (for 3 hrs in the definitive test) and centrifugation the supernatant was decanted and analyzed as described above. One desorption cycle was carried out for the soil samples, except for the highest concentration, where three desorption cycles were performed.

For establishing a parental mass balance, the radioactivity in the supernatants, the acetonitrile/water extracts, and in the remaining soil (after combustion) was determined by LSC in the pre-tests. In the definitive test, overall mass balances were established from the radioactivity recovered in supernatants and soil combustions (including residual solution).
The partition of BYI08330 was determined based on the amount of radioactivity in the supernatant due to the stability of the test item under sterile conditions ($HgCl_2$). All experiments were performed in duplicate.

2. Analytical procedures: Spirotetramat concentrations in decanted aqueous solutions were determined as radioactive residue by liquid scintillation counter. The stability of the test substance for the study period was confirmed by HPLC method. After the (last) desorption step, the soil was combusted and the trapped $^{14}CO_2$ was measured by LSC.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The overall material balance for all concentrations for individual samples was in the range of 94.8 to 115.8%, 85.1 to 102.9%, 94.8 to 105.6%, 72.0 to 99.5%, 99.0 to 125.1% of the applied radioactivity in soils [redacted], [redacted], [redacted], [redacted] and [redacted], respectively (mean: 97.6%).

B. TRANSFORMATION OF TEST ITEM

The stability of the spirotetramat in the sterile test system used was confirmed by performing HPLC analyses prior to and at the end of this study.

C. FINDINGS

After three hours of equilibration, 28.1 to 36.1%, 27.2 to 40.8%, 28.2 to 36.7%, 25.6 to 46.7%, 17.2 to 40.5% of the applied radioactivity was adsorbed to the soils [redacted], [redacted], [redacted], [redacted] and [redacted], respectively.

The calculated adsorption constants $K_{F(ads)}$ of the FREUNDLICH isotherms for the five test soils ranged from 3.70 mL/g to 4.79 mL/g. The FREUNDLICH exponents 1/n were in the range of 0.823 to 1.042, indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range.

At the end of one desorption (all concentrations) and 3 desorption phases (highest concentration only), 30.4 to 49.5%, 25.9 to 32.6%, 31.7 to 47.8%, 17.7 to 29.0% and 50.2 to 82.6% of the initially adsorbed amount were desorbed in soils [redacted], [redacted], [redacted], [redacted] and [redacted], respectively.

The desorption $K_{F(des)}$ values and the normalized $K_{OC(des)}$ values were 4 - 8 times higher than those obtained for adsorption indicating a strong binding of the test substance once adsorbed to soil.

III. CONCLUSIONS

The mobility of spirotetramat can be classified as low mobile for adsorption and immobile for desorption. For a compilation of results see the following Table 7.4.1-2.

Table 7.4.1-2: Adsorption and desorption of [^{14}C]BYI08330 on five different soils (MEF-04/373)

Soil, Origin	Soil type	Adsorption			Desorption		
		K_F [mL/g]	1/n	K_{OC} [mL/g]	K_d [mL/g]	1/n	K_{OC} [mL/g]
[redacted], Germany	Loamy Sand	4.79	1.001	201	40.69	1.207	1710
[redacted], Germany	Sandy Loam	3.78	0.892	435	22.78	0.985	2619
[redacted], Germany	Silt Loam	4.10	0.945	176	14.21	0.952	610
[redacted], Florida, USA	Sandy Loam	4.05	0.823	435	33.63	0.949	3616
[redacted], Canada	Loam	3.70	1.042	159	23.01	1.0315	988
Arithmetic mean:		4.09	0.941	281	26.87	1.082	1908

IIA 7.4.2 Adsorption & desorption of rel. metabolites, degr. & react. products

Metabolite BYI08330-enol

Chemical name (CAS): cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one; CAS #: 203312-38-3

BYI08330-enol is the major metabolite appearing shortly after application of the parent BYI08330 to soil as determined in aerobic soil studies.

Report:	**KIIA 7.4.2/01, [redacted], 2005 (IM 2000)**
Title:	**Adsorption/Desorption of BYI08330-cis-Enol in Five Different Soils**
Report No & Document No	**IM 2000 (or BAY55) M-267858-01-2**
Guidelines:	OECD Guideline No. 106; US EPA Subdivision N, Section 163-1 Canada PMRA DACO Number 8.2.4.2
GLP	Fully GLP compliant - laboratory certified by German "Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht des Landes Rheinland-Pfalz".
Testing Laboratory and dates	RLP [redacted], Germany; Experimental work: 2003-08-14 – 2005-02-21; Study completion date: 2005-09-05

EXECUTIVE SUMMARY

Freundlich adsorption and desorption constants of BYI08330-enol should have been determined in batch equilibrium experiments with five different soils using radiolabeled test substance ([azaspirodecenyl-3-^{14}C]BYI08330-enol.

BYI08330-enol revealed a highly dynamic potential for adsorption on a number of representative soils in the preliminary test. No plateau of adsorption was achieved after a shaking period of 48 hours. In addition chromatographic analysis at various time points showed that the test item degraded fast and significantly under the conditions of the test. The same applied after the use of a biocide, by which no stabilization of the BYI08330-enol in the course of the test could be achieved. BYI08330-ketohydroxy was found as a major conversion product presumably formed by an oxidation step. The degradation was accompanied by the rapid formation of non-extractable residues. As a consequence, no stability of the test item was given to fulfill the parental mass balance criterion of 90% of total applied dose as a requirement according to the actual OECD Gudeline 106.

Thus, this study indicated that the sorption characteristics of the test item BYI08330-enol to soil cannot be determined by a batch equilibrium test according to OECD Guideline 106. In order to asses the environmental behavior of the test item more suitable test methods had to be employed, i.e. see later the time-dependent sorption study (KIIA 7.4.2/02) and in section column leaching studies.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BYI08330-enol

Test Item:	BYI08330-enol Code FHN13777
Label position:	[azaspirodecenyl-3-^{14}C]BYI08330-enol
Sample ID:	BECH 0917, later BECH 1626
Specific activity:	4.54 MBq/mg (122.8 µCi/mg)
Radiochemical purity:	>98% (acc. radio-HPLC and -TLC)
Chemical purity:	>99% (HPLC, UV-detector, 210 nm)

Identity and purity of test item in the application solution was checked.

2. Soils: Five soils were used in the batch equilibrium experiments. The pH values of the soil batches were measured in 0.01 M aqueous $CaCl_2$. Three soils originated from Germany, and one from USA and Canada, each. The soils were air-dried and homogenized by sieving (≤ 2 mm). The detailed parameters of soils are shown in following Table 7.4.2-1.

Table 7.4.2-1 Physico-chemical characteristics of test soils used for adsorption/desorption study (IM 2000)

	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Origin	[redacted], Germany	[redacted], Germany	[redacted], Germany	[redacted], Florida, USA	[redacted], [redacted], Canada
Texture class (USDA) Sand: Silt: Clay:	Sandy Loam 72.4 % 22.6 % 5.0 %	Silt Loam 36.9 % 51.1 % 12.0 %	Silt Loam 12.7 % 72.5 % 14.7 %	Sandy Loam 76.0 % 16.0 % 8.0 %	Loam 27.3 % 48.3 % 24.4 %
pH (0.01 M $CaCl_2$)	6.1	6.8	6.1	6.2	4.7
Organic carbon [a)]	2.38 %	0.88 %	2.62 %	0.873 %	2.3 %
Cation exchange capacity (CEC)	11.0 meq/100 g	9.8 meq/100 g	16.0 meq/100 g	5.0 meq/100 g	19.9 meq/100g

a) % organic carbon = % organic matter / 1.724

B. STUDY DESIGN

1. Experimental conditions: Adsorption and desorption constants of BYI08330-enol should have been determined for 5 soils with batch equilibrium experiments using [azaspirodecenyl-3-^{14}C]BYI08330-enol in 0.01 M aqueous $CaCl_2$ solution at 5 different concentrations. For more details of experimental conditions see section before.

Preliminary Test I in the absence a biocide ($HgCl_2$) showed that the adsorption rate was in the range of 17.1% to 65.2% of the applied radioactivity in two tested soils after a shaking period of 24 hours. At the same time the radiochemical purity of the test item in the water phase was determined. Depending on the soil-to-solution ratio the radiochemical purity of the radioactivity in the water phase varied between 47.1 % (mean) and 77.3% (mean) for soil [redacted] and 68.6% and 90.1% for soil ([redacted], respectively.

Preliminary Test I in the presence of the biocide showed that the adsorption rates varied from 26.3% to 69.9% of the applied radioactivity after a shaking period of 24 hours. The values were therefore similar to those determined in the absence of the biocide. The radiochemical purity of BYI08330-enol in the water phase after the 24 hour shaking period was determined. Depending on the soil-to-solution ratio the radiochemical purity of the radioactivity in the water phase varied on average between 36.7% and 81.6% for soil [redacted] and 74.9% and 90.3% for soil [redacted], respectively. Based on the results of these tests a soil/solution ratio of 1:2 was used for Preliminary Test II_{-Bio} and Preliminary Test II_{+Bio}.

The Preliminary Test II_{-Bio} was conducted in order to determine the time for the establishment of the equilibrium between the test item concentration in the solution and the amount adsorbed to the soil.

It was found that the adsorption of the test item on soil was a highly dynamic process. Within the time interval from 6 hours to 24 hours the concentration of BYI08330-enol in the supernatant decreased by a factor of about 3.3 from 0.652 mg/L to 0.200 mg/L for soil [redacted]. For soil [redacted] the concentration decreased for the same interval by a factor of about 1.6 from 0.773 mg/L to 0.487 mg/L. Within a 48 hours test period no plateau was reached for the adsorption neither for soil [redacted], nor for soil [redacted].

The check on stability (parental mass balance) showed that less than 90% of the applied BYI08330-enol was recovered in all test systems in the adsorption phase. The mean values after 6 hours were 72.8% (soil [redacted]) and 88.5% (soil [redacted]) of total applied radioactivity which was recovered as BYI08330-enol, respectively. In the course of the test the mean portion of BYI08330-enol in the specimens decreased to 14.0% (48 hours) for soil [redacted], and 30.3% (48 hours) for soil [redacted], respectively.

Representative HPLC-chromatograms indicated the remarkable decline in purity of BYI08330-enol in the supernatant. After 6 hours of shaking, 95.1% (%ROI, mean value) of the radioactivity in the supernatant could be assigned to the unchanged BYI08330-enol in specimens of soil [redacted]. At the end of the shaking period (48 hours) 37.6% (%ROI, mean value) of the radioactivity were assigned to the unchanged BYI08330-enol in the supernatant. In parallel, the purity of BYI08330-enol in the soil extract was decreasing correspondingly. After 6 hours 83.6% (%ROI, mean value) were assigned to the unchanged BYI08330-enol to decrease to a mean value of 40.1% after 48 hours. The results were very similar for soil [redacted].

The results of the tests including HPLC analysis showed that BYI08330-enol was unstable under the conditions of the test (e.g. parental mass balance less than 90%). In order to stabilize BYI08330-enol Preliminary Test II_{+Bio} was conducted.

The preliminary Test II_{+Bio} was conducted in order to stabilize the test item under the conditions of the test by the presence of the biocide mercury-II-chloride ($HgCl_2$). The adsorption of the test item to soil was again a highly dynamic process as determined for the absence of biocide. Within the time interval from 6 hours to 24 hours the concentration of the test item within the supernatant decreased by a factor of about 1.9 from 0.670 mg/L to 0.361 mg/L for soil [redacted]. The results were very similar for soil [redacted]. Within the 48 hours test period no plateau was reached neither for soil [redacted] nor for soil [redacted]. The curve shows, in principle, the same shape as preliminary Test II_{-Bio}. Thus, the biocide had no influence on the adsorption process.

2. Analytical procedures: BYI08330-enol concentrations in decanted aqueous solutions were determined as radioactive residue by liquid scintillation counter. The stability of the test substance for the study period was determined by HPLC method.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Recoveries of radioactivity were within a range of 90% - 110% of AR for samples containing aqueous calcium chloride, only. After rinsing of the test vessels between 1.0% and 1.2% of the applied radioactivity were found to be adsorbed. Consequently, there was no adsorption of radioactivity to the inner surfaces of the test vessels in the absence of soil. HPLC analysis of the aqueous calcium chloride solutions showed no formation of impurities for BYI08330-enol.

However in the presence of soil, in terms of parental mass balance, less than 90% of the applied radioactivity was recovered as BYI08330-enol in all test systems in the adsorption phase. For soil [redacted] 75.8% (mean value) of the applied test item was recovered after 6 hours, for soil [redacted]

85.9% (mean value) of the applied BYI08330-enol was recovered at the same time, respectively. In the course of the test the portion of BYI08330-enol decreased significantly to 32.5% (48 hours, mean value) for soil [redacted] and 47.2% (48 hours, mean value) for soil [redacted], respectively. At the end of the shaking period (48 hours) only 64.3% (%ROI/mean value) of the radioactivity were assigned to unchanged BYI08330-enol in the supernatant. In parallel, the purity of BYI08330-enol in the soil extract was decreasing correspondingly. After 6 hours 87.7% (%ROI/mean value) were assigned to the unchanged BYI08330-enol to decrease to a mean value of 53.9% (%ROI/mean value) after 48 hours. Again the results were similar for soil [redacted].

B. TRANSFORMATION OF TEST ITEM

[Azaspirodecenyl-3-^{14}C]BYI08330-enol was shown to be stable in aqueous calcium chloride solution and did not adsorb to the test vessels. The results of PRE I_{-Bio} and PRE I_{+Bio} in the presence of soil clearly indicated a significant instability of BYI08330-enol under the test conditions.
Furthers tests showed that the adsorption equilibrium could not be reached due to the instability of BYI08330-enol, even when adding a biocide ($HgCl_2$). In addition, the effect of a fast ageing of adsorbed material is significant resulting in a strong, irreversible binding of the test item and its rapidly formed components to soil. As part of the measurable portion of degradation products, the formation of BYI08330-ketohydroxy was observed due to a microbiologically and chemically induced oxidation reaction

C. FINDINGS

BYI08330-enol revealed a highly dynamic potential for adsorption on a number of representative soils in the preliminary test. No plateau of adsorption was achieved after a shaking period of 48 hours. In addition chromatographic analysis at various time points showed that BYI08330-enol degraded fast and significantly under the conditions of the test. The same applied after the use of a biocide ($HgCl_2$). BYI08330-ketohydroxy was found as a major conversion product presumably formed by an oxidation step. The degradation was accompanied by the rapid formation of non-extractable residues. As a consequence, no stability of BYI08330-enol was given to fulfill the parental mass balance criterion of 90% of total applied dose as a requirement according to the actual OECD Guideline 106.

III. CONCLUSIONS

Because BYI08330-enol was not stable in the test system even after chemical sterilization and as a result the parental mass balance criterion of 90% of total applied dose (as a requirement according to the actual OECD TG 106) could not be fulfilled during the test, no definitive test data regarding the adsorption/desorption characteristics could be derived within this study. In order to asses the environmental behavior of the BYI08330-enol more suitable test methods have to be employed, i.e. column leaching studies.

Report:	**KIIA 7.4.2/02, [redacted], 2006 (MEF-05/222)**
Title:	**[Azaspirodecenyl-3-^{14}C]BYI08330-Enol: Time - Dependent Sorption in Soils**
Report No & Document No	**MEF-05/222** **M-268122-01-2**
Guidelines:	Supportive non-guideline study, based on parts of OECD TG Guideline 106, OECD Guideline for Testing of Chemicals, Adsorption and Desorption, Jan. 2001 US EPA Pesticide Assessment Guideline No. 163-1.
GLP	Fully GLP compliant - laboratory certified by German “Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen“.
Testing Laboratory and dates	Bayer CropScience AG – D – Metabolism / Environmental Fate, [redacted], Germany; Experimental work: 2004-07-26 - 2006-01-24; Study completion date: 2006-01-25.

EXECUTIVE SUMMARY

The time-dependent sorption of BYI08330-enol was studied using in two soils, German silt loam [redacted] and the US American sandy loam [redacted]. Incubation was performed by applying 6.2 µg of [azaspirodecenyl-3-^{14}C]BYI08330-enol to 20 g (DM) air-dried soil in a centrifugation vessel. The study was conducted in the dark at 20°C ± 1°C and at 50% of the respective maximum water holding capacity for incubation periods of 0, 1, 3, 5 and 24 hours. The desorption phase was initiated by adding 0.01 M aqueous $CaCl_2$ solution as desorption solution (in sum 20 mL of aqueous solution) to the treated 20 g (DM) of soil (soil/solution ratio = 1/1). One desorption cycle for each of the different ageing/desorption time periods of 0.03, 1.03, 5 and 24 hrs) was performed in duplicates. The aqueous supernatant after desorption step was separated by centrifugation and decantation, and the BYI08330-enol residues were analyzed by liquid scintillation counting (LSC) and High Performance Liquid Chromatography (HPLC).

The mean total recovered radioactivity of individual samples ranged from 91.1 to 98.9 % and 95.5 to 99.8 % in case of [redacted] and [redacted], respectively. Without soil (control experiment) the test item was stable in the desorption solution.

As soon as the desorption phase was started (addition of desorption solution to the treated soil) two simultaneous processes were measurable: Even within two minutes (the shortest period practically possible for application to soil directly followed by a desorption step) a significant portion (i.e. about one half) of BYI08330-enol was bound to the soil and thus could not desorbed (≡ strongly bound fraction of BYI08330-enol). This strong binding increased rapidly with time. The portion of the test item, which could be desorbed, decreased rapidly and was no longer detectable in desorption solutions after 3 and 5 hours for soil [redacted] and [redacted], respectively. In contrast, the strongly bound fraction of BYI08330-enol degraded more slowly. As a result of these processes, no stable equilibrium could be established until most of the test item was degraded. Hence it was in fact not possible to calculate adsorption parameters using the FREUNDLICH adsorption isotherm. However, using the extracted amount of BYI08330-enol in soil as the adsorbed part and the limit of detection as concentration of test item in the desorbed part, a K_{OC} calculation was performed resulting in values of approx. 1200 and 4000 for soil [redacted] and [redacted], respectively,

In conclusion, the study demonstrated that sorption and binding of BYI08330-enol to soil is extremely fast and increases very rapidly with aging time in soil. The portion not tightly bound to soil, i.e. the portion that is releasable by aqueous solution from soil (weakly sorbed), is degraded within a few hours. From these results it can be concluded that BYI08330-enol is absent from the soil pore water (either degraded or tightly bound to soil) within a very short period of time. Again, this study confirmed that

the sorption characteristics of the test item BYI08330-enol to soil cannot be determined accurately by a batch equilibrium test according to OECD TG 106.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BYI08330-enol: Code FHN13777
Label position: [azaspirodecenyl-3-^{14}C]BYI08330-enol
Sample ID: BECH 1539
Specific activity: 4.54 MBq/mg (122.8 µCi/mg)
Radiochemical purity: 97.48% (acc. radio-HPLC) before application
96.77% after application
Identity of test item in the application solution was checked.

2. Soils: The study was carried out using two different soils, the German silt loam [redacted] and the US American sandy loam [redacted]. They are representative agricultural soils and had been tested in a corresponding aerobic soil metabolism study of BYI08330-enol and the parent BYI08330, also. They were selected due to their difference in silt and sand content and pH. The pH values of the soil batches were measured in 0.01 M aqueous $CaCl_2$. The soils were air-dried and homogenized by sieving (≤ 2 mm). The detailed parameters of soils are shown in following Table 7.4.2-2.

Table 7.4.2-2: Physico-chemical characteristics of test soils used for time-dependent sorption study (MEF-05/222)

	[redacted]	[redacted]
Origin	[redacted], Germany	[redacted], Florida, USA
Texture class (USDA)	Silt Loam	Sandy Loam
Sand:	15.6 %	77.3 %
Silt:	68.1 %	12.7 %
Clay:	16.2 %	10.0 %
pH (0.01 M $CaCl_2$)	6.1	5.4
Organic carbon [a)]	2.62 %	0.8 %
Cation exchange capacity (CEC)	16.0 meq/100 g	4.2 meq/100 g

a) % organic carbon = % organic matter / 1.724

B. STUDY DESIGN

1. Experimental conditions: The time-dependent sorption of BYI08330-enol was studied in two soils. Incubation was performed by applying 6.2 µg of [azaspirodecenyl-3-^{14}C]BYI08330-enol to 20 g (DM) air-dried soil in a centrifugation vessel. The study was conducted in the dark at 20°C ± 1°C and at 50% of the respective maximum water holding capacity for incubation periods of 0, 1, 3, 5 and 24 hours. For practical reasons the 0-hrs incubation samples were aged for 10 sec each.
The desorption phase was initiated by adding 0.01 M aqueous $CaCl_2$ solution as desorption solution (in sum 20 mL of aqueous solution) to the treated 20 g (DM) of soil (soil/solution ratio = 1/1). One desorption cycle for each of the different ageing/desorption time periods was performed with duplicates. After each aging period, the samples (20 g dry soil equivalent) were overlaid with the respective volume of 0.01 M $CaCl_2$ solution resulting in a soil/solution ratio of 1:1. Then the closed vessels were shaken by means of an overhead shaker (20 ± 2 rpm) for different periods: 0.03, 1.03, 3, 5 and 24 hrs. For

practical reasons the 0-hrs desorption samples were shaken for 1 min, each. The time for preparation of centrifugation was kept to 1 min. In total the 0-hrs desorption samples were desorbed for 2 min. The aqueous supernatant after desorption step was separated by centrifugation and decantation, and the BYI08330-enol residues were analyzed by liquid scintillation counting (LSC) and High Performance Liquid Chromatography (HPLC).

2. Analytical procedures: BYI08330-enol concentrations in decanted aqueous solutions were determined as radioactive residue by liquid scintillation counter. The stability of the test substance for the study period was determined by HPLC method.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The mean total recovered radioactivity of individual samples ranged from 91.1 to 98.9 % and 96.0 to 99.0 % in case of [redacted] and [redacted], respectively. For both soils, a strong time-dependent increase of the amounts of total radioactivity bound to soil (NER) was observed, i.e. from (mean) values of 19.8 and 13.7% AR at 0.03 hrs to values of 49.4 and 43.9% AR at the 24-hrs aging time point, for soil [redacted] and [redacted], respectively.

B. TRANSFORMATION OF TEST ITEM

The ^{14}C-test item was shown to be stable in aqueous calcium chloride solution and did not adsorb to the test vessels. Despite its stability in pure $CaCl_2$ solution a fast degradation/dissipation process for BYI08330-enol was initiated as soon as it came into contact with soil, and adsorption equilibrium could not be reached. As part of the measurable portion of degradation products, the formation of BYI08330-ketohydroxy was observed due to a microbiologically and chemically induced oxidation reaction.

C. FINDINGS

As soon as desorption phase was started (addition of desorption solution to the treated soil) two simultaneous processes were measurable: Even within two minutes (the shortest period practically possible for application to soil directly followed by a desorption step) a significant portion (i.e. about one half) of BYI08330-enol was bound to the soil and thus could not be desorbed (≡ strongly bound fraction of BYI08330-enol). This strong binding rapidly increases with time. The portion of the test item which could still be desorbed decreased rapidly and was no longer detectable in desorption solutions after 3 and 5 hours for soil [redacted] and [redacted], respectively. This indicated that degradation/dissipation was slightly faster in soil [redacted] than in [redacted]. In contrast, the strongly bound fraction of BYI08330-enol degraded more slowly. As a result of these processes, no stable equilibrium could be established until most of the test item was degraded. Hence it was in fact not possible to calculate the adsorption parameters using the FREUNDLICH adsorption isotherm.
However, using the extracted amount of BYI08330-enol in soil as the adsorbed part and the limit of detection as concentration of test item in the desorbed part, a K_{OC} calculation was performed resulting in values of approx. 1200 and 4000 for soil [redacted] and [redacted], respectively, respectively.

When considering the various results received after 2 min (0.03 h ≡ time needed for shaking and centrifugation) - although no strict equilibrium was established at this time point - a clear effect of aging was apparent for both soils. Comparing the results of 0 and 1 h of aging an increase in the strongly bound fraction of BYI08330-enol was evident, already. Within the same period the portion of non-

extractable residues increased strongly as well. Later on it was not possible to measure an effect of further aging due to instability of the test item.

III. CONCLUSIONS

In conclusion, the current laboratory study demonstrated that sorption and binding of BYI08330-enol to soil is extremely fast and increases very rapidly with aging time in soil. The portion not tightly bound to soil, i.e. the portion that is releasable by aqueous solution from soil (weakly sorbed), is degraded within a few hours. From these results it can be concluded that BYI08330-enol is absent from the soil pore water (either degraded or tightly bound to soil) within a very short period of time.

Again, this study indicated that the sorption characteristics of the test item BYI08330-enol to soil cannot be determined accurately by a batch equilibrium test according to OECD TG 106.

Metabolite BYI08330-ketohydroxy

Chemical name (CAS): cis-3-(2,5-Dimethylphenyl)-3-hydroxy-8-methoxy-1-azaspiro[4.5]decan-2,4-dione

Report:	**KIIA 7.4.2/03, [redacted], 2005 (IM 2001)**
Title:	**^{14}C-BYI08330-Ketohydroxy: Adsorption/Desorption in Five Soils**
Report No & Document No	**IM 2001 (or BAY57) M-267955-02-1**
Guidelines:	OECD Guideline No. 106; US EPA Subdivision N, Section 163-1 Canada PMRA DACO Number 8.2.4.2
GLP	Fully GLP compliant - laboratory certified by German “Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht des Landes Rheinland-Pfalz“.
Testing Laboratory and dates	RLP, [redacted], Germany; Experimental work: 2004-09-20 – 2004-12-09; Study completion date: 2005-09-09, 1st Amendment dated 2006-08-30

EXECUTIVE SUMMARY

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-ketohydroxy have been determined in batch equilibrium experiments with five different soils using radiolabeled test substance ([azaspirodecenyl-3-^{14}C]BYI08330-ketohydroxy). The adsorption phase of the study (Definitive Test) was carried out using pre-equilibrated air-dried soil with BYI08330-ketohydroxy at concentrations of nominal 1, 0.3, 0.1, 0.03, and 0.01 mg/L in the dark at 20 °C ± 1 °C for 24 hours. Since significant degradation of test item was observed in a pre-test, the equilibration solution used was 0.01 M aqueous $CaCl_2$ solution spiked with 50 mg $HgCl_2$ as biocide, with a soil to solution ratio of 1:1 (soil: [redacted] and [redacted]) and 1:2 (soil: [redacted], [redacted], and [redacted]). Desorption phase of the study was carried out by supplying pre-adsorbed soil specimens with fresh 0.01 M aqueous $CaCl_2$ solution with biocide (50 mg $HgCl_2$) for one desorption cycle, except for the highest concentration, where three desorption cycles were performed. $K_{OC(ads)}$ values for the different soils were in the range of 41.0 to 99.1 mL/g with a mean K_{OC} of 63.7 mL/g (1/n = 0.922). Based on this value, BYI08330-ketohydroxy can be classified as intermediate to mobile in soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BYI08330-ketohydroxy: Code = FHN14066
Label position: [azaspirodecenyl-3-^{14}C]BYI08330-ketohydroxy
Sample ID: BECH 1588, later BECH 1619
Specific activity: 4.31 MBq/mg (116.6 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Identity and purity of test item in the application solution was checked.

2. Soils: In sum five soils were used in the batch equilibrium experiments. The pH values of the soil batches were measured in 0.01 M aqueous $CaCl_2$. Three soils originated from Germany, and each one from USA and Canada. The soils were air-dried and homogenized by sieving (≤ 2 mm). The detailed parameters of soils are shown in following Table 7.4.2-3.

Table 7.4.2-3: Physico-chemical characteristics of test soils used for adsorption/desorption study (IM 2001)

	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Origin	[redacted], Germany	[redacted], Germany	[redacted], Germany	[redacted], Florida, USA	[redacted], Canada
Texture class (USDA)	Sandy Loam	Silt Loam	Silt Loam	Sandy Loam	Clay Loam
Sand:	72.7 %	42.0 %	12.7 %	76 %	28 %
Silt:	18.4 %	44.8 %	72.5 %	16 %	44 %
Clay:	8.9 %	13.2 %	14.7 %	8 %	28 %
pH (0.01 M $CaCl_2$)	6.0	6.4	6.1	6.2	5.5
pH (in supernatant of adsorption test)	6.7	6.9	6.6	6.7	6.0
Organic carbon[a]	1.30 %	1.10 %	2.62 %	0.87 %	2.44 %
Cation exchange capacity (CEC)	6.4 meq/100 g	9.6 meq/100 g	16.0 meq/100 g	5.0 meq/100 g	19.6 meq/100g

a) % organic carbon = % organic matter / 1.724

B. STUDY DESIGN

1. Experimental conditions: Adsorption and desorption constants K_{OC} of BYI08330-ketohydroxy were determined for 5 soils with batch equilibrium experiments using [azaspirodecenyl-3-^{14}C]-BYI08330-ketohydroxy in 0.01 M aqueous $CaCl_2$ solution at 5 different concentrations. In pre-tests, the stability of the test substance, an adequate soil/solution ratio as well as appropriate adsorption and desorption equilibration times were determined.

Samples without soil were used as control. The test item did not show significant adsorption to the inner surfaces of the test vessels. Rinsing the test vessels with organic solvent did not show significant adsorption. No breakdown of the test item in pure $CaCl_2$-solutions was determined by HPLC-analysis.

Preliminary Test I showed that the adsorption rate was between 20 and 80%. The adsorption rates varied from 25.3% to 75.3% of the applied radioactivity after a shaking period of 24 hours. Based on the results of this test a soil/solution ratio of 1:1 was used for Preliminary Test II_{Bio}. Because the parental mass

balance was less than 90%, BYI08330-ketohydroxy was considered to be unstable under these test conditions. Due to the fact that the limit of parental mass balance was not achieved it was decided to conduct Preliminary Test II_{+Bio} with addition of a biocide ($HgCl_2$). There, the parental mass balance was greater than 88% within the time period of 24 hours and thus, BYI08330-ketohydroxy was considered to be stable under the sterile test conditions. The purity of BYI08330-ketohydroxy in the extracts was close to 100 % in all soils independent of the time of sampling. Thus, it was decided to conduct the adsorption phase of the Definitive Test within 24 hours without any chromatographic analysis, because the stability of BYI08330-ketohydroxy over the entire incubation period was already proven in this test. Furthermore, the test showed that equilibrium was well established after 24 hours of shaking.

The time for the establishment of the desorption equilibrium between the test item concentration adsorbed to the soil and the amount in the solution (desorbed from soil) was determined in the second part of Preliminary $Test_{+Bio}$. The test showed an established equilibrium after 4 hours of shaking. In order to assure a high stability of the test item two hours for each desorption step (i.e. in total corresponding to 6 hours for three desorption steps) were established.

For definitive adsorption test each 20 or 25 g (dry weight) of 16-hrs pre-equilibrated soil was shaken in centrifuge tubes with 20 or 50 mL aqueous 0.01 M $CaCl_2$ solution containing 50 mg/mL $HgCl_2$ and the test item. The adsorption/desorption measurements were performed with five concentrations of BYI08330-ketohydroxy in the range of 0.01 mg/L to 1.0 mg/L, covering two orders of magnitude. The tubes were closed and the suspensions were agitated using an overhead shaker at constant temperature (20 ± 1°C) and in the dark. Samples were then centrifuged and the radioactive content of the supernatants were determined by LSC. Supernatants in the pre-test were analyzed by HPLC to check the stability of the test item over the time course of the experiment.

For the desorption experiment the supernatant was completely removed after centrifugation (3000 g, 10 min) and a corresponding volume of aqueous 0.01 M $CaCl_2$ solution was added. After agitation (for 2 hrs in the definitive test) and centrifugation the supernatant was decanted and analyzed as described above. One desorption cycle was carried out for the soil samples, except for the highest concentration, where three desorption cycles were performed.

For establishing a parental mass balance, the radioactivity in the supernatants, the soil extracts, and in the remaining soil (after combustion) was determined by LSC in the pre-tests. In the definitive test, overall mass balances were established from the radioactivity recovered in supernatants and soil combustions (including residual solution).

The partitioning of BYI08330-ketohydroxy was determined based on the amount of radioactivity in the supernatant due to the stability of the test item under sterile conditions ($HgCl_2$). All experiments were performed in duplicate.

2. Analytical procedures: The aqueous supernatant after adsorption and desorption was separated by centrifugation and the BYI08330-ketohydroxy residues in the supernatant were analyzed by liquid scintillation counting (LSC). The stability of the test substance for the study period was determined by HPLC method. After the (last) desorption step, the soil was combusted and the trapped $^{14}CO_2$ was measured by LSC.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The overall material balance for all concentrations for individual specimens was in the range of 94.8 to 98.9%, 93.2 to 97.5%, 93.0 to 96.9%, 93.7 to 98.1%, and 92.5 to 98.6% of the applied radioactivity in soils [redacted], [redacted], [redacted], [redacted], and [redacted], respectively.

B. TRANSFORMATION OF TEST ITEM

The stability of the BYI08330-ketohydroxy in the sterile test systems used was confirmed by performing HPLC analyses prior to and at the end of this study.

C. FINDINGS

After 24 hours of equilibration, in the definitive adsorption test 35.0 to 43.2%, 35.5 to 44.3%, 34.1 to 42.0%, 29.8 to 39.5%, and 52.8 to 63.0% of the applied test material was adsorbed in soils [redacted], [redacted], [redacted], [redacted], and [redacted], respectively. The calculated adsorption constants $K_{F(ads)}$ of the FREUNDLICH isotherms for the five test soils ranged from 0.5158 to 2.2059 mL/g, the $K_{OC(ads)}$ ranged from 41.0 to 99.1 mL/g. The FREUNDLICH exponents 1/n were in the range of 0.9152 to 0.9287, indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range. At the end of one desorption step (and three desorption steps in case of the highest concentration), 28.6 to 37.1%, 28.5 to 31.1%, 23.3 to 29.1, 19.2 to 31.8%, and 16.7 to 26.6% of the initially adsorbed amount were desorbed in soils [redacted], [redacted], [redacted], [redacted], and [redacted], respectively.

The desorption $K_{F(des)}$ and the normalized $K_{OC(des)}$ values were 1.3 to 1.7 times higher than those obtained for adsorption phase. There was no significant correlation between pH and adsorption for the investigated soils.

III. CONCLUSIONS

Based on the soil sorption parameters measured in this study the mobility of BYI08330-ketohydroxy can be classified as intermediate to mobile for adsorption and for desorption in all soils. For a compilation of results see Table 7.4.2-4.

Table 7.4.2-4: Adsorption/desorption of [^{14}C]BYI08330-ketohydroxy on five soils (IM 2001)

Soil, Origin	Soil type	Adsorption			1st Desorption		
		K_F [mL/g]	1/n	K_{OC} [mL/g]	K_F [mL/g]	1/n	K_{OC} [mL/g]
[redacted], Germany	Sandy Loam	0.5329	0.9199	41.0	0.6679	0.9332	51.4
[redacted], Germany	Silt Loam	0.5158	0.9287	46.9	0.7133	0.9542	64.8
[redacted], Germany	Silt Loam	1.078	0.9273	41.2	1.602	0.9830	61.2
[redacted], FL, USA	Sandy Loam	0.8618	0.9177	99.1	1.4745	0.8846	169.5
[redacted], Canada	Clay Loam	2.2059	0.9152	90.4	2.8375	0.9016	116.3

Arithmetic mean:	0.9218	63.7		0.9313	92.6

Metabolite BYI08330-MA-amide

Chemical name (IUPAC): (1s,4s)-1-{[(2,5-dimethylphenyl)(hydroxy)acetyl]amino}-4-methoxy-cyclohexanecarboxylic acid

Report:	**KIIA 7.4.2/04, [redacted] & [redacted], 2005 (CX/04/070)**
Title:	**^{14}C-BYI08330-MA-Amide: Adsorption to and Desorption from Five Soils**
Report No & Document No	**CX/04/070 M-263686-01-2**
Guidelines:	OECD Guideline No. 106; US EPA Subdivision N, Section 163-1 Canada PMRA DACO Number 8.2.4.2
GLP	Fully GLP compliant - laboratory certified by Department of Health of the Government of the UK.
Testing Laboratory and dates	[redacted], UK; Experimental work: 2004-09-22 – 2005-08-22; Study completion date: 2005-09-07

EXECUTIVE SUMMARY

FREUNDLICH adsorption and desorption constants K_F and K_{OC} of BYI08330-MA-amide have been determined in batch equilibrium experiments with five different soils using ^{14}C radiolabeled test item. The adsorption phase of the definitive study was carried out using pre-equilibrated soils with BYI08330-MA-amide at concentrations of approx. 0.5, 0.1, 0.05, 0.01 and 0.005 mg/L in the dark and at 20 ± 2°C for 24 hours for all soils. The equilibration solution used was 0.01M aqueous $CaCl_2$, with a soil/solution ratio of 1:1 for all soils. The desorption phase of the study was carried out for 2 hours per cycle with fresh 0.01M aqueous $CaCl_2$ applied to pre-adsorbed soil, for one desorption cycle, with the exception of the highest concentration, where three desorption cycles were performed.
The test item was stable throughout the study for all soils. No significant breakdown of BYI08330-MA-amide was observed in any soil. The parental mass balances were determined by LSC of the supernatants after adsorption, desorption and solvent extraction, followed by HPLC analysis for the test item. In all soils >90% of AR was found to be extractable prior to combustion and 90% or greater found to be attributable to the test item.

The calculated adsorption constants K_F of the FREUNDLICH isotherms for the four test soils ranged from 0.06 to 0.18 mL/g, and the $K_{OC(ads)}$ values were in the range of 4.4 to 25.5 mL/g with a mean $K_{OC(ads)}$ of 9.3 mL/g (mean 1/n = 0.948). Based on this value, BYI08330-MA-amide can be classified as highly mobile in soil. The desorption K_{des} values were 0.13 to 0.37 mL/g and higher than those obtained for K_F in the adsorption phase indicating a slightly stronger binding once adsorbed to soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BYI08330-MA-amide: Code = AE 1786350
Label position: [hydroxy-^{14}C]BYI08330-MA-amide
Sample ID: BECH 1621

Specific activity: 4.08 MBq/mg (110.4 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Identity and purity of test item in the application solution was checked.

2. Soils: In sum five soils were used in the batch equilibrium experiments. The pH values of the soil batches were measured in 0.01 M aqueous $CaCl_2$. Three soils originated from Germany, and each one from USA and Canada. The soils were air-dried and homogenized by sieving (≤ 2 mm). The detailed parameters of soils are shown in following Table 7.4.2-5.

Table 7.4.2-5: Physico-chemical characteristics of test soils used for adsorption/desorption study (CX/04/070)

	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Origin	[redacted], Germany	[redacted], Germany	[redacted], Germany	[redacted], Florida, USA	[redacted], Canada
Texture class (USDA)	Sandy Loam	Silt Loam	Silt Loam	Loamy Sand	Loam
Sand:	72.7 %	45.0 %	12.7 %	78 %	30 %
Silt:	18.4 %	40.1 %	72.5 %	15 %	45 %
Clay:	8.9 %	14.9 %	14.7 %	7 %	25 %
pH (0.01 M $CaCl_2$)	6.3	6.7	6.5	5.7	5.4
Organic carbon [a)]	1.70 %	0.91 %	2.07 %	0.7 %	2.3 %
Cation exchange capacity (CEC)	8.9 meq/100 g	8.4 meq/100 g	14.1 meq/100 g	4.7 meq/100 g	21.2 meq/100g

a) % organic carbon = % organic matter / 1.724

B. STUDY DESIGN

1. Experimental conditions: Adsorption and desorption constants K_{OC} of BYI08330-MA-amide were determined for 5 soils with batch equilibrium experiments using [hydroxy-^{14}C]BYI08330-MA-amide in 0.01 M aqueous $CaCl_2$ solution at 5 different concentrations. In pre-tests, the stability of the test substance, an adequate soil:solution ratio as well as appropriate adsorption and desorption equilibration times were determined. Samples without soil were used as control.

The test item did not show significant adsorption to the inner surfaces of the test vessels. Rinsing the test vessels with organic solvent did not show significant adsorption. No breakdown of the test item in pure $CaCl_2$-solutions was determined by HPLC-analysis. A soil/solution ratio of 1:1 was found to be appropriate for all soils. The desorption phase of the study was carried out for 2 hours per cycle with fresh 0.01 M aqueous $CaCl_2$ applied to pre-adsorbed soil, for one desorption cycle, with the exception of the highest concentration, where three desorption cycles were performed.

For definitive adsorption test each 40 g (dry weight) of pre-equilibrated soil was shaken in centrifuge tubes with 40 mL aqueous 0.01 M $CaCl_2$ solution and the test item for 24 hours for all soils. The adsorption/desorption measurements were performed with five concentrations of [hydroxy-^{14}C]BYI08330-MA-amide of approx. 0.5, 0.1, 0.05, 0.01 and 0.005 mg/L covering two orders of magnitude. The tubes were closed and the suspensions were agitated using a rotary shaker at constant temperature (20 ± 2°C) and in the dark. The aqueous supernatant after adsorption and desorption was separated by centrifugation and analyzed by LSC. After desorption, the soil was extracted twice with acetonitrile/water 80:20 v/v (acidified to pH4 with formic acid) and once more with acetonitrile. After extraction, the soil was combusted and the trapped CO_2 analyzed by LSC. Supernatants in the pre-test

were analyzed by HPLC to check the stability of the test item over the time course of the experiment. The adsorption parameters were calculated using the FREUNDLICH isotherm.

For establishing a parental mass balance, the radioactivity in the supernatants, the soil extracts, and in the remaining soil (after combustion) was determined by LSC in the pre-tests. In the definitive test, overall mass balances were established from the radioactivity recovered in supernatants and soil combustions (including residual solution). All experiments were performed in duplicate.

2. Analytical procedures: The aqueous supernatant after adsorption and desorption was separated by centrifugation and the BYI08330-MA-amide residues in the supernatant were analyzed by liquid scintillation counting (LSC). The stability of the test substance for the study period was determined by HPLC method. After the (last) desorption step, the soil was combusted and the trapped $^{14}CO_2$ was measured by LSC.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

In all soils >90% of applied radioactivity was found to be extractable prior to combustion and 90% or greater found to be attributable to the test item
In the definitive test, the overall material balance for individual samples was in the range of 95.9 to100.7% for the [redacted] loamy sand (mean 99.4%), 94.8 to 98.3% for the [redacted] loam (mean 96.9%), 90.5 to 96.1% for the [redacted] silt loam (mean 92.8%), 96.7 to 99.8% for the [redacted] a silt loam (mean 98.5%) and 98.3 to 100.9% for the [redacted] sandy loam (mean 99.7%).

B. TRANSFORMATION OF TEST ITEM

The test item was stable throughout the study for all soils. No significant breakdown of was observed in any soil.

C. FINDINGS

In the definitive adsorption test, the amount of applied test material to be adsorbed after 24 hours of equilibration ranged from 15.1 to 17.1% in the [redacted] loamy sand, 11.0 to 15.5% in the [redacted] loam, 9.8 to 21.6% in the [redacted] silt loam, 5.6 to 7.1% in the [redacted] a silt loam and 4.7 to 6.4% in the [redacted] sandy loam, respectively.

At the end of the final desorption phase, the amount of test material desorbed, expressed as a percentage of the initial amount adsorbed, ranged from 27.1 to 55.7% for the [redacted] loamy sand, 7.6 to37.3% for the [redacted] loam, 8.4 to 34.5% for the [redacted] silt loam, 14.4 to 46.3% for the [redacted] a silt loam and 7.6 to 54.3% for the [redacted] sandy loam.

The calculated adsorption constants K_F of the FREUNDLICH isotherms for the four test soils ranged from 0.06 to 0.18 mL/g. The FREUNDLICH exponent 1/n displayed high degree of linearity in four of the five soils tested, ranging from 0.928 to 1.068, and indicating that the concentration of the test item had little effect on the adsorption behavior in the examined concentration range. The remaining soil, [redacted] silt loam, showed a less linear relationship with a 1/n value of 0.8. The $K_{OC(ads)}$ values were in the range of 4.4 to 25.5 mL/g with a mean $K_{OC(ads)}$ of 9.3 mL/g (mean 1/n = 0.948).

The desorption K_{des} values were 0.13 to 0.37 mL/g and higher than those obtained for K_F in the adsorption phase indicating a little stronger binding once adsorbed to soil.

There was no significant correlation between adsorption and any of the measured soil parameters, such

as pH, clay, organic carbon or CEC, for the investigated soils.

III. CONCLUSIONS

Based on the soil sorption parameters measured in this study the mobility of BYI08330-MA-amide can be classified as having high mobility in soil. For a compilation of results see the following Table 7.4.2-6.

Table 7.4.2-6: Adsorption/desorption of [^{14}C]BYI08330-MA-amide on five soils (CX/04/070)

Soil, Origin	Soil type	Adsorption			1st Desorption		
		K_F [mL/g]	1/n	K_{OC} [mL/g]	K_F [mL/g]	1/n	K_{OC} [mL/g]
[redacted], Germany	Sandy Loam	0.08	1.07	4.4	0.13	0.99	7.8
[redacted]a, Germany	Silt Loam	0.06	0.96	6.5	0.14	0.96	14.9
[redacted], Germany	Silt Loam	0.10	0.80	6.0	0.19	0.80	9.0
[redacted], FL, USA	Loamy Sand	0.18	0.98	25.5	0.37	0.98	52.6
[redacted], Canada	Loam	0.12	0.93	5.1	0.22	0.91	9.7
Arithmetic mean:		0.11	0.95	9.3	0.21	0.93	18.8

Metabolites BYI08330-enol-dimer 1 and -dimer 2 (ROI 6 and ROI 7)

For structures, etc. see (see Table 7.4.2-7)

In the basic study on the metabolism/degradation of BYI08330 (KIIA 7.1.1/01) the soil processing procedure was optimized for DAT-0 >90% recovery of the test item (acidic extraction conditions due to the test item's instability at pH >7). Under these conditions the major metabolite BYI08330-enol was found to be rather unstable. One route of disappearance was an oxidative BYI08330-enol dimerization leading to BYI08330-enol-dimer 1 or -enol-dimer 2, and re-entry of the BYI08330-enol after cleavage of the dimers into the other mentioned pathways (see Figure IIA 7.1-1). This BYI08330-enol dimerization is considered as of minor importance, because the formation of dimers is regarded as an artificial process, mainly caused by the hot spot application in connection with an extraction procedure in order to get high recoveries for parent compound in that laboratory test. Therefore, the concentration levels of the two BYI08330-enol-dimers 1 and 2 (ROI 6 and ROI 7) of up to 12.7% of applied radioactivity are not representative.

Due to above mentioned issues the degradation/metabolism of the BYI08330-enol in soils was investigated in a separate study (KIIA 7.1.1/03) with broadcast instead of hot spot application, and with soil processing appropriate for BYI08330-enol. The results of this study clearly showed that BYI08330-enol-dimers 1 and 2 are minor transformation products in soils, only. This was confirmed in the soils of an outdoor metabolism study (KIIA 7.1.1/02) performed under realistic climatic and GAP conditions (i.e. using formulated [^{14}C]spirotetramat) where none of the BYI08330-enol-dimers reached or exceeded 1.5% of applied radioactivity during the entire study. Since the outdoor metabolism study represents a higher tier study compared to the studies performed under artificial laboratory conditions, the BYI08330-enol-dimers 1 and 2 were only considered as minor metabolites of spirotetramat in soil. Therefore, determination of adsorption and desorption properties in soil was not triggered. However, an estimation of the adsorption behavior was done by an HPLC based estimation method as described

below. Thus, the study on the metabolism/degradation of BYI08330 (KIIA 7.1.1/01) gives just valid data related to the disappearance of active substance BYI08330, but not the true quantitative distribution pattern of the metabolites resulting from the rapidly formed major metabolite BYI08330-enol. That important information can only be drawn from the BYI08330-enol soil metabolism study (KIIA 7.1.1/03) mentioned before.

Report:	**KIIA 7.4.2/05, [redacted] & [redacted], 2006 (MEF-06/123)**
Title:	**Estimation of the Adsorption Coefficient (KOC) of BYI 08330-enol dimer 1 (ROI 6, ^{14}C-labelled) and BYI 08330-enol dimer 2 (ROI 7, ^{14}C-labelled) on Soil using High Performance Liquid Chromatography (HPLC)**
Report No & Document No	**MEF-06/123 M-274184-02-1**
Guidelines:	OECD Guideline for the Testing of Chemicals No. 121
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and dates	Bayer CropScience AG – D – Metabolism / Environmental Fate, [redacted], Germany; Experimental work: 2004-08-17 – 2004-08-30; Study completion date: 2006-05-19, and 1st amendment to report dated 2007-06-01

EXECUTIVE SUMMARY

The adsorption coefficients on soil of the metabolites BYI08330-enol dimer 1 (company code: ROI 6) and BYI08330-enol dimer 2 (company code: ROI 7) were estimated using the HPLC method according to OECD TG No. 121. Thirteen reference items for which reliable K_{OC} values are known from the literature were chromatographed in duplicate on a cyanopropyl-type column using mobile phases adjusted to pH 6 and to pH 1.7. Sodium nitrate was used to measure the chromatography system dead-time. The results under both pH conditions were nearly identical, which allows the conclusion that both test items do not have ionic (acidic or alkaline) properties. Therefore, only the values of the pH6 conditions are given in this summary. Average capacity factors (k') were derived for each compound, and a linear calibration function was established for log k' values vs. log K_{OC} values (e.g. for pH 6 slope= 3.70, intercept = 1.96, R^2 = 0.935). The capacity factors of BYI08330-enol dimer 1 and –dimer 2 were determined by replicate analyses within the same HPLC auto sampler work list as the reference items. Based on the mentioned calibration equation for pH 6, the soil adsorption coefficients of BYI08330-enol dimer 1 were estimated to be log K_{OC} = 3.23 and K_{OC} = 1708. For the BYI08330-enol dimer 2 a log K_{OC} = 3.46 and a K_{OC} = 2896 were estimated.

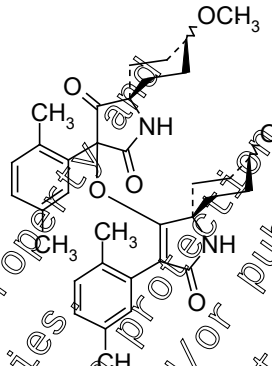
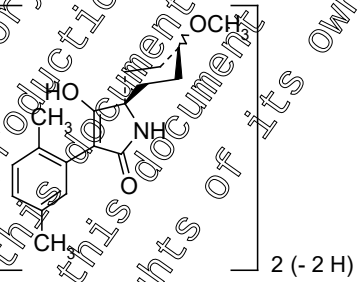
According to the Briggs' classification for the mobility of crop protection agents in soil based on their numeric adsorption coefficients, BYI08330-enol dimer 1 and BYI08330-enol dimer 2 would be categorized as immobile.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Items: The ^{14}C-labelled test items (see Table 7.4.2-7) were isolated and identified in the aerobic soil degradation / metabolism study conducted with ^{14}C-labelled BYI08330 in four different soils. The isolation procedures and spectra obtained from a metabolite production batch are described there (see earlier in Report KIIA 7.1.1/01).

Table 7.4.2-7: Description of test items (MEF-06/123)

Name	BYI08330-enol dimer 1 synonyms: WU2010, ROI 6	
IUPAC nomenclature	Not available	
CA Index name	Not available	
CAS number	Not available	
Empirical formula	$C_{36}H_{44}N_2O_6$	
Molecular weight	600 g/mol	
Specific radioactivity	0.74 MBq/mg or 44,400 dpm/µg	
Name	BYI 08330-enol dimer 2 synonyms: WU2011, ROI 7	
IUPAC nomenclature	Not available	
CA Index name	Not available	
CAS number	Not available	
Empirical formula	$C_{36}H_{44}N_2O_6$	
Molecular weight	600 g/mol	
Specific radioactivity	0.74 MBq/mg or 44,400 dpm/µg	

2. Test system: The test system was a high pressure liquid chromatography station, fitted with a pulse-free pump and a flow-through UV absorbance detector. A commercially available cyanopropyl-bonded column was employed (Zorbax CN, 5 µm (Bischoff), length = 250 mm / inner diameter = 4.6 mm; Part No.: 2546D200ZX050). The chromatography method was isocratic elution with a pre-mixed and degassed methanol / aqueous pH 6 citrate buffer eluent or with a pre-mixed and degassed methanol / aqueous phosphoric acid pH 1.7 eluent. Sodium nitrate was used to measure the chromatography system dead-time.

Thirteen reference items were employed as calibration standards for the HPLC analytical method. Reliable soil adsorption coefficients (K_{OC}) for these compounds are known from literature sources or internal guideline studies, which had been derived from batch equilibrium, soil thin-layer chromatography and aged soil column leaching experiments.

B. STUDY DESIGN

1. Experimental conditions: The adsorption coefficients on soil of the metabolites BYI08330-enol dimer 1 (company code: ROI 6) and BYI08330-enol dimer 2 (company code: ROI 7) were estimated using the HPLC method according to OECD TG No. 121. Thirteen reference items were chromatographed in duplicate on a cyanopropyl-type column using mobile phases adjusted to pH 6 and to pH 1.7.

2. Procedures: The test solutions of the reference items and of the test items were subjected to HPLC analysis, to determine the retention times (t_R) of all compounds. The analytes were injected individually

and in duplicate, together within the same auto sampler work list. Each reference item was run once before and once after the test item, to minimize influence of possible retention time drift. Injection of sodium nitrate was carried out at the beginning and at the end of the analytical series. Since sodium nitrate is unretained on the analytical column, its retention time is equal to the dead time (t_0) of the chromatography system.
In case of the both radiolabelled test items, their UV- and ^{14}C-signal were recorded during HPLC analysis. The ^{14}C-signals were used to identify and assign the UV-signals of the test items. The evaluation of the test items was based on the retention times of their UV-signals because of better and sharper peak shape of UV-signals.

The capacity factors (k') were calculated from the system dead time (t_0), and the retention times (t_R) of the test and reference items. Replicate mean log k' data (± range of the individual replicates) were then plotted versus the literature K_{OC} data of the reference items.

$$k' = \frac{t_R - t_0}{t_0}$$

t_R = HPLC retention time of test or reference items (minutes)
t_0 = HPLC system dead time, i.e. retention time of sodium nitrate (minutes)

Linear regression was used for statistical evaluation, and for calculation of log K_{OC} of the test items, BYI08330-enol dimer 1 and BYI 08330-enol dimer 2, based on its measured log k'. The replicate mean log K_{OC} from duplicate analysis was considered as the final result of determination.

$$\log K_{OC} = slope \times \log k' + y\text{-}intercept$$

slope, y-intercept = regression parameters derived from linear regression for log k' vs. log K_{OC} for the calibration references

For the evaluation and calculation of both test items under pH 6 HPLC conditions, all 13 reference items were used for the linear regression. For the evaluation and calculation of both test items under pH 1.7 HPLC conditions, one reference item was excluded from linear regression and calculation because of its atypical HPLC behavior under acidic conditions. Linear regression and calculation were performed with the remaining 12 reference items.

C. RESULTS AND DISCUSSION

The soil adsorption coefficient (K_{OC}) of the test items BYI08330-enol dimer 1 and BYI08330-enol dimer2 were estimated by the HPLC method according to OECD Test Guideline No. 121. The HPLC retention data and calculation of capacity factors (k') for the test items and all reference items were provided for both HPLC solvents, in Table 1 of report MEF-06/123 for pH 6 and in Table 2 of report MEF-06/123 for pH 1.7.

The employed reference items were heterogeneous in chemical nature, and with respect to halogenated organic moieties, in part structurally related to the test items. No trend for irregular behavior was observed for any specific compound characteristics. Comparison of the retention times indicated appropriate stability of the HPLC method throughout the auto sampler work list series. Retention time of the test item fell within the range covered by the calibration line, and individual replicate log K_{OC} deviated significantly less (0.02 log unit) than guideline repeatability quality criteria (<0.25 log unit). Thus it was concluded that the HPLC method provides a reasonable estimate of an equilibrium soil adsorption coefficient for the two test items.

For the test performed at pH 6, linear regression of the logarithms of measured k' values for all reference

items versus logarithms of their literature K_{OC} returned a slope of 3.6967, and an Y axis intercept of 1.9630. The correlation coefficient close to one ($R^2 = 0.9347$) indicated very good fit of the regression line to the experimental data. At pH 1.7, the linear regression of measured k´values yielded a line with a slope of 3.2797, an intercept of 2.1624 and a correlation coefficient R^2 of 0.9667.

Based on the above calibration functions and the log k' values determined for the test items, adsorption coefficients of BYI08330-enol dimer 1 and -dimer 2 were estimated as shown in Table 7.4.2-8.

Table 7.4.2-8: Adsorption coefficients of BYI08330-enol dimer 1 and BYI0830-dimer 2 estimated by HPLC method according to OECD TG No. 121 (MEF-06/123)

	Test performed at pH 6	
BYI 08330-enol dimer 1	log K_{OC} = 3.23	K_{OC} = 1708
BYI 08330-enol dimer 2	log K_{OC} = 3.46	K_{OC} = 2896
	Test performed at pH 1.7	
BYI 08330-enol dimer 1	log K_{OC} = 3.17	K_{OC} = 1467
BYI 08330-enol dimer 2	log K_{OC} = 3.51	K_{OC} = 4199

III. CONCLUSIONS

The log K_{OC} and the respective K_{OC} values of each test item were nearly identical at pH 6 and at pH 1.7 conditions. It can be concluded that both test items do not have ionic (acidic or alkaline) properties. According to the Briggs classification system for mobility of crop protection agents in soil based on their numeric equilibrium adsorption coefficients, BYI08330-enol dimer 1 and BYI08330-enol dimer 2 mobility would be categorized as 'immobile'.

IIA 7.4.3 Column leaching studies with the active substance

Column leaching studies were not performed for the active substance. This requirement is covered by the adsorption/desorption studies with the parent compound as presented in IIA 7.4.1.

IIA 7.4.4 Column leaching studies rel. metabolites, degr. & and react. products

Metabolite BYI08330-enol

Chemical name (CAS): cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one; CAS #: 203312-38-3

Report:	**KIIA 7.4.4/01, [redacted] (MEF-05/356)**
Title:	**[BYI08330-Enol: Soil Column Leaching**
Report No & Document No	**MEF-05/356** **M-270280-02-2**
Guidelines:	US EPA: Pesticide Assessment Guidelines, Subdivision N, Section 163-1: Leaching and Adsorption/Desorption Studies, 1982 OECD: Guideline 312: Leaching in Soil Columns, 2003 EU: Commission Directive 95/36/EC amending Council Directive 91/414/EEC (Annexes I and II, Fate and Behavior in the Environment, 1995) SETAC: Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, 1995 CAN: Environmental Chemistry and Fate, Guidelines for Registration of Pesticides, 1987
GLP	Fully GLP compliant - laboratory certified by German “Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen“.
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of Oct. 2004 to March 2005. Study completion date: 2006-03-24 (Amendment No 1 of : 2006-04-20)

EXECUTIVE SUMMARY

The mobility of radiolabelled cis-[azaspirodecenyl-3-^{14}C]BYI08330-enol (major soil metabolite of BYI08330 [common name: Spirotetramat]) was studied in a column leaching study using three German and one US soils. For these soils, K_d and K_{OC} values were calculated for the BYI08330-enol using mathematical relationships derived from the theory of chromatographic flow. The soils were identical with the soils taken for the respective aerobic soil metabolism studies using BYI08330 and BYI08330-enol as test items (t.i.).
BYI08330-enol was applied at a concentration of 30.3 µg/sectional area of the columns corresponding to the maximum recommended field application rate of about 288 g/ha per single treatment, and to an assumed (worst case) maximum amount of 50% BYI08330-enol formed from spirotetramat. The anticipated test conditions were maintained throughout the study, i.e. the test systems were leached for five days by overhead irrigation with a total solution of 1000 mL of 0.01 M $CaCl_2$ (equal to 50.8 cm/m^2 and corresponding to 20 inches of simulated rainfall). The $CaCl_2$ solution was pumped onto the soil columns and was percolated through the columns at a constant flow rate of approximately 8 mL/h under maintenance of a constant head above the soil surface (saturated flow). The study was carried out at ambient temperature (approximately 22 °C) in the dark.

Average material balances ranged from 86.9 to 96.8% of the applied amount. The total radioactivity detected in the leachates was 12.3%, 17.0%, 8.9% and 2.6% AR for the columns packed with soils [redacted], [redacted]a, [redacted] and [redacted], respectively. The total BYI08330-enol content in these leachates was 2.8%, 1.3%, 2.7% and 0.1% AR, respectively. Other leachate components formed from BYI08330-enol during the column passage were BYI08330-ketohydroxy (at maximum 7.5% AR in total leachate) and BYI08330-MAamide (at maximum 1.9% AR

in total leachate). The recovered total radioactivity from the column soil segments were 80.3%, 70.0%, 81.3% and 93.4% AR for the columns packed with soils [redacted], [redacted]a, [redacted] and [redacted], respectively. Significant amounts of radioactivity could not be extracted from the soil segments; these bound residues ranged from 29.5% (soil [redacted]) to a maximum of 42.2% AR (soil [redacted]). Radioactivity extractable from the soil segments was mainly located in the top soil segment (0-6 cm) and amounted to 29.3%, 29.6%, 33.6% and 49.7% AR for the four soils. Non-extractable portions in the top soil segments reached 40.1%, 34.6%, 37.4% and 27.9% AR for the four soils. In sum, from the top soil segments, 69.4%, 64.2%, 70.9% and 77.6% of the total applied radioactivity were recovered. BYI08330-enol was almost exclusively located in the top soil segment, and it furthermore represented the main compound detected in the extracts from the top soil segments: It amounted to 10.2%, 9.6%, 11.5% and 16.5% AR for the four soil types. Only in the case of [redacted] soil, a small amount of BYI08330-enol (1.4% AR) was detected in the second soil segment (6-12 cm); in the other three soil columns, BYI08330-enol did not move into lower segments. Other major components detected in the top soil segment extracts were BYI08330-enol-dimers 1 and 2, for which strong evidence is given, that their formation is artificial due to the respective application technique. Maximum values amounted to 11.8% AR (BYI08330-enol-dimer 1) and 10.7% AR (BYI08330-enol-dimer 2), both in soil [redacted] columns. The leachates were totally free from these.

From this study it was evident that only a small portion of applied BYI08330-enol (i.e. the equilibrium enol fraction - less than 2.8%) had a potential to leach into deeper soil layers or leave the soil column, whereas by far the major portion (strongly sorbed enol fraction) was firmly bound to the top soil layer within a soil column leaching scenario. In soil extracts, only a minor part of the applied BYI08330-enol was recovered, and an even smaller portion in the leachate, indicating immediate and strong adsorption of BYI08330-enol to the soil. Furthermore, to a small extent transformation into BYI08330-enol-derived degradates and significant formation of bound residues occurred during the progress of the study.

Since only partial degradation of the test item occurred during the course of the study, the test system allowed the calculation of adsorption constants for the test item in four different soils. For the strongly bound BYI08330-enol fraction Koc values between 828 and 1711 mL/g were calculated, resulting in a mean value of 1187 mL/g over four soil types. For the mobile BYI08330-enol fraction Koc values between 27 and ca. 99 mL/g were calculated, resulting in a mean value of 55 mL/g over four soil types.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BYI08330-enol, within the study also called "enol"
Identity and purity of test item in the application solution were checked
Label position = [Azaspirodecenyl-3-^{14}C] (sample ID: BECH 1610)
Specific activity 4.54 MBq/mg (122.8 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

2. Soil: The mobility of BYI08330-enol was studied in three EU soils and one US soil in the dark. The selected soils were identical with the soils taken for the respective aerobic soil metabolism studies using BYI08330 and BYI08330-enol as test items (t.i.) and meet the guidelines' requirements (for data see Table IIA 7.4.4-1). All soils were taken freshly from the A horizon (ca. 0-20 cm depth) of the respective field. Stones and plant material were removed, and soil moisture was partially reduced by spreading the soil at ambient temperature to allow for sieving to a particle size of 2 mm (approximately two weeks prior to the start of the pre-incubation for the degradation tests). Finally, the soil batches were each

mixed thoroughly for optimal batch homogeneity.

Table IIA 7.4.4-1: Physicochemical properties of test soils (MEF-05/356)

Designation	Source	Soil Type (USDA)	pH ($CaCl_2$)	Organic Carbon [%]	Texture Analysis [% sand/silt/clay]
[redacted]	[redacted], Germany	Sandy loam	6.1	2.0	69 / 21 / 10
[redacted]	[redacted], Germany	Loam	6.5	1.2	49 / 31 / 20
[redacted]	[redacted], Germany	Silt loam	6.4	2.4	19 / 63 / 18
[redacted]	[redacted], Florida, USA	Sandy loam	5.4	0.8	77 / 13 / 10

The cation exchange capacity (CEC) ranged between 4 to 14 meq/100 g DM.
Measurement of initial & final soil biomass (mg microbial C/kg soil DM) indicated that the soils were viable throughout the study.

The test system consisted of each two poured soil columns (duplicates) of ca. 30 cm in length and 5 cm in diameter. The glass columns were closed at the lower end with cotton wool, followed by a layer of washed sea sand in the conical part of the column up to a level just reaching the cylindrical part of the glass tube. The columns were dry-packed with 2 mm-sieved soils (dry weight between 583 and 836, corresponding to 760 to 900 g fresh weight) in portions, while gently vibrating, to a height of approximately 30 cm. Then, the columns were saturated with the help of a peristaltic pump with an overnight upward flow of 0.01 M $CaCl_2$ solution to minimize air entrapment in soil pores. The entire volume of the $CaCl_2$ solution needed to just cover the soil in each case was determined (saturation volume). After a soaking period for approximately eight hours, the $CaCl_2$ solutions were allowed to drain, and the volumes of drained water (dripping volume) were determined after a dripping period overnight (about 16 hours). The columns were re-watered from the bottom with $CaCl_2$ solution to just cover the soil. For more information of experimental design see also Table 2 and Table 3 of report MEF-05/356 and next paragraph.

B. STUDY DESIGN

1. Experimental conditions: The mobility of radiolabelled BYI08330-enol was studied in a column leaching study using three German and one US soils. For these soils, K_d and K_{OC} values were calculated for the BYI08330-enol using mathematical relationships derived from the theory of chromatographic flow.

BYI08330-enol was applied on top of the water-saturated soil columns at a concentration of 30.3 µg/sectional area of the columns corresponding to the maximum recommended field application rate of about 288 g/ha per single treatment, and to an assumed (worst case) maximum amount of 50% BYI08330-enol formed from spirotetramat.

The test systems were leached for five days by overhead irrigation with a total solution of 1000 mL of 0.01 M $CaCl_2$ (equal to 50.8 cm/m^2 and corresponding to 20 inches of simulated rainfall). The $CaCl_2$ solution was pumped onto the soil columns and was percolated through the columns at a constant flow rate of approximately 8 mL/h under maintenance of a constant head above the soil surface (saturated flow). The study was carried out at ambient temperature (approximately 22 °C) in the dark.

Since, formation of volatile ^{14}C-components during the relatively short course of the study was not a major issue, collecting trap attachments were not used (open system). Nevertheless, material balances

were calculated.

The study setup does not represent a realistic leaching scenario, but is designed to provide mobility parameters (sorption characteristics) of BYI08330-enol. The set-up allowed control and maintenance of a constant level of ponding water on top of the soil to guarantee saturated flow conditions as a prerequisite for the estimation of K_{OC} values according to the chromatographic theory. The height of ponding water in this study was approximately 10 mm

2. Sampling: The mobility of the test item and derived residues in the columns was investigated by analyzing extracts of each five 6-cm soil segments and eleven leachate fractions (ten 100 mL fractions during flow through plus one final dripping fraction). The pH values of all leachate fractions were measured, and the radioactivity content of the leachate fractions was determined by LSC.
At the end of the leaching period the soil columns were frozen and then slightly thawed using a fan in order to push the frozen cores out of the glass tubes. The 30 cm columns were cut into five segments of 6 cm each using a knife. The bottom segment extended up to some millimeters more or less than 6 cm depending on the entire column length and included the sea sand plus the cotton wool plug. The top segments were extracted immediately after slicing, and the lower segments were stored frozen and extracted later. After thawing the soil segment was extracted three times with 120 mL of acetonitrile/water (1:1; containing 0.1% ammonium chloride [w/v] and 0.06% ammonia [v/v]; pH ca. 8.5) at room temperature on a mechanical shaker for 30 min. After each shaking step the suspension was centrifuged for about 15 minutes (about 5000 x g), and the clear supernatant was passed through a paper filter. The conventional (ambient) organic/aqueous extracts were combined and the volume and radioactivity content was determined.
In order to obtain some information on the nature of bound residues, the soil already extracted using the ambient procedure was additionally subjected to an aggressive extraction with 120 mL of acetonitrile/water (1:1; containing 0.1% ammonium chloride [w/v] and 0.06% ammonia [v/v]; pH ca. 8.5) using a microwave extractor for 10 min at 70 °C. After cooling down to room temperature, the suspension was centrifuged for about 15 minutes (about 5000 x g), and the clear supernatant was passed through the paper filter used already for the ambient extraction. The volume and radioactivity content was determined.

3. Description of analytical procedures: All leachate fractions with a radioactivity content of approximately greater 0.3% of the applied radioactivity (equivalent to approximately 385 Bq/100 mL of leachate) were analyzed by RP-18 HPLC. All conventional soil segment extracts were analyzed by RP-18 HPLC, also. Only the "aggressive" extracts obtained from the top soil segment were analyzed by RP-18 HPLC, since the RA content in the others was very low.
Leachate aliquots (20 mL aliquots of 100 mL fractions) were concentrated to 1-2 mL using a SpeedVac vacuum concentrator (with sample cooling caused by solvent evaporation). The concentrated samples were sonicated, their volume measured and their radioactivity content determined by LSC. Prior to HPLC analysis, they were centrifuged for 15 min at approximately 13000 rpm. Recoveries for all leachate fractions from all soil columns were calculated.
Aliquots of the ambient organic/aqueous extracts and the "aggressive" organic/aqueous extracts were concentrated from 10 mL to approximately 1-2 mL by SpeedVac evaporation. After addition of approximately 0.5 mL of methanol, the extracts were sonicated, centrifuged and analyzed by HPLC. Recoveries for all ambient and "aggressive" extracts from all soil columns were calculated.
Identification of test item and related residues was performed by co-chromatography with authentic reference substances and by LC-MS and LC-MS/MS spectrometry.
The amount of NER (bound residues) after aggressive extraction was determined by combustion of small aliquots (approx. 1 g) after homogenization (by milling) of the entire amount of air-dried soil. The

filter papers were divided into four parts, pressed into pills and combusted in order to determine their radioactivity content, which was added to the bound residues.

All the calculations performed in the study (i.e. those for estimating of K_{OC} values for a compound remaining in the soil column or leaving the soil column) were described in detail in paragraph 3.7 of report MEF-05/356.

II. RESULTS AND DISCUSSION

A. DATA

The anticipated test conditions were maintained throughout the study. The respective results for the four soils are compiled in Table IIA 7.4.4-2.

B. MASS BALANCE

Average material balances ranged from 86.9 to 96.8% of the applied amount (mean 91.4%). A complete mass balance (i.e. approx. 100% recovery) was not to be expected since an open system was used in this study mainly focusing on mobility in soil.

C. BOUND AND EXTRACTABLE RESIDUES

The total radioactivity detected in the leachates was 12.3%, 17.0%, 8.9% and 2.6% AR for the columns packed with soils [redacted], [redacted]a, [redacted] and [redacted], respectively.

The recovered total radioactivity from the column soil segments was 80.3%, 70.0%, 81.3% and 93.4% AR for the columns packed with soils [redacted], [redacted]a, [redacted] and [redacted], respectively. Significant amounts of radioactivity could not be extracted (NER) from the soil segments; these bound residues ranged from 29.5% (soil [redacted]) to a maximum of 42.2% AR (soil [redacted]). Radioactivity extractable from the soil segments was mainly located in the top soil segment (0-6 cm) and amounted to 29.3%, 29.6%, 33.6% and 49.7% AR for the four soils. Non-extractable portions in the top soil segments reached 40.1%, 34.6%, 37.4% and 27.9% AR for the four soils. In sum, from the top soil segments, 69.4%, 64.2%, 70.9% and 77.6% of the total applied radioactivity were recovered.

D. VOLATILIZATION

N/A

E. TRANSFORMATION OF TEST ITEM

The total BYI08330-enol content in the leachates was 2.8%, 1.3%, 2.7% and 0.1% AR, respectively. Other leachate components formed from BYI08330-enol during the column passage were BYI08330-ketohydroxy (at maximum 7.5% AR in total leachate) and BYI08330-MA-amide (at maximum 1.9% AR in total leachate).

BYI08330-enol was almost exclusively located in the top soil segment, and it furthermore represented the main compound detected in the extracts from the top soil segments: It amounted to 10.2%, 9.6%, 11.5% and 16.5% AR for the four soil types. Only in the case of [redacted] soil, a small amount of BYI08330-enol (1.4% AR) was detected in the second soil segment; in the other three soil columns, BYI08330-enol did not move into lower segments. Other major components detected in the top soil segment extracts were BYI08330-enol-dimers 1 and 2, for which strong evidence is given, that their

formation is artificial due to the respective application technique. Maximum values amounted to 11.8% AR (BYI08330-enol-dimer 1) and 10.7% AR (BYI08330-enol-dimer 2), both in soil [redacted] columns. The leachates were totally free from these.

Table IIA 7.4.4-2: Column leaching results of BYI08330-enol in for soils (values expressed as means of two columns for each soil) (MEF-05/356)

Soil	[redacted]	[redacted] a	[redacted]	[redacted]
	Sandy loam	Loam	Silt loam	Sandy loam
C_{org} (%)	2.0	1.2	2.4	0.8
	% of applied radioactivity (AR)			
Soil extractable (post leaching)				
Total	37.8	33.5	41.3	63.8
Top soil segment	29.3	29.6	33.6	49.7
Soil non-extractable (post leaching)				
Total	42.2	36.5	40.0	29.5
Top soil segment	40.1	34.6	37.4	27.9
Enol in column				
Total	10.2	9.6	11.6	17.9
Top soil segment	10.2	9.6	11.5	16.5
Leachate	12.3	17.0	8.9	2.6
Enol in leachate	2.8	1.3	2.7	0.1
Mass balance	92.4	87.0	90.2	96.0

F. ADSORPTION PARAMETERS OF TEST ITEM

Since only partial degradation of the test item occurred during the course of the study, the test system allowed the calculation of adsorption constants for the test item in four different soils. For the strongly sorbed BYI08330-enol fraction K_{OC} values between 828 and 1711 mL/g were calculated, resulting in a mean value of 1187 mL/g over four soil types (see Table IIA 7.4.4-3). For the equilibrium BYI08330-enol fraction K_{OC} values between 27 and ca. 99 mL/g were calculated, resulting in a mean value of 55 mL/g over four soil types (see Table IIA 7.4.4-4).

III CONCLUSIONS

From this study it was evident that only a small portion of applied BYI08330-enol (i.e. the equilibrium enol fraction - less than 2.8%) had a potential to leach into deeper soil layers or leave the soil column, whereas by far the major portion (strongly sorbed enol fraction) was firmly bound to the top soil layer within a soil column leaching scenario. In soil extracts, only a minor part of the applied BYI08330-enol was recovered, and an even smaller portion in the leachate, indicating immediate and strong adsorption of BYI08330-enol to the soil.

Furthermore, to a small extent transformation into BYI08330-enol-derived degradates and significant formation of bound residues occurred during progress of study.

Table IIA 7.4.4-3: Estimated adsorption constants of strongly sorbed BYI08330-enol fraction*) in soils (MEF-05/356)

Soil	[redacted]	[redacted]a	[redacted]	[redacted]
1) Calculation according to Lambert et al. [1]				
K_d value	14.5	13.7	16.5	11.6
K_{oc} value	723	1138	688	1455
2) Calculation according to Hamaker/McCall et al. [2] [3]				
K_d value	20.1	18.7	23.2	15.7
K_{oc} value	1004	1557	967	1966
Mean over methods 1) & 2)				
K_d	17.3	16.2	19.9	13.7
K_{oc}	863	1347	828	1711
Mean over 4 soils:	K_d = 16.8	K_{OC} = 1187		

*): Strongly sorbed BYI08330-enol fraction: Probably oligo- or polymeric structures releasing free enol due to the extraction process of soil.

[1] Lambert, S. M., Porter, P. E. & Schieferstein, R. H. (1965). Movement and sorption of chemicals applied to the soil, Weeds 13, 185-190
[2] Hamaker, J. W. (1975). The interpretation of soil leaching experiments, in: Environmental dynamics of pesticides (Eds. R. Haque & V. H. Freed, Plenum Press, New York), pp 115-132
[3] McCall, P. J., Laskowski, D. A., Swann, R. L. & Dishburger, H. J. (1981). Measurement of sorption coefficients of organic chemicals and their use in environmental fate analysis, in: Test protocols for environmental fate and movement of toxicants; Proceedings of AOAC Symposium, AOAC, Washington, DC, pp 89-109

Table IIA 7.4.4-4: Estimated adsorption constants of equilibrium BYI08330-enol fraction) in soils (MEF-05/356)**

Soil	[redacted]	[redacted]a	[redacted]	[redacted]
Calculation according to Ketelle et al. [1] & Swoboda et al. [2]				
K_d value	0.34	0.78	0.70	0.79
K_{oc} value	27.0	65.0	29.0	98.8
Mean over 4 soils:	K_d = 0.7	K_{OC} = 55		

** Equilibrium BYI08330-enol fraction. Occurs already in soil prior to the extraction procedure and is very fast degradable

[1] Ketelle, B. H. & Boyd, G. E. (1947). The exchange adsorption of ions from aqueous solutions by organic zeolithes. IV. The separation of the Yttrium group rare earths, J. Amer. Chem. Soc. 69, 2800-2812
[2] Swoboda, A. R. & Thomas, G. W. (1968). Movement of parathion in soil columns, J. Agric. Food Chem. 16, 923-927

IIA 7.4.5 Aged residue column leaching

Aged column leaching studies were not performed. This requirement is covered by the adsorption/desorption studies with the parent compound and metabolites as presented in IIA 7.4.1 and IIA 7.4.2.

IIA 7.4.6 Leaching (TLC)

Such data describing the mobility in soil were supplied by higher tier studies shown in all other sections of point IIA 7.4.

IIA 7.4.7 Lysimeter studies

The mobility of the spirotetramat residues in soil has been assessed on the basis of the adsorption/desorption studies with the parent compound and metabolites (as presented in IIA 7.4.1 and IIA 7.4.2) and a modeling study of the $PEC_{Ground\ Water}$ values following the maximum annual use for citrus, oranges, mandarins, lemons, limes etc. in EU-South and for lettuce in EU-North and EU-South (KIIIA 9.6.1/01, [redacted], 2006).

Based on the results indicating no concern with regard to groundwater contamination – it is concluded that the mobility of spirotetramat residues in soil is sufficiently understood after its use in the EU. A lysimeter study is considered not necessary.

IIA 7.4.8 Field leaching studies

Based on the results of laboratory and modeling studies mentioned in the chapter before it is concluded that the mobility of spirotetramat residues in soil is sufficiently understood after its intended use, and no concern with regard to groundwater contamination is indicated. This was supported by the terrestrial field dissipation studies performed in the USA, since there was no movement measured below a 15-cm depth of soil. Thus, field leaching studies were considered not necessary.

Summary: Mobility of spirotetramat in soil

Freundlich adsorption and desorption constants K_F and K_{OC} of spirotetramat have been determined in batch equilibrium experiments with five different soils using radiolabeled test substance ([azaspirodecenyl-3-^{14}C]BYI08330). Since significant degradation of test item was observed in a pre-test, the main test was performed with sterilized soil. K_{OC} values for the different soils were in the range of 159 to 435 mL/g with a mean K_{OC} of 281 mL/g (1/n = 0.941). Based on this value, spirotetramat can be classified as low mobile in soil.

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-enol, the major metabolite in soil, was attempted in batch equilibrium experiments with five different soils using radiolabeled test substance ([azaspirodecenyl-3-^{14}C]BYI08330-enol. However, the study showed that the sorption characteristics of BYI08330-enol to soil cannot be determined by a batch equilibrium test according to OECD Guideline 106. In order to asses the environmental behavior of the test item more suitable test methods had to be employed. Another option, i.e. a so-called time-dependent sorption study, demonstrated that sorption and binding of BYI08330-enol to soil is extremely fast and increases very rapidly with aging time in soil. The portion not tightly bound to soil, i.e. the portion that is releasable by aqueous solution from soil (weakly sorbed), is degraded within a few hours. From these results it can be concluded that BYI08330-enol is expected to be generally absent from the soil pore water (either degraded or tightly bound to soil) within a very short period of time. This study also indicated that the sorption characteristics of the test item BYI08330-enol to soil cannot be determined accurately by a batch equilibrium test according to OECD TG 106. The adsorption constant of BYI08330-enol was however calculated using the results from a column leaching study conducted on four soils. For the

strongly sorbed BYI08330-enol fraction K_{OC} values between 828 and 1711 mL/g were calculated, resulting in a mean value of 1187 mL/g over four soil types. For the equilibrium BYI08330-enol fraction K_{OC} values between 27 and ca. 99 mL/g were calculated, resulting in a mean value of 55 mL/g over four soil types. Based on the classification of soil mobility potential according to Briggs, the strongly sorbed BYI08330-enol fraction is classified as immobile, and the equilibrium BYI08330-enol fraction has an intermediate potential to leach through soil.

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-ketohydroxy, a major metabolite in soil, have been determined in batch equilibrium experiments with five different soils using [azaspirodecenyl-3-^{14}C]BYI08330-ketohydroxy. Since significant degradation of test item was observed in a pre-test, the equilibration solution used was 0.01 M aqueous $CaCl_2$ solution spiked with 50 mg $HgCl_2$ as biocide. $K_{OC(ads)}$ values for the different soils were in the range of 41.0 to 99.1 mL/g with a mean K_{OC} of 63.7 mL/g (1/n = 0.922). Based on this value, BYI08330-ketohydroxy can be classified as intermediate to mobile in soil.

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-MA-amide, a major metabolite in soil, have been determined in batch equilibrium experiments with five different soils using [hydroxy-^{14}C]BYI08330-MA-amide. The calculated adsorption constants K_F of the FREUNDLICH isotherms for the four test soils ranged from 0.06 to 0.18, and the $K_{OC(ads)}$ values were in the range of 4.4 to 25.5 mL/g with a mean $K_{OC(ads)}$ of 9.3 mL/g (mean 1/n = 0.948). Based on this value, BYI08330-MA-amide can be classified as high mobile in soil. The desorption K_{des} values were 0.13 to 0.37 and higher than those obtained for K_F in the adsorption phase indicating a little stronger binding once adsorbed to soil.

Despite an isomerization leading to the BYI08330-enol dimers 1 and 2 is regarded as of minor importance for use of spirotetramat in the field according to the GAP, its adsorption coefficients on soil were estimated, i.e. by using the HPLC method according to OECD TG No. 121. Since the results under both pH conditions tested were nearly identical, which allows the conclusion that both test items do not have ionic (acidic or alkaline) properties, only the values of the pH6 conditions are given in this summary. The soil adsorption coefficients of BYI08330-enol dimer 1 were estimated to be log K_{OC} = 3.23 and K_{OC} = 1708. For the BYI08330-enol dimer 2, a log KOC = 3.46 and a K_{OC} = 2896 were estimated. According to the Briggs' classification for the mobility of crop protection agents in soil based on their numeric adsorption coefficients, BYI08330-enol dimer 1 and BYI08330-enol dimer 2 would be categorized as immobile.

From all the before mentioned laboratory studies it is concluded that the mobility of spirotetramat residues in soil is sufficiently understood. The available data can be adequately used to perform long-term leaching simulations with validated computer programs.

IIA 7.4.9 Volatility - laboratory study

In accordance with Points IIA 2.3.1 and IIA 2.3.2, the vapor pressure and Henry's law constant of spirotetramat were determined. Based on the results of these studies it was concluded that significant volatilization of spirotetramat in the environment is not to be expected. Therefore, no further laboratory experiments were considered necessary. Short summaries of the studies on vapor pressure and Henry's law constant are given below.

Vapour pressure

Report:	**KIIA 2.3.1/01; KIIA 7.4.9/01, [redacted] (20040156.02)**
Title:	**BYI 08330, Mix-Batch 08045/0003 - Vapor pressure**
Report No &	**20040156.02**
Document No	M-066171-01-1
Guidelines:	EC Directive 92/69/EEC Method A4; OECD Guideline 104 (1995)
GLP	yes
Testing Laboratory and dates	Sicherheitstechnik [redacted], D-[redacted], GER; Experimental work: 2004-03-15 – 2004-03-16; Study completion date (including amendment): 2004-03-26

The vapor pressure of the test substance BYI08330 was experimentally determined using the vapor pressure balance (effusion method). No consistent weight losses were recorded from samples exposed at temperatures of 34 up to 89 °C. The vapor pressure was measured in the temperature range of 34 °C to 146 °C. Above 96 °C a vapor pressure could be measured, and the following vapor pressure values (p) were extrapolated:

T in °C	p in hPa	p in Pa
20	5.6 x 10E-14	5.6 x 10E-09
25	1.5x 10E-10	1.5 x 10E-08
50	1.5x 10E-08	1.5 x 10E-06

Based on this vapor pressure, considerable volatilization of spirotetramat when applied to soil surfaces or leaves is not to be expected. This evaluation is also confirmed by the rating of trigger values for volatilization as described in the model EVA 2.0 developed from the FOCUS Air group. There, for compounds with a vapor pressure < 10^{-4} Pa at 20 °C volatilization from soil surfaces or with a vapor pressure < 10^{-5} Pa at 20 °C volatilization from plant surfaces is not considered relevant.

Henry's Law constant

Report:	**KHA 2.3.2/01; KHA 7.4.9/02, [redacted] (AF05/085)**
Title:	**Henry's Law Constant of BYI 08330 (AE 1302943) at pH 4, pH 7 and pH 9**
Report No &	**AF05/085**
Document No	**M-262215-01-1**
Guidelines:	not recorded in the report
GLP	not applicable (calculation)
Testing Laboratory and dates	Bayer CropScience GmbH, Product Technology-Analytics – [redacted], D-[redacted], GER Performed and completed on December 08, 2005

The Henry law constant describes the tendency of a compound being solved in water to volatilize from the aqueous solution. The Henry law constant of spirotetramat has been calculated according to the following formula:

$$H = \frac{p \times MolarMass}{S} \left[\frac{Pa \times m^3}{mol} \right]$$

Where: p = vapor pressure at 20 °C = 5.6 x 10E-09 Pa
molar mass of BYI08330 = 373.45 g/mol
S = water solubility at 20 °C in [g/m³] = 33.5 g/m³ in buffer of pH 4,
= 29.9 g/m³ in buffer of pH 7
= 19.1 g/m³ in buffer of pH 9

A Henry law constant of H = 6.24 x 10^{-8} to 1.09 x 10^{-7} Pa m^3/mol was calculated for pH 4 to pH 9, respectively. Based on this range, significant volatilization of spirotetramat from aqueous solutions like soil pore water is not expected.

IIA 7.5 Hydrolysis rate of relevant metabolites at pH values 4, 7 and 9

In accordance with Point IIA 2.9.1, tests on hydrolysis of spirotetramat using radiolabelled test substance in sterile buffer solutions at pH 4.0, 7.0, and 9.0 in the absence of light were submitted. A summary of this study is repeated here, focusing on formation and hydrolytic degradation of metabolites. In addition, a study in order to determine the hydrolysis rate of the relevant metabolite BYI08330-enol is summarized in this chapter.

Report:	**KIIA 7.5/01, [redacted], 2004 (MEF-04/176)**
Title:	**[Azaspirodecenyl-3-^{14}C]- and [Azaspirodecenyl-5-^{14}C]BYI08330: Hydrolytic Degradation**
Report No & Document No	**MEF-04/176 M-093124-01-2**
Guidelines:	OECD Guideline No. 114 Guidelines 94/37/EC, 95/36/EC USA EPA Subdivision N, Section 161-1 Canada PMRA DACO Number 8.2.3.2 Japan MAFF New Test Guideline, 12 Nousan 8147
GLP	Fully GLP compliant laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of January to June 2004. Study completion date: 2004-09-08

EXECUTIVE SUMMARY

Hydrolytic degradation of spirotetramat (code BYI08330; two different radiolabels) at 1 mg/L was studied in the dark at 20°C, 25°C, 50°C (pH 9: 30°C) in sterile aqueous buffered solutions at pH 4 (0.01 M acetate buffer), pH 7 (0.01 M TRIS buffer), and pH 9 (0.01 M borate buffer) for a maximum of 31 days.

The results showed that BYI08330 is hydrolytically labile under acidic, neutral and alkaline conditions at ambient temperature. The fastest degradation was observed at pH 9 with a half-life of 7.6 hours at pH 9 (25°C). This was confirmed by the pre-test performed at 30°C (DT50 of 3.3 hours). 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 with half-lives of 32.5 days (25°C) and 48 days (20°C) at pH 4.

The hydrolytic degradation was strongly temperature dependent. One major degradation product, the BYI08330-enol, occurred and its concentration increased towards the end of the incubations at all pH ranges tested (pH 4 to 9).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat (Code = BYI08330), CAS no. 203313-25-1 (unlabelled substance)
Identity and purity of test item in the application solutions were checked.

Label #1 Label position = [azaspirodecenyl-3-^{14}C]BYI08330
Specific activity 3.71 MBq/mg (100.2 µCi/mg)
Radiochemical purity: 99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

Label #2 Label position = [azaspirodecenyl-5-^{14}C]
Specific activity 4.03 MBq/mg (108.8 µCi/mg)
Radiochemical purity: 98% (acc. radio-HPLC and -TLC)
Chemical purity: >98% (HPLC, UV detection at 210 nm)

2. Buffer solution: The water used for preparation of buffer solutions was highly pure water, purified in a Milli-Q unit. The conductivity of the water was 18.2 MΩ cm, hardness was 0°H, and total organic carbon was 13 ppb. Chemicals of HPLC-grade quality from Merck (or other manufacturer) were used to prepare the buffers. Hydrolytic reactions were carried out using 10 mL of 0.01 M sterile buffer solution at three pH values:

pH 4.0 – acetate buffer
pH 7.0 – TRIS [tris(hydroxymethyl)aminomethane] buffer
pH 9.0 – borate buffer

B. STUDY DESIGN

1. Experimental conditions: Hydrolytic degradation of spirotetramat was investigated by Test 1 (Pre-Test) at 50°C for pH 4 and 7, and at 30°C for pH 9. Each 10-mL portions of test solution was pipetted into the 10 mL crimp-top vials under sterile conditions. Six vessels (one for each label and pH) were used as time zero samples for analytical investigations (LSC, HPLC, TLC, and pH) and were then stored in a refrigerator.
The test design of test 2 (main test, 25°C) was the same as described for Test 1. The duration of the main test was approximated to the data obtained by test 1. Prior to the start of test 2, the purity of the test substance was checked again. Test 2 of pH 7 was repeated, because two samples were found to be non-sterile. The results of the repetition were reported only. The data of the 1st attempt were archived within the raw data.
The test design of test 3 (Optional Test, 20°C) was the same as described before. Test 3 was performed at 20°C for pH 4 and pH 7 only, as the test item is not stable at pH 9 according to OECD [1]. The sampling intervals were reduced from the results of test 1. Prior to the start of test 3, the purity of the test substance was checked again.

2. Sampling: In case of Test 1 individual vessels were withdrawn from the water bath at 0 h, 6 h, 3, 4, 5, 6, 7, 10 and 12 days for pH 4 at 50°C, at 0, 2, 4, 8, 10, 24, 30, and 48 hours for pH 7 at 50°C and at 0, 1, 2, 3, 4, 5, 6 and 7 hours for pH 9 at 30°C after treatment.
In case of Test 2 individual vessels were withdrawn from the water bath 0, 3, 7, 10, 14, 17, 21, 24 and 31 days for pH 4, at 0, 1, 3, 7, 13, 20, 24 and 29 days for pH 7, and at 0, 1, 2, 3, 4, 6, 8, 10, 24, and 30 hours for pH 9 after treatment.
Test 3 was performed at 20°C for pH 4 and pH 7 only, as the test item is not stable at pH 9 Individual vessels were withdrawn from the water bath at 0, 5, 8, 13, 16, 19, 23, 26 and 30 days after treatment.
No attempt was made to trap volatiles, since volatilization of RA from the solutions was not expected. This was confirmed by the complete material balances calculated for each sampling interval.

3. Analytical procedures: At each interval, one sample of each label and pH was removed from the water bath, 0.1 mL aliquots were counted on the liquid scintillation counting (LSC) and 0.5 mL of each sample was directly analyzed by radio-HPLC methods, using reference standards for co-chromatography for the identification of the components. For the investigation of the samples of tests 1 through 3, a HPLC method was used as a primary method and a radio-TLC method was used as a confirmatory method for representative samples.
Mean values of the replicates were calculated for each system. Degradation curve and regression analysis was calculated with the evaluation program ModelManager® (Environmental Kinetics), Version 1.1, developed and published by Cherwell Scientific Ltd, Oxford, UK. The model was run in the mode "use standard data" as well as "use existing parameter estimates".

II. RESULTS AND DISCUSSION

A. DATA

The measured pH of the selected samples confirmed the pH levels were constant to the set-up values within <0.1 pH units. The sterility tests demonstrated that sterile conditions could be maintained throughout the test period. No contamination was observed in the test solutions, except for two samples (1st attempt of test 2 pH 7: label #1, samples day 21 and 30). For that reason, test 2 at pH 7 was repeated. The incubation temperature in the dark was maintained constant at 50°C ± 0.02°C (pH 9: 30°C ± 0.03°C) throughout test 1, 25°C ± 0.03°C throughout test 2, and 20°C ± 0.03°C throughout test 3. The resulting data based on LSC and analyses are shown in Table IIA 7.5-1 to Table IIA 7.5-4.

B. MASS BALANCE

For this study the AR (100% of applied radioactivity) was defined as the amount of radioactivity recovered in the day 0 sample (mean of label #1 and #2). Based on the results of LSC an RA balance was established for each buffer solution at each sampling interval. A summary of the total recoveries of the radioactivity is given in the following Table IIA 7.5-1.

Table IIA 7.5-1: Mass balance in the different test series expressed as % of AR (MEF-04/176)

Test Solution	Balance (MIN)	Balance (MAX)	Balance (MEAN)	Relative Standard Deviation [%]
Test 1				
pH 4 / 50°C	97.6	105.2	101.2	2.0
pH 7 / 50°C	100.0	104.5	102.3	1.6
pH 9 / 30°C	95.5	100.0	98.0	1.3
Test 2				
pH 4 / 25°C	98.8	102.7	100.4	1.2
pH 7 / 25°C	99.1	103.4	101.3	1.3
pH 9 / 25°C	98.3	103.3	100.6	1.4
Test 3				
pH 4 / 20°C	97.3	101.0	99.5	0.8
pH 7 / 20°C	99.5	102.8	101.3	1.0

The complete material balances found in all solutions demonstrated that no radioactivity dissipated from the solutions by means of volatilization or was lost during sampling/processing.

C. BOUND AND EXTRACTABLE RESIDUES

N/A

D. VOLATILIZATION

Since the test system was sealed and a loss in material balance was not observed, no attempt was made to trap volatiles.

E. TRANSFORMATION OF TEST ITEM

Since the test solutions were analyzed directly, other distinctive fractions did not occur. The amounts of BYI08330 and BYI08330-enol were quantified. The results for all tests expressed as percent AR are given in Table IIA 7.5-2 to Table IIA 7.5-4, the results for all tests expressed as ppb (mean of replicates) can be found in Table 7 of report MEF-04/176.

From the % values of total AR the concentrations of known degradation products (expressed as µg/L) were calculated based on their molecular weight (MW); the concentrations of unidentified components were calculated based on the MW of the active substance and expressed as µg/L a.s. equivalents. The results of test 1 (pre-test at 50°C, pH 9: 30°C) indicated that >10% of the applied BYI08330 was hydrolyzed after 5 days at each pH (Table IIA 7.5-2). Therefore a main test at 25°C was carried out. At the end of the pre-test, the remaining level of the test substance at pH 4, pH 7, and pH 9 amounted to 22.8%, 10.6%, and 21.8% of AR after 12 days, 2 days, and 7 hours, respectively. Beside the main metabolite BYI08330-enol several other HPLC peaks were formed but were not identified on account of low amounts (<5% of AR).

Also at 25°C (test 2, main test) BYI08330 was degraded under the hydrolytic conditions at pH 4, 7 and 9. Greatest instability was observed at alkaline pH. At the end of the main test, the remaining level of the test substance amounted to 52% (pH 4, day 31), 9.6% (pH 7, day 29) and 7.1% (pH 9, 30 hours). At the end of test 3 at 20°C, the remaining level of the test substance amounted to 64.1% (pH 4, day 30), and 20.5% (pH 7, day 30).

F. KINETICS OF TEST ITEM DEGRADATION

The duration and number of sampling intervals was sufficient to determine the hydrolytic behavior of BYI08330. There was a clear tendency of decrease of the concentration of the test substance in the buffer solutions during the incubation period. Especially in alkaline aqueous solution, the compound disappeared rapidly. The calculated simple first order degradation kinetics of BYI08330 is summarized in Table IIA 7.5-5. BYI08330 can be considered to be hydrolytically labile under environmental conditions, especially at higher pH values.

Table IIA 7.5-2: Distribution of the active substance and degradates after application of [^{14}C]-BYI08330 to sterile buffer solutions - data given for Test 1 as means of both radiolabels in % of AR (MEF-04/176)

a) Test 1: sterile acetate buffer pH 4.0, incubation at 50°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	 0.25	 3	 4	 5	 6	 7	 10	 12
BYI08330	Mean SD	100.0 ± 0.0	98.9 ± 1.0	70.4 ± 0.8	61.4 ± 1.1	53.5 ± 1.2	49.8 ± 1.2	43.7 ± 0.3	30.0 ± 0.2	22.8 ± 0.4
-enol	Mean SD	n.d.	3.2 ± 0.2	29.6 ± 0.5	39.0 ± 0.9	43.7 ± 0.2	52.1 ± 0.6	55.8 ± 2.9	61.8 ± 9.6	72.4 ± 3.0
ROI2	Mean SD	n.d.	n.d.	1.5 ± 1.5	n.d.	1.0 ± 1.0	1.4 ± 1.4	1.3 ± 0.2	3.3 ± 2.3	1.7 ± 0.1
ROI3	Mean SD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.0 ± 1.0	4.4 ± 4.4	2.5 ± 2.5
Unidentified radioactivity	Mean SD	n.d.	0.4 ± 0.4	0.6 ± 0.6	0.5 ± 0.5	0.7 ± 0.7	n.d.	0.3 ± 0.3	1.3 ± 1.3	0.7 ± 0.7
Total dissolved RA	Mean SD	100.0 ± 0.0	102.5 ± 0.4	102.1 ± 1.3	100.9 ± 1.5	98.9 ± 1.3	103.2 ± 2.0	102.1 ± 2.2	100.8 ± 1.8	100.1 ± 0.4
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	102.5 ± 0.4	102.1 ± 1.3	100.9 ± 1.5	98.9 ± 1.3	103.2 ± 2.0	102.1 ± 2.2	100.8 ± 1.8	100.1 ± 0.4

b) Test 1: sterile TRIS buffer pH 7.0, incubation at 50°C

Compound	Mean [%] SD [%]	Sampling Times [hours] 0	2	4	8	10	24	30	48
BYI08330	Mean	100.0	89.6	82.3	69.0	62.6	42.3	24.9	10.6
	SD	± 0.0	± 0.8	± 0.4	± 0.1	± 0.8	± 0.3	± 0.1	± 0.4
-enol	Mean	n.d.	10.4	18.7	33.0	39.5	71.1	78.7	93.4
	SD		± 0.8	± 0.1	± 0.6	± 1.0	± 1.0	± 0.8	± 0.0
Unidentified radioactivity	Mean	n.d.	0.2	0.5	0.7	n.d.	n.d.	0.4	0.4
	SD		± 0.2	± 0.5	± 0.7			± 0.4	± 0.4
Total dissolved RA	Mean	100.0	100.2	101.5	102.7	102.1	103.5	104.0	104.4
	SD	± 0.0	± 0.2	± 0.1	± 0.2	± 0.2	± 0.7	± 0.5	± 0.0
$^{14}CO_2$ and volatile organics	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Bound to Apparatus Walls	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Total recovery of RA	Mean	100.0	100.2	101.5	102.7	102.1	103.5	104.0	104.4
	SD	± 0.0	± 0.2	± 0.1	± 0.2	± 0.2	± 0.7	± 0.5	± 0.0

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

c) Test 1: sterile borate buffer pH 9.0, incubation at 50°C

Compound	Mean [%] SD [%]	Sampling Times [hours] 0	1	2	3	4	5	6	7
BYI08330	Mean	97.1	77.6	63.5	51.8	41.3	32.5	27.3	21.8
	SD	± 0.2	± 1.8	± 2.0	± 1.2	± 0.3	± 1.0	± 1.0	± 0.5
-enol	Mean	2.7	19.6	34.2	45.3	57.2	64.8	69.4	75.7
	SD	± 0.4	± 2.0	± 2.3	± 2.4	± 0.3	± 0.4	± 1.3	± 0.3
Unidentified radioactivity	Mean	0.2	n.d.	0.2	0.4	0.4	0.4	0.5	n.d.
	SD	± 0.2		± 0.2	± 0.2	± 0.4	± 0.4	± 0.5	
Total dissolved RA	Mean	100.0	97.2	97.9	97.3	98.9	97.7	97.3	97.5
	SD	± 0.0	± 0.3	± 0.5	± 1.0	± 0.4	± 0.9	± 1.8	± 0.7
$^{14}CO_2$ and volatile organics	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Bound to Apparatus Walls	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Total recovery of RA	Mean	100.0	97.2	97.9	97.3	98.9	97.7	97.3	97.5
	SD	± 0.0	± 0.3	± 0.5	± 1.0	± 0.4	± 0.9	± 1.8	± 0.7

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Table IIA 7.5-3: Distribution of the active substance and degradates after application of [^{14}C]-BYI08330 to sterile buffer solutions - data given for Test 2 as means of both radiolabels in % of AR (MEF-04/176)

a) Test 2 (Main Test): sterile acetate buffer pH 4.0, incubation at 25°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	3	7	10	14	17	21	24	31
BYI08330	Mean	100.0	95.2	86.1	79.5	74.3	68.9	64.9	60.0	52.0
	SD	± 0.0	± 1.2	± 0.4	± 0.1	± 0.3	± 0.9	± 0.5	± 0.6	± 1.0
-enol	Mean	n.d.	6.6	14.0	19.0	25.8	30.4	36.2	38.6	48.8
	SD		± 0.3	± 0.0	± 0.2	± 0.3	± 0.0	± 0.2	± 0.3	± 0.0
Unidentified radioactivity	Mean	n.d.	n.d.	0.7	0.5	0.3	0.5	0.3	1.0	n.d.
	SD			± 0.3	± 0.5	± 0.3	± 0.1	± 0.3	± 0.3	
Total dissolved RA	Mean	100.0	101.8	100.8	99.9	100.3	99.8	101.4	99.6	100.8
	SD	± 0.0	± 0.9	± 0.0	± 0.2	± 0.4	± 0.8	± 0.0	± 0.7	± 2.0
$^{14}CO_2$ and volatile organics	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD									
Bound to Apparatus Walls	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD									
Total recovery of RA	Mean	100.0	101.8	100.8	99.9	100.3	99.8	101.4	99.6	100.8
	SD	± 0.0	± 0.9	± 0.0	± 0.2	± 0.4	± 0.8	± 0.0	± 0.7	± 2.0

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

b) Test 2 (Main Test): sterile TRIS buffer pH 7.0, incubation at 25°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	1	3	7	13	20	24	29
BYI08330	Mean	99.8	90.3	78.5	55.8	35.6	19.5	14.5	9.6
	SD	± 0.2	± 0.6	± n.a.	± 0.2	± 0.3	± 0.3	± 0.5	± 0.7
-enol	Mean	n.d.	9.5	23.1	45.0	65.9	82.9	85.8	91.8
	SD		± 0.1	± 0.1	± 0.3	± 0.6	± 0.1	± 1.7	± 0.4
ROI1	Mean	n.d.	n.d.	n.d.	n.d.	0.5	0.3	0.5	0.6
	SD					± 0.5	± 0.3	± 0.5	± 0.6
Unidentified radioactivity	Mean	0.2	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	SD	± 0.2	± 0.5						
Total dissolved RA	Mean	100.0	100.3	101.5	100.8	101.9	102.7	100.8	102.0
	SD	± 0.0	± 0.9	± 1.1	± 0.6	± 0.9	± 0.7	± 1.8	± 0.4
$^{14}CO_2$ and volatile organics	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Bound to Apparatus Walls	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Total recovery of RA	Mean	100.0	100.3	101.5	100.8	101.9	102.7	100.8	102.0
	SD	± 0.0	± 0.9	± 1.1	± 0.6	± 0.9	± 0.7	± 1.8	± 0.4

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

c) Test 2 (Main Test): sterile borate buffer pH 9.0, incubation at 25°C

Compound	Mean [%] SD [%]	Sampling Times [hours] 0	1	2	3	4	6	8	10	24	30**
BYI08330	Mean SD	97.8 ± 0.2	87.5 ± 0.3	80.0 ± 1.0	72.9 ± 0.5	65.2 ± 0.3	56.9 ± 1.3	46.6 ± 0.7	38.7* --	11.2 ± 1.3	7.1 --
-enol	Mean SD	2.2 ± 0.2	11.7 ± 0.0	20.5 ± 0.1	27.8 ± 0.0	34.8 ± 0.2	43.8 ± 0.3	54.0 ± 1.8	64.1* --	89.8 ± 0.2	92.6 --
Unidentified radioactivity	Mean SD	n.d.	n.d.	n.d.	0.2 ± 0.2	0.3 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	0.5* --	0.6 ± 0.0	0.6 --
Total dissolved RA	Mean SD	100.0 ± 0.0	99.2 ± 0.3	100.5 ± 0.8	100.9 ± 0.7	100.3 ± 0.4	101.0 ± 1.9	100.9 ± 1.3	100.8 ± 2.5	101.6 ± 1.1	100.3 --
$^{14}CO_2$ and vol. organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	99.2 ± 0.3	100.5 ± 0.8	100.9 ± 0.7	100.3 ± 0.4	101.0 ± 1.9	100.9 ± 1.3	100.8 ± 2.5	101.6 ± 1.1	100.3 --

n.d.: not detected, n.a.: not analyzed, SD: standard deviation
* no valid analysis result of sample of label #1 was available
** no replicate sample (i.e. of label #1) was investigated

Table IIA 7.5-4: Distribution of the active substance and degradates after application of [^{14}C]-BYI08330 to sterile buffer solutions - data given for Test 3 as means of both radiolabels in % of AR (MEF-04/176)

a) Test 3 (Optional Test): sterile acetate buffer pH 4.0, incubation at 20°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	5	8	13	16	19	23	26	30
BYI08330	Mean SD	99.6 ± 0.4	91.6 ± 0.1	88.7 ± 0.4	82.8 ± 0.5	78.8 ± 0.2	75.7 ± 0.2	70.8 ± 0.9	68.0 ± 0.8	64.1 ± 0.6
-enol	Mean SD	n.d.	7.1 ± 0.1	10.9 ± 0.1	17.1 ± 0.5	20.7 ± 0.4	24.4 ± 0.5	27.5 ± 0.1	30.8 ± 0.2	34.5 ± 0.6
Unidentified radioactivity	Mean SD	0.4 ± 0.4	0.2 ± 0.2	n.d.	0.4 ± 0.4	n.d.	0.3 ± 0.3	0.3 ± 0.3	0.2 ± 0.2	0.3 ± 0.3
Total dissolved RA	Mean SD	100.0 ± 0.0	98.9 ± 0.2	99.6 ± 0.4	100.3 ± 0.4	99.4 ± 0.2	100.4 ± 0.6	98.6 ± 1.3	99.1 ± 0.8	98.9 ± 0.4
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	98.9 ± 0.2	99.6 ± 0.4	100.3 ± 0.4	99.4 ± 0.2	100.4 ± 0.6	98.6 ± 1.3	99.1 ± 0.8	98.9 ± 0.4

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

b) Test 3 (Optional Test): sterile TRIS buffer pH 7.0, incubation at 20°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	5	8	13	16	19	23	26	30
BYI08330	Mean SD	100.0 ± 0.0	76.3 ± 1.1	65.4 ± 0.1	49.4 ± 0.9	43.2 ± 1.3	36.2 ± 0.4	29.5 ± 0.8	25.7 ± 0.7	20.5 ± 0.0
-enol	Mean SD	n.d.	24.5 ± 0.5	36.1 ± 0.2	52.4 ± 0.6	58.8 ± 1.6	64.7 ± 0.8	71.9 ± 0.5	74.6 ± 0.8	80.5 ± 0.6
Unidentified radioactivity	Mean SD	n.d.	n.d.	n.d.	0.6 ± 0.6	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	n.d.
Total dissolved RA	Mean SD	100.0 ± 0.0	100.8 ± 0.6	101.4 ± 0.3	102.4 ± 0.4	102.3 ± 0.1	101.3 ± 0.8	101.8 ± 0.7	100.6 ± 1.1	101.0 ± 0.5
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	100.8 ± 0.6	101.4 ± 0.3	102.4 ± 0.4	102.3 ± 0.1	101.3 ± 0.8	101.8 ± 0.7	100.6 ± 1.1	101.0 ± 0.5

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Table IIA 7.5-5: Calculated simple first order degradation kinetics of [^{14}C]BYI08330 in sterile buffer solutions (MEF-04/176)

Test No / Temperature	pH	Simple First Order Kinetics DT50	DT75	DT90	K [1/time]	R^2
Test 1 / 50°C	4	137 h (5.7 d)	11.4 d	18.9 d	0.122	0.999
Test 1 / 50°C	7	15 h (0.62 d)	30 h	50 h	0.046	1.00
Test 1 / 30°C	9	3.3 h (0.14 d)	6.5 h	10.8 h	0.212	1.00
Test 2 / 25°C	4	32.5 d	65 d	108 d	0.021	0.998
Test 2 / 25°C	7	8.6 d	17 d	29 d	0.080	1.00
Test 2 / 25°C	9	7.6 h	15 h	25 h	0.092	0.999
Test 3 / 20°C	4	48 d	95 d	158 d	0.015	0.999
Test 3 / 20°C	7	13 d	26 d	44 d	0.053	1.00

III. CONCLUSIONS

From the current laboratory study it is concluded that hydrolysis is relevant for the degradation of BYI08330 in the environment, especially under neutral and alkaline conditions. The hydrolytic half-life at pH 7 and 25°C (20°C) is expected to be in the range of 8.6 days (13 days).

One major degradation product occurred. In the total pH range tested (pH 4 to 9) the formation of BYI08330-enol as a common hydrolysis product was observed. The concentration of BYI08330-enol increased towards the end of the incubations at all pH ranges tested. Thus, the degradation and kinetics of the major metabolite BYI08330-enol was evaluated in a separate study (see below).

Metabolite BYI08330-enol

Chemical name (CAS): cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one; CAS #: 203312-38-3

Report:	**KIIA 7.5/02, [redacted], 2004 (MEF-04/311)**
Title:	**[Azaspirodecenyl-3-^{14}C]- and [Azaspirodecenyl-5-^{14}C]BYI08330-Enol: Hydrolytic Degradation**
Report No & Document No	**MEF-04/311** **M-242999-01-2**
Guidelines:	OECD Guideline No. 111 Guidelines 94/37/EC, 95/36/EC USA EPA Subdivision N, Section 161-1 Canada PMRA DACO Number 8.2.3.2 Japan MAFF New Test Guideline, 12 Nousan 8147
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of June to August 2004. Study completion date: 2004-12-03

EXECUTIVE SUMMARY

Hydrolysis of [^{14}C]BYI08330-enol (two radiolabels) at 1 mg/L was studied in the dark at 25°C and 50°C in sterile aqueous buffered solutions at pH 4 (0.01 M acetate buffer), pH 7 (0.01 M TRIS buffer), and pH 9 (0.01 M borate buffer) for a maximum of 31 days. The BYI08330-enol residues were analyzed directly without any step of extraction or enrichment by LSC, HPLC and TLC.
Complete material balances were found, and the results showed that BYI08330-enol is hydrolytically stabile under acidic, neutral and alkaline conditions at ambient temperature. DT50 values of BYI08330-enol derived from all pH values of the test at 25 °C were > 1 year.
Considering the hydrolytic behavior determined under environmental pH conditions it is expected that hydrolytic processes will not contribute to the degradation of BYI08330-enol in the environment.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Item: BYI08330-enol, CAS #: 203312-38-3 (unlabelled substance)
Identity and purity of test item in the application solutions were checked

Label #1 Label position = [azaspirodecenyl-3-^{14}C]BYI08330-enol: BECH 1564 / BECH 0265-2
Specific activity 4.54 MBq/mg (122.8 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

Label #2 Label position = [azaspirodecenyl-5-^{14}C]BYI08330-enol: BECH 1565 / BECH 0278-1
Specific activity 4.99 MBq/mg (135.0 µCi/mg)
Radiochemical purity: ≥99% (acc. radio-HPLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

2. Buffer solution: The water used for preparation of buffer solutions was highly pure water, purified

in a Milli-Q unit. The conductivity of the water was 18.2 MΩ cm, hardness was 0°H, and total organic carbon was 8 ppb. Chemicals of HPLC-grade quality from Merck (or other manufacturer) were used to prepare the buffers. Hydrolytic reactions were carried out using 10 mL of 0.01 M sterile buffer solution at three pH values:

pH 4.0 – acetate buffer
pH 7.0 – TRIS [tris(hydroxymethyl)aminomethane] buffer
pH 9.0 – borate buffer

B. STUDY DESIGN

1. Experimental conditions: Hydrolytic degradation of BYI08330-enol was investigated for pH 4, 7 and 9 by Test 1 (Pre-Test) at 50°C and by the main test at 25 °C. The test systems consisted of 10 mL glass crimp-top vials closed using crimp caps with Teflon®-faced septa. The test systems were kept in a temperature-controlled, covered water bath. For this study, labels #1 and #2 were regarded as duplicates. The radiolabeled test substances were applied to sterile oxygen-free aqueous buffers. The vessels were filled with 10-mL buffer solution (= buffer plus stock solution). The procedures described herein were performed under sterile conditions using a clean-bench. The crimp caps of the samples were marked to distinguish between the labels used and pH values. Nine vessels were prepared for each label, pH and temperature.

2. Sampling: In case of Test 1 (Pre-Test, 50°C) individual vessels were withdrawn from the water bath at 0, 1, 2, and 5 days after treatment. In case of Test 2 (Main Test, 25°C) individual vessels were withdrawn from the water bath at 0, 3, 7, 12, 17, 24 and 31 days after treatment.
No attempt was made to trap volatiles, since volatilization of RA from the solutions was not expected. This was confirmed by the complete material balances calculated for each sampling interval.

3. Analytical procedures: At each interval, one sample of each label and pH was removed from the water bath, 0.1 mL aliquots were counted on the liquid scintillation counting (LSC) and 0.5 mL of each sample was directly analyzed by radio-HPLC methods, using reference standards for co-chromatography for the identification of the components. For the investigation of the samples of tests 1 through 3, a HPLC method was used as a primary method and a radio-TLC method was used as a confirmatory method for representative samples.
Mean values of the replicates were calculated for each system. Degradation curve and regression analysis was calculated with the evaluation program ModelManager® (Environmental Kinetics), Version 1.1, developed and published by Cherwell Scientific Ltd. Oxford, UK. The model was run in the mode "use standard data" as well as "use existing parameter estimates".

II. RESULTS AND DISCUSSION

A. Data

The measured pH of the selected samples confirmed the pH levels were constant to the set-up values within <0.1 pH units. The sterility tests demonstrated that sterile conditions could be maintained throughout the test period. No contamination was observed in the test solutions. The incubation temperature was maintained constant at 50°C ± 0.02°C throughout test 1 and 25°C ± 0.03°C throughout test 2. The resulting data of the test series based on LSC and analyses are shown in Table IIA 7.5-6 to Table IIA 7.5-8.

B. Mass Balance: For this study the AR (100% of applied radioactivity) was defined as the amount of radioactivity recovered in the day 0 sample (mean of label #1 and #2). Based on the results of LSC a

mass balance was established for each buffer solution at each sampling interval. A summary of the total recoveries of the radioactivity is given in the following Table IIA 7.5-6.

Table IIA 7.5-6: Mass balance in the different series expressed as % of AR (MEF-04/311)

Test Solution	Balance MIN	Balance MAX	Balance MEAN	Relative Standard Deviation [%]
Test 1:				
pH 4 / 50°C	98.9	100.5	99.8	0.5
pH 7 / 50°C	100.0	102.6	100.9	0.9
pH 9 / 50°C	99.6	102.2	100.4	0.8
Test 2:				
pH 4 / 25°C	99.5	100.9	100.0	0.4
pH 7 / 25°C	98.9	101.1	100.0	0.6
pH 9 / 25°C	97.5	101.3	99.4	1.0

The complete material balances found in all solutions demonstrated that no radioactivity dissipated from the solutions by means of volatilization or was lost during sampling/processing.

C. BOUND AND EXTRACTABLE RESIDUES

N/A

D. VOLATILIZATION

Since the test system was sealed and a loss in material balance was not observed, no attempt was made to trap volatiles.

E. TRANSFORMATION OF TEST ITEM

Since the test solutions were analyzed directly, other distinctive fractions did not occur. The amounts of BYI08330-enol were quantified. The results for all tests expressed as percent AR are given in Table IIA 7.5-7 to Table IIA 7.5-8, the results for all tests expressed as ppb (mean of replicates) can be found in Table 7 of report MEF-04/311. The concentrations of unidentified components were calculated based on the MW of the test item and expressed as µg/L t.i. equivalents.
Since in the pre-test less than 10% of hydrolysis was observed after 5 days, the chemical was considered hydrolytically stable, and no additional testing was required. Nevertheless, in order to cover US-EPA requirements additional testing was performed at 25°C and pH 4, 7, and 9 (test 2).

F. KINETICS OF TEST ITEM DEGRADATION

The duration and number of sampling intervals was sufficient to determine the hydrolytic behavior of BYI08330-enol. As shown in the tables mentioned before BYI08330-enol showed no decline in the buffer solutions during the entire incubation period. Therefore, BYI08330-enol was regarded as hydrolytically stable at the conditions mentioned. The calculated simple first order degradation kinetics of BYI08330 is summarized in Table IIA 7.5-9. BYI08330-enol can be considered to be hydrolytically

stabile under environmental conditions. DT50 values of BYI08330-enol derived from all pH values of test 2 were > 1 year.

Table IIA 7.5-7: Distribution of the test item and degradates after application of [^{14}C]BYI08330-enol to sterile buffer solutions - data given for Test 1 as means of both radiolabels in % of AR (MEF-04/311)

a) Test 1: sterile acetate buffer pH 4.0, incubation at 50°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	1	2	5
BYI08330-enol	Mean SD	99.6 ± 0.0	99.2 ± 0.3	99.9 ± 0.3	98.2 ± 0.0
Unidentified radioactivity	Mean SD	0.4 ± 0.0	n.d.	n.d.	1.9 ± 0.3
Total dissolved RA	Mean SD	100.0 ± 0.0	99.2 ± 0.3	99.9 ± 0.3	100.1 ± 0.4
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	99.2 ± 0.3	99.9 ± 0.3	100.1 ± 0.4

b) Test 1: sterile TRIS buffer pH 7.0, incubation at 50°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	1	2	5
BYI08330-enol	Mean SD	99.7 ± 0.3	100.4 ± 0.0	101.2 ± 0.2	100.0 ± 0.3
Unidentified radioactivity	Mean SD	0.3 ± 0.3	n.d.	n.d.	2.0 ± 0.4
Total dissolved RA	Mean SD	100.0 ± 0.0	100.4 ± 0.0	101.2 ± 0.2	101.9 ± 0.7
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	100.4 ± 0.0	101.2 ± 0.2	101.9 ± 0.7

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

c) Test 1: sterile borate buffer pH 9.0, incubation at 50°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	1	2	3
BYI08330-enol	Mean SD	99.3 ± 0.0	100.1 ± 0.4	100.2 ± 0.4	99.8 ± 0.1
Unidentified radioactivity	Mean SD	0.7 ± 0.0	n.d.	n.d.	1.6 ± 0.7
Total dissolved RA	Mean SD	100.0 ± 0.0	100.1 ± 0.4	100.2 ± 0.4	101.4 ± 0.8
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	100.1 ± 0.4	100.2 ± 0.4	101.4 ± 0.8

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Table IIA 7.5-8: Distribution of the test item and degradates after application of [^{14}C]BYI08330-enol to sterile buffer solutions - data given for Test 2 as means of both radiolabels in % of AR (MEF-04/311)

a) Test 2 (Main Test): sterile acetate buffer pH 4.0, incubation at 25°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	3	7	12	17	24	31
BYI08330-enol	Mean SD	99.4 ± 0.0	99.9 ± 0.3	100.5 ± 0.4	99.7 ± 0.3	99.5 ± 0.4	99.4 ± 0.4	99.6 ± 0.1
Unidentified radioactivity	Mean SD	0.6 ± 0.0	0.2 ± 0.2	n.d.	0.6 ± 0.6	0.4 ± 0.4	0.2 ± 0.2	n.d.
Total dissolved RA	Mean SD	100.0 ± 0.0	100.1 ± 0.1	100.5 ± 0.4	100.3 ± 0.3	99.9 ± 0.1	99.6 ± 0.1	99.6 ± 0.1
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	100.1 ± 0.1	100.5 ± 0.4	100.3 ± 0.3	99.9 ± 0.1	99.6 ± 0.1	99.6 ± 0.1

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

b) Test 2 (Main Test): sterile TRIS buffer pH 7.0, incubation at 25°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	3	7	12	17	24	31
BYI08330-enol	Mean SD	99.7 ± 0.3	100.1 ± 0.3	100.1 ± 0.1	100.9 ± 0.2	99.4 ± 0.5	99.5 ± 0.1	99.0 ± 0.1
Unidentified radioactivity	Mean SD	0.3 ± 0.3	0.2 ± 0.2	0.1 ± 0.1	n.d.	0.3 ± 0.3	n.d.	0.2 ± 0.2
Total dissolved RA	Mean SD	100.0 ± 0.0	100.3 ± 0.2	100.3 ± 0.0	100.9 ± 0.2	99.7 ± 0.1	99.5 ± 0.1	99.2 ± 0.3
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	100.3 ± 0.2	100.3 ± 0.0	100.9 ± 0.2	99.7 ± 0.1	99.5 ± 0.1	99.2 ± 0.3

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

c) Test 2 (Main Test): sterile borate buffer pH 9.0, incubation at 25°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	3	7	12	17	24	31
BYI08330-enol	Mean SD	99.6 ± 0.4	99.3 ± 0.5	99.8 ± 0.9	100.0 ± 0.8	99.1 ± 0.7	98.0 ± 0.5	98.8 ± 0.5
Unidentified radioactivity	Mean SD	0.4 ± 0.4	n.d.	0.3 ± 0.3	n.d.	n.d.	n.d.	0.2 ± 0.2
Total dissolved RA	Mean SD	100.0 ± 0.0	99.3 ± 0.5	100.1 ± 1.2	100.0 ± 0.8	99.1 ± 0.7	98.0 ± 0.5	98.9 ± 0.3
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to Apparatus Walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	99.3 ± 0.5	100.1 ± 1.2	100.0 ± 0.8	99.1 ± 0.7	98.0 ± 0.5	98.9 ± 0.3

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Table IIA 7.5-9: Calculated simple first order degradation kinetics of [^{14}C]BYI08330-enol in sterile buffer solutions (MEF-04/311)

Test no / Temperature	pH	DT50
Test 2 / 25°C	4	> 1 year
Test 2 / 25°C	7	> 1 year
Test 2 / 25°C	9	> 1 year

III. CONCLUSIONS

From the current laboratory study it is concluded that hydrolysis is not relevant for the degradation of BYI08330-enol in the environment. The hydrolytic half-life at pH 4, 7 and 9 at 25°C is expected to be > 1 year.

Metabolite BYI08330-ketohydroxy

Chemical name (CAS): cis-3-(2,5-Dimethylphenyl)-3-hydroxy-8-methoxy-1-azaspiro[4.5]decan-2,4-dione

Report:	**KIIA 7.5/03, [redacted], 2009 (MEF-04/311)**
Title:	**[Azaspirodecenyl-3-^{14}C]BYI08330-ketohydroxy: Hydrolytic Degradation**
Report No & Document No	**MEF-09/120 M-347361-01-1**
Guidelines:	OECD Guideline No. 111 EU Directives 94/37/EC and 95/36/EC Japan MAFF New Test Guideline, 12 Nousan 8147
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Environmental Safety Metabolism/ADME and Environmental Fate (former Metabolism and Environmental Fate), D-[redacted], GER, conducted the study during the period of November 2008 to Februars 2009. Study completion date: 2009-05-12

EXECUTIVE SUMMARY

Hydrolysis of [azaspirodecane-3-^{14}C]BYI08330-ketohydroxy was studied in the dark at 50°C, 25°C and 20°C in sterile aqueous buffered solutions at pH 4 (0.01 M acetate buffer), pH 7 (0.01 M TRIS buffer), and pH 9 (0.01 M borate buffer) for a maximum of 30 days. The test concentration was 1.0 mg test item/L.

The BYI08330-ketohydroxy residues were analyzed directly without any step of extraction or enrichment by LSC, HPLC and TLC.

Complete material balances were found, and the results showed that BYI08330- ketohydroxy is hydrolytically stabile under acidic conditions, but labile especially under alkaline conditions in buffered solutions. The fastest degradation was observed at pH 9 conditions. The experimental half-lives of BYI08330-ketohydroxy at pH 7 were 32.7 hours (50°C), 82.7 days (25°C) and 333 days (20°C). The experimental half-lives of BYI08330-ketohydroxy at pH 9 were 71.3 minutes (50°C), 4.9 days (25°C) and 15.6 days (20°C). The hydrolytic degradation of the test item strongly depended on the temperature and pH value.

One major degradation product was formed in neutral and alkaline buffer solutions. The formation of BYI08330-MA-amide as a common hydrolysis product was observed at pH 7 and pH 9. The concentration of the hydrolysis product BYI08330-MA-amide increased towards the end of the incubation period at pH 7 and pH 9

Considering the hydrolytic behavior determined under alkaline pH conditions it is expected that hydrolytic processes will contribute to the degradation of BYI08330-ketohydroxy in the environment.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Item: BYI08330-ketohydroxy, CAS #: not available (unlabelled substance)
Identity and purity of test item in the application solutions were checked

Label position = [azaspirodecenyl-3-^{14}C]BYI08330- ketohydroxy: KATH 6595 / KML 3260-1
Specific activity: 4.54 MBq/mg (122.8 µCi/mg)
Radiochemical purity: >99% and 98% (acc. radio-HPLC and TLC scan)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

2. Buffer solution: The water used for preparation of buffer solutions was highly pure water, purified in a Milli-Q unit. Chemicals of HPLC-grade quality from Merck (or other manufacturer) were used to prepare the buffers. Hydrolytic reactions were carried out using 10 mL of 0.01 M sterile buffer solution at three pH values:

pH 4.0 – acetate buffer
pH 7.0 – TRIS [tris(hydroxymethyl)aminomethane] buffer
pH 9.0 – borate buffer

After adjustment of the pH value, nitrogen was bubbled through the buffer solutions. The solutions were then sterilized in an autoclave.

B. STUDY DESIGN

1. Experimental conditions: The test systems consisted of 10 mL glass crimp-top vials closed using crimp caps with Teflon®-faced septa. The test systems were kept in a temperature-controlled, covered water bath. The radiolabeled test item was applied to sterile aqueous buffer solutions separately. The vessels were filled with 5 mL of buffer solution (= buffer plus application solution). The procedures described herein were performed under sterile conditions using a clean-bench.
Duplicate samples for each pH were used for analytical investigations (LSC, HPLC, pH). In addition at selected time points TLC confirmatory analysis and sterility check were performed. Subsequently the samples were stored in a refrigerator.
The total applied amount of radioactivity was determined by LSC measurements of the day 0 samples. This radioactivity was set to 100% of the applied radioactivity (AR) and was used for further calculations. The test concentration was 1.0 mg test item/L.

2. Sampling: Individual vessels were withdrawn from the water bath analyzed directly without any processing at 0, 2, 5, 7 days (pH 4), 0, 6, 24, 28, 32, 48, 72 hours (pH 7) and 0, 30, 60, 80, 100, 120, 180, 240 minutes (pH 9) after incubation (test 1 = pre-test, 50°C) and 0, 3, 8, 10, 15, 21, 30 days (pH 4, pH 7) after incubation and 0, 0.25, 1.25, 2, 4, 10, 21, 30 days after incubation (pH 9) (test 2 = main test, 25°C) and 0, 3, 7, 10, 15, 21, 30 days after incubation (pH 7, pH 9) (test 3 = optional test, 20°C).
No attempt was made to trap volatiles, since volatilization of RA from the solutions was not expected. This was finally confirmed by the complete material balances calculated for each sampling interval.

3. Analytical procedures: At each interval, duplicate samples of each pH were removed from the water bath, 0.1 mL aliquots in triplicate were counted by the liquid scintillation counting (LSC) and 500 µL of each sample was directly analyzed by HPLC, using reference standards for co-chromatography for the identification of the components.
The quantitative evaluation of ^{14}C-labeled zones (ROIs) was performed by reversed phase HPLC chromatography with radio-detection (primary method). The minimum limit of detection (LOD) in HPLC for a single peak in the aqueous solutions was in the range of 0.5% of the AR.

In addition, samples after application (day 0) and at the end of the of incubation period were analyzed by normal phase TLC for confirmatory purposes. Identification of the radiolabeled transformation product BYI08330-MA-amide was accomplished by HPLC-MS, HPLC-MS/MS (ESI negative) and by accurate mass measurement (FT-Orbitrap-MS in the positive ESI mode).
Amounts of test item and metabolite were calculated as percent of the applied radioactivity [% of AR]. Values are presented as single values and as means if replicates were made.
DT_{50} and DT_{90} values were determined for the degradation of the test item BYI08330-ketohydroxy. DT_{50} and DT_{90} values for metabolites of BYI08330-ketohydroxy were not calculated. The determination of the kinetic values followed the recommendations of FOCUS rules according to the FOCUS guidance document on degradation kinetics. The kinetic evaluations and the statistical calculations for the quality checks were conducted with software KinGUI v1.1.

II. RESULTS AND DISCUSSION

A. Data

The sterility tests demonstrated that sterile conditions could be maintained throughout the test period. No contamination was observed in the test solutions.
The incubation temperature was maintained constant at 50°C throughout test 1, at 25°C throughout test 2 and at 20°C throughout test 3.
The measured pH of the selected samples confirmed the pH levels were constant to the set-up values within <0.1 pH units.
The resulting data of the test series based on LSC and analyses are shown in Table IIA 7.5-10 to Table IIA 7.5-12.

B. Mass Balance: The total radiocarbon recovery ranged from 97.0% to 106.3% of the applied amount. The complete material balances found in all solutions demonstrated that no RA dissipated from the solutions by means of volatilization or was lost during sampling/processing.

C. BOUND AND EXTRACTABLE RESIDUES

N/A

D. VOLATILIZATION

Since the test system was sealed and a loss in material balance was not observed, no attempt was made to trap volatiles.

E. TRANSFORMATION OF TEST ITEM

Since the test solutions were analyzed directly, other distinctive fractions did not occur. The amounts of BYI08330-ketohydroxy were quantified. The results for all tests expressed as percent AR are given in Table IIA 7.5-10 to Table IIA 7.5-12.
Since in the pre-test (see Table IIA 7.5-10) less than 10% of hydrolysis was observed after 5 days, the chemical was considered hydrolytically stable at pH 4, and no additional testing was required. However, additional testing for pH 7 and 9 was performed at 25°C (test 2, see Table IIA 7.5-11), and at 20°C (test 3, see Table IIA 7.5-12).

Table IIA 7.5-10: Distribution of the test item and degradates after application of [^{14}C]BYI08330-ketohydroxy to sterile buffer solutions - data given for Test 1 as means of both radiolabels in % of AR (MEF-09/120)

Test 1: sterile acetate buffer pH 4.0, incubation at 50°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	2	5	7
BYI08330-ketohydroxy	Mean SD	100.0 ± 0.0	100.2 ± 0.2	100.7 ± 0.7	100.7 ± 1.6
Unidentified radioactivity	Mean SD	n.d.	n.d.	n.d.	1.1 ± 0.1
Total dissolved RA	Mean SD	100.0 ± 0.0	100.2 ± 0.2	100.7 ± 0.7	101.8 ± 1.6
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.
Bound to apparatus walls	Mean SD	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.0	100.2 ± 0.2	100.7 ± 0.7	101.8 ± 1.6

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Test 1: sterile TRIS buffer pH 7.0, incubation at 50°C

Compound	Mean [%] SD [%]	Sampling Times [hours] 0	6	24	28	32	48	72
BYI08330-ketohydroxy	Mean SD	99.5 ± 0.2	90.5 ± 0.2	60.8 ± 0.7	54.7 ± 0.6	52.4 ± 0.6	36.4 ± 0.4	21.0 ± 0.3
BYI08330-MA-amide	Mean SD	n.d.	12.1 ± 0.2	41.8 ± 0.1	45.1 ± 0.3	51.4 ± 0.7	66.4 ± 0.7	78.6 ± 0.3
Reg 3	Mean SD	n.d.	n.d.	1.2 ± 0.1	1.3 ± 0.0	1.2 ± 0.0	1.5 ± 0.0	1.5 ± 0.1
Unidentified radioactivity	Mean SD	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.0	1.1 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.0
Total dissolved RA	Mean SD	100.0 ± 0.3	103.3 ± 0.5	104.6 ± 0.5	101.7 ± 0.4	105.9 ± 0.0	105.0 ± 0.3	101.6 ± 0.1
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to apparatus walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.3	103.3 ± 0.5	104.6 ± 0.5	101.7 ± 0.4	105.9 ± 0.0	105.0 ± 0.3	101.6 ± 0.1

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Test 1: sterile borate buffer pH 9.0, incubation at 50°C

Compound	Mean [%] SD [%]	Sampling Times [minutes] 0	30	60	80	100	120	180	240
BYI08330-ketohydroxy	Mean	100.0	79.9	56.3	45.9	38.6	31.3	17.8	9.2
	SD	± 0.0	± 0.3	± 0.6	± 0.2	± 0.2	± 0.2	± 0.4	± 0.1
BYI08330-MA-amide	Mean	n.d.	23.8	47.1	57.9	65.4	72.1	87.8	94.9
	SD		± 0.3	± 0.7	± 0.2	±1.0	± 0.0	± 0.2	± 0.1
Unidentified radioactivity	Mean	n.d.	0.6	0.8	0.9	0.7	0.8	0.7	0.7
	SD		± 0.0	± 0.0	± 0.1	± 0.0	± 0.0	± 0.1	± 0.0
Total dissolved RA	Mean	100.0	104.4	104.2	104.7	104.7	104.3	106.3	104.7
	SD	± 0.0	± 0.1	± 0.1	± 0.1	± 0.8	± 0.2	± 0.2	± 0.1
$^{14}CO_2$ and volatile organics	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Bound to apparatus walls	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Total recovery of RA	Mean	100.0	104.4	104.2	104.7	104.7	104.3	106.3	104.7
	SD	± 0.0	± 0.1	± 0.1	± 0.1	± 0.8	± 0.2	± 0.2	± 0.1

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Table IIA 7.5-11: Distribution of the test item and degradates after application of [^{14}C]BYI08330-ketohydroxy to sterile buffer solutions - data given for Test 2 as means of both radiolabels in % of AR (MEF-09/120)

Test 2 (Main Test): sterile acetate buffer pH 4.0, incubation at 25°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	3	8	10	15	21	30
BYI08330-ketohydroxy	Mean	100.0	102.3	102.0	102.2	103.4	101.5	100.6
	SD	± 0.2	± 0.1	± 0.2	± 0.3	± 0.5	± 0.6	± 0.6
Unidentified radioactivity	Mean	n.d.	n.d.	n.d.	n.d.	<LOD	<LOD	<LOD
	SD							
Total dissolved RA	Mean	100.0	102.3	102.0	102.2	103.5	101.9	101.1
	SD	± 0.2	± 0.1	± 0.2	± 0.3	± 0.4	± 0.6	± 0.6
$^{14}CO_2$ and volatile organics	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD							
Bound to apparatus walls	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD							
Total recovery of RA	Mean	100.0	102.3	102.0	102.2	103.5	101.9	101.1
	SD	± 0.2	± 0.1	± 0.2	± 0.3	± 0.4	± 0.6	± 0.6

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Test 2 (Main Test): sterile TRIS buffer pH 7.0, incubation at 25°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	3	8	10	15	21	30
BYI08330-ketohydroxy	Mean	100.0	98.2	94.7	92.9	90.4	82.9	78.4
	SD	± 0.3	± 0.4	± 0.4	± 0.1	± 0.0	± 1.9	± 0.1
BYI08330-MA-amide	Mean	n.d.	2.7	6.5	8.3	11.7	14.6	21.7
	SD		± 0.1	± 0.0	± 0.0	± 0.1	± 1.0	± 0.3
Unidentified radioactivity	Mean	n.d.	0.8	1.0	1.1	1.2	1.4	1.3
	SD		± 0.0	± 0.0	± 0.0	± 0.0	± 0.3	± 0.0
Total dissolved RA	Mean	100.0	101.7	102.1	102.4	103.4	98.9	101.5
	SD	± 0.3	± 0.5	± 0.4	± 0.0	± 0.1	± 2.9	± 0.4
$^{14}CO_2$ and volatile organics	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD							
Bound to apparatus wWalls	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD							
Total recovery of RA	Mean	100.0	101.7	102.1	102.4	103.4	98.9	101.5
	SD	± 0.3	± 0.5	± 0.4	± 0.0	± 0.1	± 2.9	± 0.4

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Test 2 (Main Test): sterile borate buffer pH 9.0, incubation at 25°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	0.25	1.25	2	4	10	21	30
BYI08330-ketohydroxy	Mean	100.0	95.1	81.0	73.4	50.1	26.1	10.3	6.2
	SD	± 0.3	± 0.3	± 0.0	± 1.8	± 0.6	± 7.1	± 0.1	± 0.1
BYI08330-MA-amide	Mean	n.d.	5.0	19.0	28.3	50.7	76.6	86.0	94.1
	SD		± 0.1	± 0.1	± 1.8	± 0.7	±7.3	± 0.2	± 0.1
Unidentified radioactivity	Mean	n.d.	0.8	0.6	0.6	0.8	0.6	0.7	0.7
	SD		± 0.0	± 0.1	± 0.0	± 0.1	± 0.1	± 0.0	± 0.1
Total dissolved RA	Mean	100.0	100.8	101.2	102.3	101.7	102.9	97.0	100.9
	SD	± 0.3	± 0.4	± 0.1	± 0.0	± 0.0	± 0.2	± 0.1	± 0.2
$^{14}CO_2$ and volatile organics	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Bound to apparatus walls	Mean	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	SD								
Total recovery of RA	Mean	100.0	100.8	101.2	102.3	101.7	102.9	97.0	100.9
	SD	± 0.3	± 0.4	± 0.1	± 0.0	± 0.0	± 0.2	± 0.1	± 0.2

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Table IIA 7.5-12: Distribution of the test item and degradates after application of [^{14}C]BYI08330-ketohydroxy to sterile buffer solutions - data given for Test 3 as means of both radiolabels in % of AR (MEF-09/120)

Test 3 (Main Test): sterile TRIS buffer pH 7.0, incubation at 20°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	3	7	10	15	21	30
BYI08330-ketohydroxy	Mean SD	100.0 ± 0.1	97.4 ± 0.1	96.5 ± 0.2	94.2 ± 0.2	95.1 ± 0.6	95.1 ± 0.0	92.6 ± 0.5
BYI08330-MA-amide	Mean SD	n.d.	0.8 ± 0.0	1.7 ± 0.0	2.5 ± 0.0	3.4 ± 0.2	3.9 ± 0.1	6.0 ± 0.2
Unidentified radioactivity	Mean SD	n.d.	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	n.d.	0.8 ± 0.1
Total dissolved RA	Mean SD	100.0 ± 0.1	98.9 ± 0.1	99.0 ± 0.2	97.5 ± 0.4	99.3 ± 0.4	99.0 ± 0.1	99.4 ± 0.2
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to apparatus walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.1	98.9 ± 0.1	99.0 ± 0.2	97.5 ± 0.4	99.3 ± 0.4	99.0 ± 0.1	99.4 ± 0.2

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

Test 3 (Main Test): sterile borate buffer pH 9.0, incubation, incubation at 20°C

Compound	Mean [%] SD [%]	Sampling Times [days] 0	3	7	10	15	21	30
BYI08330-ketohydroxy	Mean SD	100.0 ± 0.1	82.9 ± 0.6	73.5 ± 1.6	58.2 ± 4.7	55.8 ± 1.2	48.5 ± 10.4	15.0 ± 0.0
BYI08330-MA-amide	Mean SD	n.d.	17.5 ± 0.6	26.6 ± 1.1	42.9 ± 5,2	44.5 ± 1.2	54.9 ± 0.6	85.8 ± 0.2
Unidentified radioactivity	Mean SD	n.d.	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	n.d.	n.d.
Total dissolved RA	Mean SD	100.0 ± 0.1	101.0 ± 0.1	100.9 ± 0.3	101.9 ± 0.5	101.2 ± 0.0	103.5 ± 0.3	100.8 ± 0.2
$^{14}CO_2$ and volatile organics	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bound to apparatus walls	Mean SD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total recovery of RA	Mean SD	100.0 ± 0.1	101.0 ± 0.1	100.9 ± 0.3	101.9 ± 0.5	101.2 ± 0.0	103.5 ± 0.3	100.8 ± 0.2

n.d.: not detected, n.a.: not analyzed, SD: standard deviation

F. KINETICS OF TEST ITEM DEGRADATION

The duration and number of sampling intervals was sufficient to determine the hydrolytic behavior of BYI08330-ketohydroxy. The calculated simple first order degradation kinetics of BYI08330-ketohydroxy is summarized in Table IIA 7.5-13.

Table IIA 7.5-13: Calculated simple first order degradation kinetics of [^{14}C]BYI08330-ketohydroxy in sterile buffer solutions (MEF-09/120)

Test No / Temperature	pH	Single First Order Kinetics [days]		
		DT50	DT90	Chi^2 Error
Test 1 / 50°C	4	No calculation, no degradation observed within incubation period.		
Test 1 / 50°C	7	32.7 hours	109 hours	1.5
Test 1 / 50°C	9	71.3 minutes	237 minutes	2.7
Test 2 / 25°C	4	No calculation, no degradation observed within incubation period		
Test 2 / 25°C	7	82.7	275	0.8
Test 2 / 25°C	9	4.9	16.3	4.9
Test 3 / 20°C	7	333	>1000	0.9
Test 3 / 20°C	9	15.6	51.8	8.2

III. CONCLUSIONS

From the current study it is concluded that BYI08330-ketohydroxy is hydrolytically stabile under acidic conditions but labile at a pH above 7. The fastest degradation was observed at pH 9.
The experimental half-lives of BYI08330-ketohydroxy at pH 7 were 82.7 days (25°C) and 333 days (20°C). The experimental half-lives of BYI08330-ketohydroxy at pH 9 were 4.9 days (25°C) and 15.6 days (20°C). Hydrolysis is relevant for the degradation of BYI08330-ketohydroxy in the environment, at pH values above 7. Thereby, one major degradation product is formed. The formation of BYI08330-MA-amide as a common hydrolysis product was observed at pH 7 and pH 9. That metabolite is considered as hydrolytically stable since its concentration increased towards the end of the incubation period at pH 7 and pH 9.

IIA 7.6 Direct phototransformation of relevant metabolites in water

In accordance with Points IIA 2.9.2 and IIA 2.9.3, tests on photolysis (direct photo-transformation) of spirotetramat (BYI08330) in water are also presented here to provide a complete and comprehensive overview on the fate and behavior of this substance in this corresponding section IIA 7 of the dossier. For the respective route of phototransformation study (compare Point IIA 2.9.2) radiolabeled BYI08330 was investigated in pure buffer. A summary of these studies is repeated here, focusing on formation and degradation of transformation products.

In addition, the determination of the quantum yield of direct phototransformation in water and the derived environmental half lives in surface water are also given for active substance (compare Point IIA 2.9.3) and its major direct photo-transformation products in water.

Report:	**KIIA 7.6/01, [redacted], H.-P., 2005 (MEF-05/206)**
Title:	**BYI08330: Phototransformation of BYI08330 in Sterile Water**
Report No & Document No	**MEF-05/206** **M-266695-01-2**
Guidelines:	Commission Directive 95/36/EC amending Council Directive 91/414/EEC, 1995 Pesticide Assessment Guidelines, Subdivision N, Environmental Fate, US EPA, 162-1: Aqueous Photolysis Studies on Soil, 1982 Canada PMRA, DACO No. 8.2.3.3.2
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of Nov. 2003 to Feb. 2004. Study completion date: 2005-11-15

EXECUTIVE SUMMARY

The phototransformation of [azaspirodecenyl-3-^{14}C]- and -[azaspirodecenyl-5-^{14}C]-BYI08330 (labels #1 and #2) was studied in sterile 0.01 M aqueous acetate buffer solution of pH 5 at 25°C at an initial concentration of 1 mg/L. The test solutions were kept in quartz glass vessels connected to traps for the collection of CO_2 and organic volatiles and continuously exposed to artificial irradiation (xenon lamp with <290 nm cut-off filter). In addition, dark controls were set up. Test solutions were analyzed at 0, 1, 2, 3, 4, 6 and 7 days directly without extraction by LSC and reversed phase HPLC with radioactivity detection.

The mass balance based on the means of both labels was 102.8% ± 3.0% and 99.2% ± 2.2% of the applied radioactivity (AR) in irradiated and dark samples, respectively. No significant amount of volatiles was detected. BYI08330 was well degraded under the influence of simulated sunlight. In the irradiated test system, BYI08330 decreased from an average of 99.2% at day 0 to 14.4% of the AR after 7 days exposure to light. Since test item and major phototransformation products were detected with both labels, mean values were calculated for each sampling interval.

Four major phototransformation products were found and identified as products of re-arrangement reactions. BYI08330-photo-cyclopentyl (P6) was the main metabolite and increased to max. 39.2% of the AR at DAT-7. BYI08330-photo-2-hydroxymethyl (P7) was max. 22.1% at DAT-4. BYI0330-photo-2-formyl (P8) increased to 11.1% after 3 days exposure to light and decreased then to 5.3% at the end. BYI08330-photo-2-methyl carbonate (P9) was max. 18.2% of the AR at DAT-6. Five other minor metabolites were detected, but were not further identified. None exceeded 5.5% of the applied radioactivity (i.e. P5 at DAT-3). For structures see Figure IIA 7.6-1. In the dark test system, BYI08330-enol was formed due to the already well known hydrolysis reaction and increased (as it was expected) to 13.8% of AR at DAT-7. No other transformation product was detected thereby.
Based on the experimental DT50 of 2.7 days for BYI08330 the predicted environmental DT50 is calculated to be e.g. 12.9 solar summer days at [redacted], AZ, USA, or 19.9 summer days at [redacted], Greece. Under dark conditions the half life under the prevailing experimental conditions was 26 days, which is regarded as hydrolysis half-life. From this study, it is considered that photo-degradation of BYI08330 is a route for the elimination of this compound from water. But the test was performed under sterile conditions in highly purified buffer of pH 5. It is to be expected that the behavior will be different in natural aqueous systems, due to faster hydrolysis with increasing pH and indirect reactions as well as biodegradation might compete with the re-arrangement reactions observed in the prevailing study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat: Code = BYI08330
Identity and purity of test item in the application solutions were checked

Label #1: Label position = [azaspirodecenyl-3-^{14}C] (sample ID: BECH 0950)
Specific activity 3.67 MBq/mg (99.1 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

Label #2: Label position = [azaspirodecenyl-5-^{14}C] (sample ID: BECH 0952)
Specific activity 4.03 MBq/mg (108.8 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Chemical purity: >98% (HPLC, UV detection at 210 nm)

2. Test System: This aqueous photolysis study was conducted using aqueous 0.01 M acetate buffer at pH 5 to help distinguish between hydrolytic and photolytic reactions. The test item was not sufficient stable at pH 7. The water used had a total organic carbon content of 13 ppb and an electrical resistance of 18.2 MΩ x cm. BYI08330 was found to be most stable in aqueous buffer pH 5. Before use the buffer solution was sterilized in an autoclave. The content of acetonitrile in the test solutions was minimized to 0.1% (v/v), only.

B. STUDY DESIGN

1. Experimental conditions: The tests were performed using individual static test systems held at aerobic conditions at 25 ±1 °C for a maximum period of 7 experimental days. They consisted of 30 quartz glass vessels [50 mm x 26 mm x 16 mm (height)] each containing 10 mL of the test solution (buffer solution + test item), and were closed (except in case of time 0) with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds. All containers and glassware, as well as the buffer solution were sterilized in an autoclave in order to prevent biodegradation of the test solutions during the study.

The test systems were either incubated in the dark as controls or continuously exposed to artificial irradiation (Suntest® unit equipped with a xenon lamp and <290 nm cut-off filter). Thus, the spectral distribution of the light intensity was similar to the distribution of natural sunlight. The light intensity was constant throughout the study, and the samples were constantly irradiated for 24 hours/day. The experimental light intensity of 1709 W/m² of continuous irradiation was in a way that 7 days of irradiation is equivalent to 33.3 solar days in June under extreme sunlight conditions at [redacted], AZ (USA) or to 51.7 days in June under extreme European conditions in Greece.

2. Sampling: The test systems were processed for analysis immediately after the application of the kinetic treatment solution on day 0. Subsequently, samples of both irradiated and dark test systems were processed at 1, 2, 3, 4, 6 and 7 days post-application. The trap attachments for $^{14}CO_2$ and volatile organics were stored at ambient temperature until processing for analysis.

3. Description of analytical procedures: The radioactivity of the test solutions was radio-assayed by triplicate 100-µL aliquots. Chromatographic analyses by the primary method (reversed phase HPLC equipped with a radioactivity detector) were performed within one day after sampling. Analyses by the confirmatory method (radio-TLC) were performed immediately after sampling. Analyzed samples were stored deep-frozen at approximately -15°C or below until further investigations. Amounts of the test

items and the transformation products were calculated as percentage of the applied radioactivity. Values were presented as single values and as means if replicates (label #1 and #2) were possible. Quantification of test item was based on the radioactivity measured in the solutions. Calculations were performed using the computer software Microsoft Excel® 97. Identification and confirmation of the parent compound and transformation products was done by co-chromatography. Three main metabolites were found and investigated by LC-MS/MS and NMR techniques.

C. DETERMINATION OF DEGRADATION KINETICS

A linear regression analysis was used to determine the radioactive detector response (Microsoft Excel® 97). Arithmetic means were used for all LS measurements, and for the mentioned replicates. Outlier rejection criteria were not used.

A simple first-order (SFO) degradation rate constant (k) was determined by the software program (ModelManager® 1.1) using a nonlinear optimization method. The percentage of AR as BYI08330 was plotted against time. The equation for the simple first-order degradation relationship is:

$$C_t = C_0 \times e^{-kt}$$

where C_0 and C_t are the BYI08330 concentrations at time 0 and t (days), respectively. Based on the above relationship the DT_{50} (or $T_{1/2}$) and DT_{90} (or $T_{1/10}$) values, in days, were calculated as follows:

$$T_{1/2} = \ln (0.5)/-k$$

$$T_{1/10} = \ln (0.1)/-k$$

II. RESULTS AND DISCUSSION

A. DATA

The pH of the test solutions (pH 5) and the oxygen saturation (>95%) were constant throughout the incubation period. No contamination by micro-organisms was observed from the sterility test.

The analytical data for the irradiated test systems and the dark controls are shown in Table IIA 7.6-1 and Table IIA 7.6-2 for each radiolabel, a compilation (means of both radiolabels) is given by Table IIA 7.6-3.

B. MASS BALANCE

For irradiated test systems, the material balance ranged from 98.2% to 108.4% of AR for individual samples, the overall mean was 102.8 ± 3.0% for both radiolabels A and B. For dark test systems, the material balance ranged from 93.8% to 104.7% of the AR, with an overall mean of 99.2 ± 2.2%. The material balance found at all sampling intervals demonstrated that no significant radioactivity dissipated from the vessels or was lost during processing. The slight tendency of increase in case of irradiated samples may be due to evaporation of some water. The distribution of radioactive residues in the irradiated and dark test systems is summarized in Table IIA 7.6-1 to Table IIA 7.6-3.

C. BOUND AND EXTRACTABLE RESIDUES

N/A

D. VOLATILIZATION

For the irradiated systems testing label #1 the $^{14}CO_2$ formation increased up to max 2.6% of the AR at DAT-6. For irradiated systems testing label #2 the $^{14}CO_2$ formation was only max 0.2% of the AR at DAT-7. For dark test systems of both radiolabels $^{14}CO_2$ formation was not measured.

Organic volatile formation was negligible throughout the study (< 1%) for irradiated samples and was not measured in case of dark samples.

E. TRANSFORMATION OF TEST ITEM

The parent compound was quickly degraded (for synopsis of results see Table IIA 7.6-4). The distribution and composition of residues in the irradiated and dark test systems are presented as a percentage of the AR in Table IIA 7.6-1 to Table IIA 7.6-3. The proposed transformation pathway is shown in Figure IIA 7.6-1.

In the irradiated test system, BYI08330 decreased from an average of 99.2% at day 0 to 14.4% of the AR after 7 days exposure to light. Four major phototransformation products were formed and were fully identified. BYI08330-photo-cyclopentyl (P6) was the main metabolite and increased to max. 39.2% of the AR at DAT-7. BYI08330-photo-2-hydroxymethyl (P7) was max. 22.1% at DAT-4. BYI0330-photo-2-formyl (P8) increased to 11.1% after 3 days exposure to light and decreased then to 5.3% at the end. BYI08330-photo-2-methyl carbonate (P9) was max. 18.2% of the AR at DAT-6. Five other minor metabolites were detected, but were not further identified. None exceeded 5.5% of the applied radioactivity (i.e. P5 at DAT-3).

In the dark test system, BYI08330 decreased from 101.1% of the AR to 84.8% at the end of the incubation period. BYI08330-enol was formed due to hydrolysis and increased to 13.8% of AR at DAT-7. No other transformation product was detected.

Comparing both, dark and irradiated test systems, four major products were formed due to direct phototransformation processes. These products are formed by re-arrangement reactions which occur under light exposure, only. Under dark conditions the hydrolysis product BYI08330-enol was formed even under the most stable conditions at pH 5, as it was expected from the knowledge on hydrolysis (see report KIIA 7.5/01 in the section before).

F. KINETICS OF TEST ITEM DEGRADATION

The first-order experimental DT50 values of BYI08330 in irradiated and in dark samples are summarized in Table IIA 7.6-4.

Based on the experimental DT50 of 2.7 days for BYI08330 the predicted environmental DT50 is calculated to be e.g. 12.9 solar summer days at [redacted], AZ, USA, or 19.9 summer days at [redacted], Greece. Under dark conditions the half life under the prevailing experimental conditions was 26 days, which is regarded as hydrolysis half-life.

Table IIA 7.6-1: Transformation of BYI08330 in buffer pH 5, mean values of radiolabel #1 expressed as % of AR (MEF-05/206)

Compound		Sampling Times [days] 0	1	2	3	4	6	7
BYI08330	Irradiated	100.2	72.9	64.0	36.4	34.6	16.8	16.7
	Dark	100.9	102.4	93.5	90.5	88.5	84.5	84.6
Reg1	Irradiated							1.7
	Dark							
P3	Irradiated						2.6	4.2
	Dark							
P4	Irradiated						4.8	5.2
	Dark							
P5	Irradiated				5.1		2.6	3.0
	Dark							
Photo-cyclopentyl (P6)	Irradiated		3.1	5.8	19.2	24.9	37.6	35.4
	Dark							
Photo-2-hydroxymethyl (P7)	Irradiated		16.9	18.4	16.2	22.2	15.8	15.5
	Dark							
Photo-2-formyl (P8)	Irradiated			2.8	10.7	4.9	3.5	6.3
	Dark							
Photo-2-methyl carbonate (P9)	Irradiated		7.2	9.5	14.0	16.1	19.3	17.0
	Dark							
BYI08330-enol	Irradiated							
	Dark		2.3	4.2	6.5	9.0	11.7	13.7
Total RA in test solution	Irradiated	100.2	100.1	100.7	101.6	102.7	103.1	105.0
	Dark	100.9	104.7	97.7	97.0	97.6	96.1	98.3
$^{14}CO_2$	Irradiated	n.m.	0.1	0.2	0.8	1.3	2.6	2.5
	Dark	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Volatile organics	Irradiated	n.m.	0.1		0.1			0.8
	Dark	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Total recovery of RA	Irradiated	100.2	100.2	100.9	102.5	103.9	105.7	108.4
	Dark	100.9	104.7	97.7	97.0	97.6	96.1	98.3

Blanks represent values below LOD (for radio-HPLC: LOD = 1 %; for volatiles: LOD = 0.1 %)

Table IIA 7.6-2: Transformation of BYI08330 in buffer pH 5, mean values of radiolabel #2 expressed as % of AR (MEF-05/206)

Compound		Sampling Times [days]						
		0	1	2	3	4	6	7
BYI08330	Irradiated	98.2	78.3	64.5	39.3	41.1	32.7	12.0
	Dark	101.4	97.5	97.3	86.6	89.5	89.1	85.1
Reg1	Irradiated							2.6
	Dark							
P2	Irradiated			1.4	2.6	5.3	4.4	5.2
	Dark							
P4	Irradiated				2.3			3.4
	Dark							
P5	Irradiated				3.5			3.0
	Dark							
Photo-cyclopentyl (P6)	Irradiated		2.3	5.4	15.1	16.6	25.3	42.9
	Dark							
Photo-2-hydroxymethyl (P7)	Irradiated		13.7	18.1	15.4	21.9	22.9	13.8
	Dark							
Photo-2-formyl (P8)	Irradiated		0.0	2.3	11.5	3.9	3.6	4.3
	Dark							
Photo-2-methyl carbonate (P9)	Irradiated		5.5	8.8	11.7	14.1	17.2	18.4
	Dark							
BYI08330-enol	Irradiated							
	Dark		2.6	4.2	6.9	9.8	12.5	13.8
Total RA in test solution	Irradiated	98.2	99.7	100.5	103.4	103.4	106.2	105.7
	Dark	101.4	100.1	101.6	93.8	99.4	101.6	98.9
$^{14}CO_2$	Irradiated	n.m.				0.1	0.1	0.2
	Dark	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Volatile organics	Irradiated	n.m.	0.3					0.1
	Dark	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Total recovery of RA	Irradiated	98.2	100.0	100.5	103.4	103.5	106.2	106.0
	Dark	101.4	100.1	101.6	93.8	99.4	101.6	98.9

Blanks represent values below LOD (for radio-HPLC: LOD = 1 %; for volatiles: LOD = 0.1 %)

Table IIA 7.6-3: Transformation of BYI08330 in buffer pH 5, mean values of both radiolabels #1 and B expressed as % of AR (MEF-05/206)

Compound		Sampling Times [days]						
		0	1	2	3	4	6	7
BYI08330	Irradiated	99.2	75.6	64.3	37.9	37.8	24.8	14.4
	SD	± 1.4	± 3.8	± 0.3	± 2.1	± 4.6	± 11.3	± 3.3
	Dark	101.1	99.9	95.4	88.7	89.0	86.8	84.8
	SD	± 0.4	± 3.5	± 2.7	± 2.6	± 0.7	± 3.3	± 0.3
Photo-cyclopentyl (P6)	Irradiated		2.7	5.6	17.1	20.7	31.5	39.2
	SD		± 0.6	± 0.3	± 2.9	± 5.9	± 8.6	± 5.8
	Dark							
	SD							
Photo-2-hydroxymethyl (P7)	Irradiated		15.3	18.3	15.8	22.1	19.4	14.6
	SD		± 2.3	± 0.2	± 0.6	± 0.2	± 5.0	± 1.2
	Dark							
	SD							
Photo-2-formyl (P8)	Irradiated			2.5	11.1	4.4	3.5	5.3
	SD			± 0.4	± 0.5	± 0.7	± 0.0	± 1.4
	Dark							
	SD							
Photo-2-methyl carbonate (P9)	Irradiated		6.4	9.2	12.8	15.4	18.2	17.7
	SD		± 0.2	± 0.5	± 1.6	± 1.0	± 1.5	± 0.9
	Dark							
	SD							
BYI08330-enol	Irradiated							
	SD							
	Dark		2.4	4.2	6.7	9.4	12.1	13.8
	SD		± 0.2	± 0.0	± 0.3	± 0.6	± 0.6	± 0.1
Total RA in test solution	Irradiated	99.2	99.9	100.6	102.5	103.1	104.6	105.4
	SD	± 1.4	± 0.3	± 0.1	± 1.3	± 0.5	± 2.2	± 0.5
	Dark	101.1	102.4	99.6	95.4	98.5	98.9	98.6
	SD	± 0.4	± 3.3	± 2.8	± 2.3	± 1.2	± 3.9	± 0.5
Total recovery of RA	Irradiated	99.2	100.1	100.7	103.0	103.7	105.9	107.2
	SD	± 1.4	± 0.2	± 0.3	± 0.7	± 0.3	± 0.4	± 1.7
	Dark	101.1	102.4	99.6	95.4	98.5	98.9	98.6
	SD	± 0.4	± 3.3	± 2.8	± 2.3	± 1.2	± 3.9	± 0.5

Blanks represent values below LOD (for radio-HPLC: LOD = 1 %; for volatiles: LOD = 0.1 %)

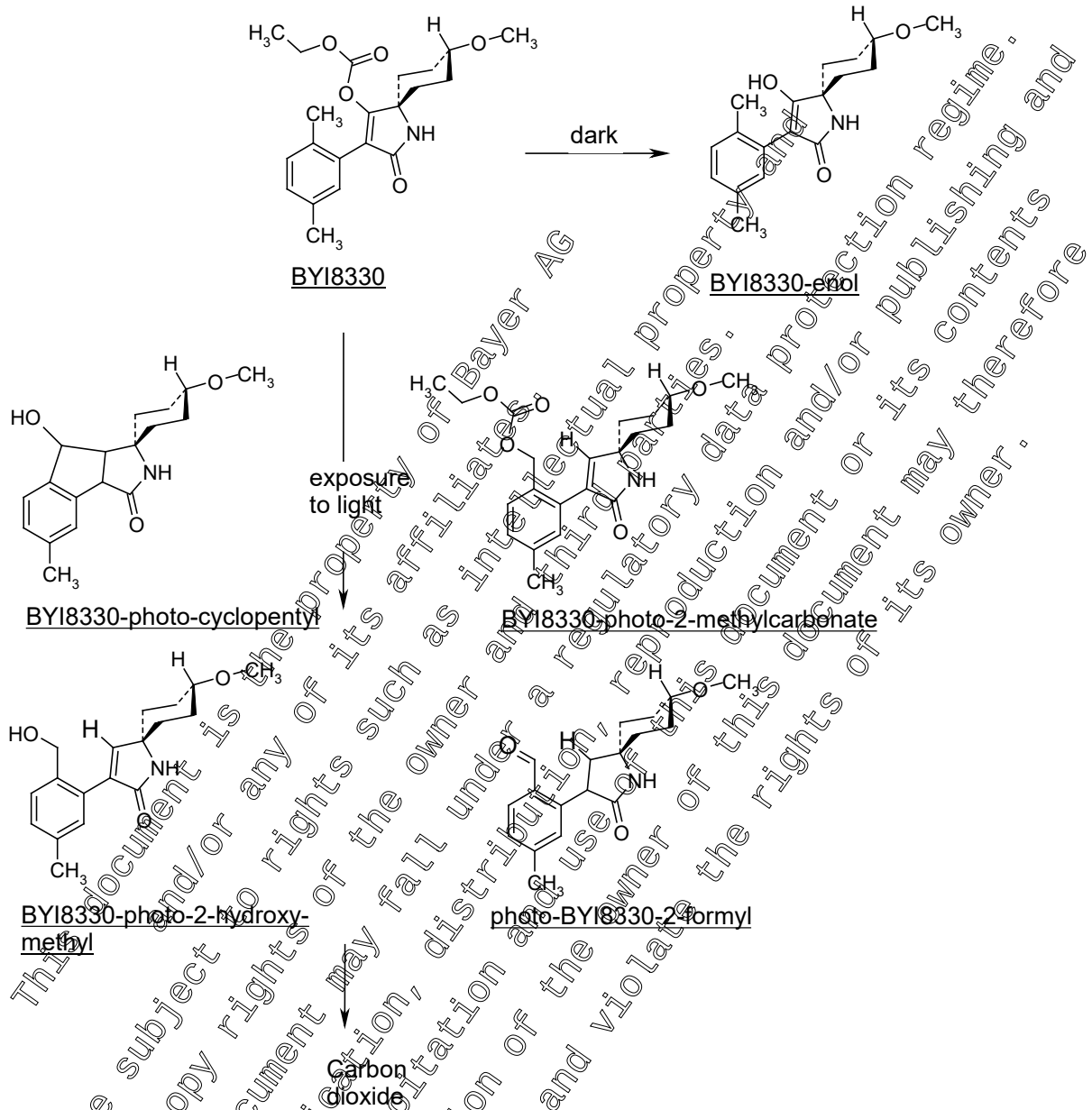


Figure IIA 7.6-1: Proposed Pathway of Direct Phototransformation for Spirotetramat (BYI08330) in Pure Water, i.e. Buffer of pH 5 (MEF-05/206)

Table IIA 7.6-4: Synopsis of transformation of BYI08330 in pure buffer of pH 5 (MEF-05/206)

Soil	Evaluation of Means of [azaspirodecenyl-3-^{14}C] and [azaspirodecenyl-5-^{14}C]BYI08330	
Type	Irradiated	Dark
k (1/d)	0.257	0.027
Experimental 1st order DT_{50} [d]	2.7	26.2
R^2	0.980	0.897
Environmental DT_{50} [d] in June at [redacted], AZ (USA)	12.9	N/A
Environmental DT_{50} [d] in June at [redacted] (Greece)	19.9	N/A
Major transformation products *)	Photo-cyclopentyl (P6) Photo-2-hydroxymethyl (P7) Photo-2-formyl (P8) Photo-2-methyl carbonate (P9)	Enol
Minor transformation products	Some not identified, each <5.5%	N/A

*): Criteria for term "major": >10% of AR at any DAT or >5% of AR at two successive DATs

III CONCLUSIONS

Based on the experimental DT50 of 2.7 days for BYI08330 and related predicted environmental DT50 (e.g. of 12.9 solar summer days at [redacted], AZ, USA or 19.9 summer days at [redacted], Greece) it is concluded that photo-transformation of BYI08330 in aqueous systems is a significant route for the elimination of this compound in sterile pure buffered water.

However, this test was performed under sterile conditions in highly purified buffer of pH 5, in order to help distinguish between hydrolytic or and biotic and photolytic reactions. Thus, it is to be expected that the behavior will be different in natural aqueous systems. Then, biodegradation will happen and hydrolysis will be faster with increasing pH, as well as indirect reactions might compete with the re-arrangement reactions observed in the prevailing study.

From all this facts it was concluded better to investigate the phototransformation of [azaspirodecenyl-3-^{14}C] and -[azaspirodecenyl-5-^{14}C]BYI08330 (labels #1 and #2) in sterile natural water by a supportive study that is described below.

Supportive study: Phototransformation of BYI08330 in natural water

Report:	**KIIA 7.6/02, [redacted], H.-P., 2005 (MEF-05/262)**
Title:	**[Azaspirodecenyl-3-^{14}C]BYI08330 and [Azaspirodecenyl-5-^{14}C]BYI08330: Phototransformation in Natural Water**
Report No &	**MEF-05/262**
Document No	**M-266753-01-2**
Guidelines:	Not a required guideline study but in accordance with: Commission Directive 95/36/EC amending Council Directive 91/414/EEC, 1995 Pesticide Assessment Guidelines, Subdivision N, Environmental Fate, US EPA, 162-1: Aqueous Photolysis Studies on Soil, 1982 Canada PMRA, DACO No. 8.2.3.3.2
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period of Sep. 2004 to Feb. 2005. Study completion date: 2005-11-15

EXECUTIVE SUMMARY

The phototransformation of [azaspirodecenyl-3-^{14}C] and [azaspirodecenyl-5-^{14}C]BYI08330 (labels #1 and #2) was studied in sterile natural water from the river Rhine at 25°C at an initial concentration of 1 mg/L. The test solutions were kept in quartz glass vessels connected to traps for the collection of CO_2 and organic volatiles and continuously exposed to artificial irradiation (xenon lamp with <290 nm cut-off filter). In addition, dark controls were set up. Test solutions were analyzed at 0, 1, 2, 3, 6, 8 and 10 days directly without extraction by LSC and reversed phase HPLC with radioactivity detection.

The mass balance was for label #1 102.0% ± 2.4% / 100.6% ± 2.3% and for label #2 101.8% ± 2.2% / 99.9% ± 2.7% of the applied radioactivity (AR) in irradiated / dark samples, respectively. No significant amount of volatiles was detected. In the irradiated test system, BYI08330 decreased fast and BYI08330-enol was formed to max. 80.1% ± 1.8% of AR (mean of both labels) after one day of exposure to light. At the same time a multitude of photoproducts was formed. Subsequently, BYI08330-enol decreased to 15.5% ± 1.3% of AR (mean of both labels) at the end of the test period (i.e. after 10 days).

Two major phototransformation products were formed when using radiolabel #2, and the structures were identified as BYI08330-methoxy-cyclohexylamino-caboxylic acid (ID: PB1) and BYI08330-methoxycyclo-hexanone (ID: PB3). These products were not found with label #1 and were therefore formed by cleavage of the 5-membered ring system. BYI08330-methoxy-cyclohexylamino-carboxylic acid increased to max. 11.3% and BYI08330-methoxycyclohexanone increased to max. 17.5% of the AR at day 8 of the irradiation period. For structures see Figure IIA 7.8.3-2. In the dark test system, BYI08330-enol was formed due to hydrolysis. At the end of the test period BYI08330-enol was 102.4% ± 0.4%. No other transformation product was detected thereby.

Comparing both, dark and irradiated test systems, degradation of the test item was faster under exposure to light. Based on the experimental DT50 of 0.2 days for BYI08330 the predicted environmental DT50 is calculated to be e.g. 0.6 solar summer days at [redacted], AZ, USA or 1.0 summer days at [redacted], Greece. Under dark conditions the half life under experimental conditions is 1.5 days.

From this study it is concluded that photo-degradation of BYI08330 is a significant route for the elimination of this compound from natural water. This test performed in a natural water (as it is quite

common to be neutral or slightly alkaline) clearly showed that competition of hydrolysis and indirect photo-reactions does not allow the light-induced re-arrangement reactions of parent compound observed in highly purified buffer of pH 5. Together with the well-known fast biodegradation this was the justification to consider the products formed in the prevailing study for the overall pathway of spirotetramat degradation in water (see Figure IIA 7.8.3-2), but not the re-arrangement photo-products (see Figure IIA 7.6-1) found in the highly artificial study performed in sterile pure buffer.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat: Code = BYI08330
Identity and purity of test item in the application solutions were checked

Label #1: Label position = [azaspirodecenyl-3-^{14}C] (sample ID: BECH 0950)
Specific activity = 3.67 MBq/mg (99.1 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

Label #2: Label position = [azaspirodecenyl-5-^{14}C] (sample ID: BECH 0952)
Specific activity = 4.03 MBq/mg (108.8 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Chemical purity: >98% (HPLC, UV detection at 210 nm)

2. Test System: This aqueous photolysis study was conducted using natural water from the Rhine River. It was freshly collected on 2004-10-04. About 4 L water was carefully sampled in distance of about 1.5 m from the right bank (location km 713-714) in a water depth of about 30 cm. The pH of the water was pH 7.9, the oxygen saturation was 98.4% at 16.5 °C, and it contained 25.8 mg/L of suspended solid and 2 mg/L of total organic carbon. The total evaporation residue was 273.3 mg/L. Hardness and conductivity were measured to be 523 µS/cm and 10.6 °dH, the total nitrate and phosphorus were 8.8 mg and 0.1 mg per liter, respectively. The UV absorption measured in the range of 290 to 800 nm was < 0.05 (1 cm layer) for natural water and for the test solution with test item. The water was used in this study without further filtration. However, before application of test item the water was sterilized by steam pressure sterilization to avoid biotic degradation of the test substance. The content of acetonitrile in the test solutions was minimized to 0.1% (v/v), only.

B. STUDY DESIGN

1. Experimental conditions: The tests were performed using individual static test systems held at aerobic conditions at 25 ±1 °C for a maximum period of 7 experimental days. They consisted of 30 quartz glass vessels [50 mm x 26 mm x 16 mm (height)] each containing 10 mL of the test solution (buffer solution + test item), and were closed (except in case of time 0) with a trap attachment (permeable for oxygen) containing soda lime for absorption of $^{14}CO_2$ and a polyurethane foam plug for adsorption of volatile organic compounds. All containers and glassware, as well as the buffer solution were sterilized in an autoclave in order to prevent biodegradation of the test solutions during the study.

The test systems were either incubated in the dark as controls or continuously exposed to artificial irradiation (Suntest® unit equipped with a xenon lamp and <290 nm cut-off filter). Thus, the spectral distribution of the light intensity was similar to the distribution of natural sunlight. The light intensity was constant throughout the study, and the samples were constantly irradiated for 24 hours/day. The experimental light intensity of 1209 W/m² of continuous irradiation was in a way that 10 days of

irradiation is equivalent to 33.7 solar days in June under extreme sunlight conditions at [redacted], AZ (USA) or to 52.2 days in June under extreme European conditions in Greece.

2. Sampling: The test systems were processed for analysis immediately after the application of the kinetic treatment solution on day 0. Subsequently, samples of both irradiated and dark test systems were processed at 1, 2, 3, 6, 8 and 10 days post-application. The trap attachments for $^{14}CO_2$ and volatile organics were stored at ambient temperature until processing for analysis.

3. Description of analytical procedures: The radioactivity of the test solutions was radio-assayed by triplicate 100-µL aliquots. Chromatographic analyses by the primary method (reversed phase HPLC equipped with a radioactivity detector) were performed within one day after sampling. Analyses by the confirmatory method (radio-TLC) were performed immediately after sampling. Analyzed samples were stored deep-frozen at approximately -15°C or below until further investigations. Amounts of the test items and the transformation products were calculated as percentage of the applied radioactivity. Values were presented as single values and as means if replicates (label #1 and #2) were possible. Quantification of test item was based on the radioactivity measured in the solutions. Calculations were performed using the computer software Microsoft Excel® 97. Identification and confirmation of the parent compound and transformation products was done by co-chromatography. Four main metabolites were found and the complex structures of the rearrangement products were investigated by LC-MS/MS and NMR techniques.

C. DETERMINATION OF DEGRADATION KINETICS

A linear regression analysis was used to determine the radioactive detector response (Microsoft Excel® 97). Arithmetic means were used for all LS measurements, and for the mentioned replicates. Outlier rejection criteria were not used.

A simple first-order (SFO) degradation rate constant (k) was determined by the software program (ModelManager® 1.1) using a nonlinear optimization method. The percentage of AR as BYI08330 was plotted against time. The equation for the simple first-order degradation relationship is:

$$C_t = C_0 \times e^{-kt}$$

where C_0 and C_t are the BYI08330 concentrations at time 0 and t (days), respectively. Based on the above relationship the DT_{50} (or $T_{1/2}$) and DT_{90} (or $T_{1/10}$) values, in days, were calculated as follows:

$$T_{1/2} = \ln(0.5)/-k$$

$$T_{1/10} = \ln(0.1)/-k$$

II. RESULTS AND DISCUSSION

A. DATA

The pH of the test solutions was about pH 8 at the beginning of the experiment. For dark samples the pH remained constant, but the pH increased for irradiated samples to pH 9.4, probably due phototransformation and the lack of buffer capacity in the natural water. The oxygen saturation was constant on a high level throughout the incubation period (>94%). The test water maintained sterile throughout the test period. No contamination by micro-organisms was observed from the sterility test.

The analytical data for the irradiated test systems and the dark controls are shown in Table IIA 7.6-6 and Table IIA 7.6-7, a compilation is given by Table IIA 7.6-5.

B. MASS BALANCE

The mass balance expressed as % of AR was in case of label #1 102.0% ± 2.4% / 100.6% ± 2.3% and for label #2 101.8% ± 2.2% / 99.9% ± 2.7% of the applied radioactivity (AR) in irradiated / dark samples, respectively. Due to the lack of significant formation of volatiles, mean values were calculated from both labels for each sampling interval and irradiated and dark samples. Based on this the overall mean was 101.9 ± 2.3% of AR for irradiated samples. For dark test systems, the overall material balance was 100.3% ± 2.5%. The distribution of radioactive residues in the irradiated and dark test systems is summarized in Table IIA 7.6-5 to Table IIA 7.6-7.

C. BOUND AND EXTRACTABLE RESIDUES

N/A

D. VOLATILIZATION

For the irradiated systems testing label #1 the $^{14}CO_2$ formation increased up to max 1.1% of the AR, for irradiated systems testing label #2 the $^{14}CO_2$ formation was only max 0.3% of the AR at DAT-10. For dark test systems of both radiolabels $^{14}CO_2$ formation was not measured.

Organic volatile formation was negligible throughout the study (max. 1.5%) for irradiated samples and was not measured in case of dark samples.

E. TRANSFORMATION OF TEST ITEM

The parent compound was quickly degraded (for synopsis of results see Table IIA 7.6-8). The distribution and composition of residues in the irradiated and dark test systems are presented as a percentage of the AR in Table IIA 7.6-5 to Table IIA 7.6-7. The proposed transformation pathway is included Figure IIA 7.8.3-2.

In the irradiated test system, BYI08330 decreased fast and BYI08330-enol was formed to max. 80.1% ± 1.8% of AR (mean of both labels) after one day of exposure to light, already. At the same time a multitude of photoproducts were formed. Using radiolabel #1 33 minor metabolites were observed besides BYI08330-enol, which could not all be separated completely by HPLC chromatography. The maximum amount of a single region PA3 was 9.6% of AR at DAT-8 and decreased to 9.1% at DAT-10. Using radiolabel #2 27 metabolites were detected. Two major phototransformation products were formed and the structures were identified as BYI08330-methoxy-cyclohexylamino-caboxylic acid (ID: PB1) and BYI08330-methoxycyclohexanone (ID: PB3). These products were not found with label #1 and were therefore formed by cleavage of the 5-membered ring system. BYI08330-methoxy-cyclohexylamino-carboxylic acid increased to max. 11.3% and BYI08330-methoxycyclohexanone increased to max. 17.5% of the AR at day 8 of the irradiation period. None of the minor photo-products exceeded 5.6% of AR. BYI08330-enol decreased to 15.5% ± 1.3% of AR (mean of both labels) at the end of the test period.

In the dark test system, BYI08330-enol was formed due to hydrolysis. At the end of the test period BYI08330-enol was 102.4% ± 0.4%. No other transformation product was detected.

F. KINETICS OF TEST ITEM DEGRADATION

The first-order experimental DT50 values of BYI08330 in irradiated and in dark samples are summarized in Table IIA 7.6-8.

Based on the experimental DT50 of 0.2 days for BYI08330 the predicted environmental DT50 is

calculated to be e.g. 0.6 solar summer days at [redacted], AZ, USA or 1.0 summer days at [redacted], Greece. Under dark conditions the half life under the prevailing experimental conditions was 1.5 days, which is regarded as hydrolysis half-life.

Table IIA 7.6-5: Transformation of BYI08330 in Rhine River water, mean values of both radiolabels #1 and B (if possible) expressed as % of AR (MEF-05/262)

Compound		Sampling Times [days] 0	1	2	3	6	8	10
BYI08330	Irradiated	78.9	2.0					
	SD	± 1.5	± 0.5					
	Dark	86.1	59.6	35.5	21.4	3.8	1.5	0.0
	SD	± 2.1	± 2.1	± 0.1	± 0.5	± 0.0	± 0.0	± 0.0
BYI08330-enol	Irradiated	17.8	80.1	70.1	57.4	32.4	19.2	15.5
	SD	± 1.5	± 1.8	± 2.1	± 0.9	± 0.3	± 2.2	± 1.3
	Dark	9.4	39.3	64.8	79.0	98.0	100.8	102.4
	SD	± 1.5	± 1.6	± 0.1	± 0.1	± 0.6	± 0.0	± 0.4
Total RA in test solution	Irradiated	96.8	103.2	103.2	102.0	101.8	101.6	101.4
	SD	± 0.0	± 0.0	± 0.0	± 0.3	± 0.1	± 0.3	± 0.8
	Dark	95.5	98.8	100.3	100.4	101.8	102.7	102.4
	SD	± 0.7	± 0.5	± 0.0	± 0.6	± 0.6	± 0.3	± 0.4
$^{14}CO_2$ *	Irradiated	n.m.	0.0	0.0	0.0	0.2	0.4	0.7
	SD		± 0.0	± 0.0	± 0.0	± 0.1	± 0.3	± 0.4
	Dark	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
	SD							
Volatile organics *	Irradiated	n.m.	0.0	0.0	0.8	0.4	0.6	0.4
	SD		± 0.0	± 0.0	± 0.7	± 0.3	± 0.4	± 0.3
	Dark	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
	SD							
Total recovery of RA	Irradiated	96.8	103.2	103.2	102.8	102.4	102.6	102.4
	SD	± 0.0	± 0.0	± 0.0	± 0.4	± 0.5	± 0.1	± 0.1
	Dark	95.5	98.8	100.3	100.4	101.8	102.7	102.4
	SD	± 0.7	± 0.5	± 0.0	± 0.6	± 0.6	± 0.3	± 0.4

Blanks represent values below LOD: for radio-HPLC: LOD = 0.5 % (for metabolites depending o the peak shape), for volatiles: LOD = 0.1 %)

*: Formation is different for the labels on a very low level; Means were calculated to give an overview.

Table IIA 7.6-6: Transformation of BYI08330 in Rhine River water, mean values of radiolabel #1 expressed as % of AR (MEF-05/262)

Irradiated:	Sampling Times [days]						
Compound	0	1	2	3	6	8	10
BYI08330	80.4	2.6					
PA1		0.3	1.6	1.9	5.1	6.7	8.8
RegA2						1.4	2.2
RegA3				2.1	3.4	3.8	6.4
RegA4		0.4	1.0		4.0	5.9	6.0
PA2		1.0	1.8	1.9	4.1	5.3	6.3
RegA6		0.6	1.9	2.3	5.4	2.5	4.3
RegA7		1.1					3.7
RegA8		1.1	0.8	1.2	2.3	2.9	2.5
RegA9						0.9	1.2
RegA10		0.3	1.0	0.9	2.3	2.8	1.3
RegA11						2.6	1.0
PA3		0.4	1.6	2.9	7.7	9.6	9.1
RegA13		0.5		0.4		1.3	1.3
RegA14		1.1	1.9	2.5	3.0	3.1	3.0
RegA15						1.3	0.6
RegA16		0.9	1.7	2.3	2.5	2.9	2.3
RegA17		0.3				1.5	1.5
RegA18		1.6	2.1	2.6	3.5	3.1	2.3
RegA19		1.3	2.7	2.0	2.4	2.2	1.5
RegA20		0.7			1.5	1.2	1.7
RegA21		0.7	1.1	2.0	2.9		2.4
RegA22		1.1	1.2	0.9	1.8		1.6
RegA23					0.7		0.9
RegA24							0.7
RegA25		0.9	1.6	1.0	0.6		0.7
BYI08330-enol (PA4)	16.3	78.3	68.0	56.5	32.7	21.5	14.1
RegA27		0.8	2.0	3.0	3.3	4.8	3.6
RegA28		1.6	1.7	3.0	1.6	1.4	1.0
RegA29		2.0	2.5	3.6	2.6	1.6	1.1
RegA30						0.9	0.6
PA5		1.4	2.2	3.5	4.2	4.5	3.2
RegA32						0.3	0.4
PA6		1.0	2.5	4.0	4.3	4.5	3.4
RegA34		1.4	2.3	1.1	0.0	0.5	0.0
Total RA in test solution	96.7	103.2	103.1	101.6	101.9	101.0	100.6
$^{14}CO_2$	n.m.	0.0	0.0	0.1	0.3	0.7	1.1
Volatile organics	n.m.	0.0	0.0	1.5	0.7	1.0	0.7
Total recovery of RA	96.7	103.3	103.2	103.2	102.9	102.7	102.3

Blanks represent values below LOD: for radio-HPLC: LOD = 0.5 % (for metabolites depending o the peak shape), for volatiles: LOD = 0.1 %)

Continued:

Dark: Compound	Sampling Times [days] 0	1	2	3	6	8	10
BYI08330	88.3	61.7	35.4	21.9	3.7	1.5	0.0
BYI08330-enol (PA4)	7.9	37.7	64.8	79.1	98.6	100.8	102.7
Total RA in test solution	96.2	99.3	100.3	101.0	102.4	102.3	102.7
$^{14}CO_2$	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Volatile organics	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Total recovery of RA	96.2	99.3	100.3	101.0	102.4	102.3	102.7

Blanks represent values below LOD: for radio-HPLC: LOD = 0.5 % (for metabolites depending o the peak shape), for volatiles: LOD = 0.1 %)

Table IIA 7.6-7: Transformation of BYI08330 in Rhine River water, mean values of radiolabel #2 expressed as % of AR (MEF-05/262)

Irradiated Compound	Sampling Times [days] 0	1	2	3	6	8	10
BYI08330	77.5	1.5					
RegB1		0.2			3.1	2.2	2.0
RegB2						2.7	3.4
RegB3						1.9	1.7
RegB4		0.3		1.9	2.3	2.6	2.9
BYI08330-methoxy-cyclo-hexylamino-caboxylic acid (PB1)		0.5	1.5	2.7	6.1	9.9	11.3
RegB6					3.5	2.7	3.0
RegB7				1.7		2.4	2.8
PB2		1.9	2.8	2.1	4.3	5.2	5.5
RegB9					0.5	1.0	1.9
BYI08330-methoxycyclo-hexanone (PB3)		3.9	6.0	8.1	13.0	17.5	16.0
RegB11				1.1	2.1	2.3	1.6
RegB12							1.0
RegB13		0.6		1.6	3.3	5.1	4.6
RegB14							0.5
RegB15		1.2	1.1	1.6	2.2	3.1	1.2
RegB16						0.6	0.4
RegB17		1.9	1.0	2.5	2.8	2.4	2.5
RegB18		1.6	2.2	1.8	2.6	1.7	0.9
RegB19			1.0	0.8		1.1	0.7
RegB20		0.8	1.4	1.9	3.3	3.1	3.0
RegB21		0.6	2.0	0.7	1.2	1.8	1.4
RegB22		0.4		0.8	1.8	1.7	1.3
BYI08330-enol (PB4)	19.3	31.9	72.2	58.3	32.1	17.0	16.8
RegB24		0.5	0.9	0.9	1.1	0.8	1.0
RegB25		1.9	2.5	2.8	1.7	2.8	3.0
RegB26		2.0	2.8	2.9	2.3	2.1	
PB5		1.1	2.9	4.1	5.0	4.1	5.2
PB6		2.0	2.9	4.0	5.2	4.6	5.6
Total RA in test solution	96.8	103.2	103.2	102.3	101.7	102.2	102.2
$^{14}CO_2$	n.m.	0.0	0.0	0.0	0.1	0.2	0.3
Volatile organics	n.m.	0.0	0.0	0.1	0.1	0.1	0.1
Total recovery of RA	96.8	103.2	103.2	102.4	101.9	102.5	102.5

Blanks represent values below LOD: for radio-HPLC: LOD = 0.5 % (for metabolites depending o the peak shape), for volatiles: LOD = 0.1 %)

Continued:

Dark Compound	Sampling Times [days] 0	1	2	3	6	8	10
BYI08330	84.0	57.5	35.6	20.9	3.8	1.5	0.0
BYI08330-enol (PA4)	10.9	40.9	64.7	78.9	97.4	100.8	102.0
Total RA in test solution	94.8	98.4	100.3	99.8	101.2	103.0	102.0
$^{14}CO_2$	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Volatile organics	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Total recovery of RA	94.8	98.4	100.3	99.8	101.2	103.0	102.0

Blanks represent values below LOD: for radio-HPLC: LOD = 0.5 % (for metabolites depending o the peak shape); for volatiles: LOD = 0.1 %)

Table IIA 7.6-8: Synopsis of transformation of BYI08330 in Rhein River water (MEF-05/262)

Soil	Evaluation of Means of [azaspirodecenyl-3-^{14}C] and [azaspirodecenyl-5-^{14}C]BYI08330	
Type	Irradiated	Dark
k (1/d) Experimental 1st order DT_{50} [d] R^2	3.68 0.19 1.000	0.45 1.54 0.996
Environmental DT_{50} [d] in June at [redacted], AZ (USA)	0.6	N/A
Environmental DT_{50} [d] in June at [redacted] (Greece)	1.0	N/A
Major transformation products *)	BYI08330-enol BYI08330-methoxy-cyclohexylamino carboxylic acid BYI08330-methoxy-cyclohexanone	BYI08330-enol
Minor transformation products	A multitude of not identified minor photoproducts	N/A

*): Criteria for term "major": >10% of AR at any DAT

III CONCLUSIONS

Based on the experimental DT50 of 0.2 days for BYI08330 and related predicted environmental DT50 (e.g. of 0.6 solar summer days at [redacted], AZ, USA or 1.0 summer days at [redacted], Greece) it is concluded that photo-transformation of BYI08330 in aqueous systems is a significant route for the elimination of this compound in natural water. This test performed in a sterilized natural water (as it is quite common to be neutral or slightly alkaline) clearly showed that competition of hydrolysis and indirect photo-reactions does not allow the light-induced re-arrangement reactions of parent compound observed in highly purified buffer of pH 5. Together with the well-known fast biodegradation this was the justification to consider the products formed in the prevailing study for the overall pathway of spirotetramat degradation in water (see Figure IIA 7.8.3-2), but not the re-arrangement photo-products (see Figure IIA 7.6-1) found in the highly artificial study performed in sterile pure buffer.

Quantum Yield and Assessment of the Environmental Half-life of the Direct Photodegradation in Water

Report:	**KIIA 7.6/03, [redacted], 2004 (MEF-04/080)**
Title:	**BYI08330: Determination of the Quantum Yield and Assessment of the Environmental Half-life of the Direct Photodegradation in Water**
Report No &	**MEF-04/080**
Document No	**M-092941-01-2**
Guidelines:	European Chemical Industry Ecology and Toxicology Centre (ECETOC) Technical Report No. 3 (1981) and Technical Report No. 12 (1984) Federal Agency of Environment (UBA) of Germany: Test Guideline Phototransformation of Chemicals in Water, Part A. (December 1992) US EPA Guideline No. 161-2 SUPP
GLP	Fully GLP compliant - laboratory certified by German “Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen“.
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during January 2004. Study completion date: 2004-09-28

EXECUTIVE SUMMARY

The quantum yield for direct photodegradation of BYI08330 in aqueous solution was determined in a merry-go-round apparatus and a polychromatic mercury arc lamp. Light with a wavelength of less than 290 nm was filtered off. BYI08330 was dissolved in pure water at a concentration of 5.0 mg/L corresponding to 1.34 10^{-5} mol/L. The content of ACN was less than 0.4% (v/v). 3-mL quartz glass cells of an optical pathway of 1 cm containing the solution of the test substance were irradiated at 25°C for various time intervals up the 240 min. The remaining concentration of the test substance was determined by quantitative HPLC-UV. The intensity of the incident light was determined by uranyl oxalate actinometer method. In addition, absorption data (molar extinctions coefficients) for the interesting wavelength range of 290 to 490 nm were taken from an UV/VIS absorption spectrum.

A degradation of BYI08330 of approx. 56% was measured during the maximum irradiation period of 240 minutes in water. Using the UV absorption data and the degradation kinetics of both the experiments a mean quantum yield of $\Phi = 0.00571$ was calculated.

Based on this intrinsic value of the test substance environmental half lives for direct photolysis in surface water could be calculated employing modeling according to Zepp and Cline (using the program GCSOLAR) or to Frank and Kloepffer. Zepp and Cline assumed a cloudless sky for spring, summer, fall and winter at 30, 40, 50 and 60° northern latitude. Frank and Kloepffer assumed a low, medium and high cloudiness for the twelve months of the year and 50°N. The estimates were well comparable when using the identical marginal conditions. "Environmental direct phototransformation half-lives" of BYI08330 of 0,5 days to about one week during the period of main use (late spring to summer) can be assessed. Thus, direct phototransformation in water does contribute to elimination of BYI08330 in the environment. This assessment does not consider any indirect mechanisms, which may enhance the photodegradation in natural water.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat, code = BYI08330: (ID: AZ10811, Batch M26802). Identity of test item

in the application solutions was checked. Chemical purity was 99.2%.

2. Test System: This aqueous photolysis study was conducted using highly pure water (taken from a Milli-Q-unit, Millipore Co.): conductivity = 18.2 mΩ cm; TOC = 13 ppb, hardness = 0 °dH. UV-VIS spectra were measured in aqueous test solution as well as in 0.01 mole/L aqueous buffer solutions: acetate pH 4, phosphate pH 7 and borate pH 9 were used in the study. Two photodegradation experiments were performed in pure water containing the test item and less than 0.4 % of acetonitrile.

B. STUDY DESIGN / TEST METHOD

The quantum yield for direct phototransformation of BYI08330 in aqueous solution was determined in a merry-go-round apparatus (Type 13/150 Mangels Co.) that was equipped with a mercury arc lamp (Type TQ 150 Original Hanau Co.) in a Duran® 50 filter and cooling finger. The filter absorbed light with a wavelength below 290 nm and let pass the polychromatic light above this cut-off wavelength. The test substance was dissolved in pure water at test concentration of 5.0 mg/L corresponding to 0.0134 mmol/L. After the treatment less than 0.4 % of acetonitrile were contained in the test solution.

Each 3 mL of this test solution was filled into quartz glass cells with an optical pathway of 1 cm. 10 quartz glass cells were placed at different positions in the merry-go-round after an equilibration time of approx. 30 min. The experiment was conducted in duplicate. The cells were irradiated at 25 ± 1°C for different time intervals up to 240 min while the cells in the merry-go-round turned around the centered lamp. The concentration of the test substance in cells was determined by HPLC (RP18 column and a gradient of water plus 0.1 % phosphoric acid and acetonitrile, UV detector operated at 250 nm, direct injection of 100 µL sample solution). The calibration curve of the UV detector signal versus concentration showed an excellent linearity (correlation coefficient R^2 = 0.9996) between a concentration range of 0.50 mg/L (= LOQ) and 14.99 mg/L). Peak area was evaluated against external standard, t_R = 12.6 min (RSD: Area = 0.49%, t_R = 0.06%; tested at 5.00 mg/L).

The light intensity was determined actinometrically employing a light sensitive solution of 0.01 M uranyl nitrate and 0.05 M oxalic acid. In this solution, light between 295 and 490 nm is absorbed by the uranyl ions and transferred to the oxalate ions that are consequently degraded to carbon monoxide and carbon dioxide. The amount of degraded oxalate was determined by back-titration (titration prior to and after the irradiation) with 0.01 M sodium permanganate solution. Using this method the intensity of the incident light (number of photons per area and time unit) could be determined from the amount of degraded oxalate employing the quantum yield of the uranyl oxalate actinometer of Φ_{act} = 0.5 – 0.6 (mean Φ_{act} = 0.55; 295 – 450 nm) as published in the literature[2].
The quantum yield Φ for direct photodegradation of the test compound is defined by the following equation

$$\Phi = \frac{\text{Number of degraded molecules per unit of time and area}}{\text{Total number of absorbed photons during the same unit of time and area}} \leq 1$$

The number of degraded molecules was derived from the photodegradation experiment assuming pseudo-first order degradation kinetic with

$$c(t) = c_0 \times e^{-kt} \quad \text{or} \quad N(t) = N_0 \times e^{-kt}$$

[2] Brackett, F. P. Jr. and Forbes, G. S., Actinometry with uranyl oxalate at λλ 278, 253 and 208 mµ, including a comparison of periodically intermittent and continuous radiation, J. Amer. Chem. Soc. **55**, 4459 – 4466 (1933) and European Photochemical Association's Newsletter No. 29, March 1987, IUPAC Commission on Photochemistry, Project Chemical Actinometers

c(t) or N(t) are the concentration or the number of molecules after the time t. c_0 or N_0 are the initial concentration or initial number of molecules and k is the rate constant of the degradation. Usually, only the first 10 % of the degradation were considered for photochemical degradation taking into account the formation of potentially light absorbing photoproducts that may tamper the number of photons absorbed by the test substance. Therefore, 10-% degradation is achieved after the time $t_{10\%}$ (DT10):

$$t_{(10\%)} = DT10 = \ln 0.9 / -k \qquad \text{applying for } N(t) = 0.9 \times N_0$$

The number of absorbed photons can be derived from the UV/VIS absorption spectrum of the test substance. However, it has to be considered that the light energy E carried with a photons depends on the wavelength λ according to the equation $E = h \times c \times 1/\lambda$ with h being the Planck's quantum of action and c the speed of light. Therefore, the absorbed light energy I_{abs} had to be integrated over the wavelength range of absorption (290 to 490 nm) or for simplification summed up in 5-nm increments to yield $\Sigma I_{abs}.(\lambda)$ with the following wavelength-depended contributions

$$I_{abs}(\lambda) = Io(\lambda) \times (1 - 10^{-OD(\lambda)}) = Io(\lambda) \times (1 - 10^{-\varepsilon(\lambda) Co\ 1cm})$$

Io (λ) is the incident light energy at the wavelength λ and OD(λ) is the optical density at λ. The optical density is defined by the product of the molar extinction coefficient ε (taken from the UV/VIS absorption curve), the molar concentration of the test substance c_0 and the length of the optical pathway (1 cm) in the absorption experiment.

Using the basic equations describing the rate of photodegradation with the quantum yield Φ assuming to be independent of the wavelength λ the photodegradation rate constant k can be replaced by the remaining concentration (or number of molecules) after 10% degradation and the time interval needed for this degradation using the equation above that describes the pseudo first order degradation kinetic. In this case the following formula results for the quantum yield Φ

$$\Phi = \frac{N_{10\%}}{t_{10\%} * 60 * 0.95 * \sum I_{abs}(\lambda)}$$

The factor 0.95 accounts for a 5 % loss of light energy due to reflections as noted by the manufacturer of the used lamp. The factor 60 accounts for a transformation of the used units of degradation time, i.e. minutes into usual unit used in photochemistry, i.e. seconds.

Applying the known quantum yield Φ in a similar formula with a 50-% degradation of the initial amount $N_{50\%}$ (or $c_{50\%}$) the environmental half life $t_{50\%}$ (= DT50) for photolysis in surface waters can be calculated for a known sunlight intensity at a distinct geographical latitude and temporal season. However some additional aspects, e.g. angle of impinging sunlight, reflection losses and matrix-specific marginal conditions have to be taken into account as described by Zepp and Cline (1977)[3] as well as Frank and Kloepffer (1985)[4].

[3] Zepp, R. G and Cline, D. M. (1977), Environm. Sci. Technol. **11**, 359 ff (1997) and program GCSOLAR
[4] Frank, R. and Kloepffer, W. (1985), UBA Research Report N. 10602046 (1985)

II. RESULTS AND DISCUSSION

A. DATA

From the actinometer measurements a photon flux of 6.94 x 10^{16} and 6.66 x10^{16} photons s^{-1} 3-mL^{-1} was derived for the wavelength range of 295 to 490 nm in two parallel experiments. Irradiation of 3 mL test solution of BYI08330 at this light intensity resulted in photodegradation as shown in Table IIA 7.6-9.

Table IIA 7.6-9: Photodegradation of BYI08330 in aqueous solution (MEF-04/080)

Duration of Irradiation [min]	Experiment #1 Concentration [mg/L]	Experiment #2 Concentration [mg/L]
0	5.00	5.00
24	4.43	4.40
48	3.88	3.20
72	3.81	3.43
96	4.42	3.48
120	3.19	2.88
144	3.04	3.09
168	2.77	2.84
192	2.64	2.72
216	2.21	2.34
240	2.21	2.21

Regression analysis of the degradation data resulted in a time interval needed for 10-% degradation of $t_{10\%}$ (DT10) time of 30.74 and 36.63 min in the two parallel experiments with a correlation coefficient of -0.962 and -0.937. The corresponding DT50 amounted to 202.3 and 241.0 min. By using the DT10 values and the sum of absorbed photons (calculated from incident light and the molar extinction coefficients) the quantum yield for direct photodegradation of BYI08330 in aqueous solution could be calculated as presented in Table IIA 7.6-10.

Table IIA 7.6-10: Quantum yield (Φ) for direct photolysis of BYI08330 in aqueous solution (MEF-04/080)

Experiment #1	Experiment #2	Mean
6.0886 x 10^{-3}	5.3207 x 10^{-3}	5.71 x 10^{-3}

Using the mean quantum yield for direct photolysis environmental half lives of BYI08330 were calculated using two methods, Zepp and Cline modeling and Frank and Kloepffer modeling. The Zepp and Cline model does not consider any clouds at the sky. In contrast to Zepp and Cline, the Frank and Kloepffer model takes into consideration the condition of cloudiness in Central Europe. Nevertheless, both models resulted in a similar half life for the spring and summer season at 50°N and minimum DT50 in the Frank and Kloepffer model (means low cloudiness). The results of both models are presented in Table IIA 7.6-11 and Table IIA 7.6-12.

Table IIA 7.6-11: Environmental half lives (given in days) for direct photolysis of BYI08330 according to Zepp and Cline modeling (MEF-04/080)

Season/Latitude	30°N	40°N	50°N	60°N
Spring	0.53	0.56	0.62	0.73
Summer	0.48	0.48	0.49	0.52
Fall	0.73	0.92	1.32	2.41
Winter	0.96	1.44	2.80	8.71

Marginal conditions: pure surface water at 0-5 cm depth, 10th degree eastern longitude, clear sky, typical ozone concentrations in the atmosphere, half-lives integrated over the entire day. The column of the 50th degree of northern latitude is more or less relevant to the conditions of Central Europe.

Table IIA 7.6-12: Environmental half lives (given in days) for direct photolysis of BYI08330 according to Kloepffer modeling (MEF-04/080)

Month	Photolysis Constant [1/sec]	Minimum (d)	Mean (d)	Maximum (d)
January	0.121×10^{-5}	3.2	6.6	30
February	0.245×10^{-5}	1.6	3.3	14
March	0.469×10^{-5}	0.90	1.7	7.1
April	0.775×10^{-5}	0.58	1.0	4.1
May	0.980×10^{-5}	0.51	0.82	3.3
June	0.109×10^{-4}	0.49	0.73	2.9
July	0.977×10^{-5}	0.55	0.82	2.7
August	0.962×10^{-5}	0.56	0.83	2.8
September	0.563×10^{-5}	0.84	1.4	5.3
October	0.316×10^{-5}	1.3	2.5	12
November	0.144×10^{-5}	2.4	5.6	28
December	0.796×10^{-6}	4.7	10	52

Marginal conditions: pure stagnant surface water at 0-5 cm depth, geographic and climatic conditions of Germany (50th degree northern latitude), no contribution of another mono- or bimolecular elimination process.

III CONCLUSIONS

A degradation of BYI08330 of approx. 56% was measured by HPLC-UV during the maximum irradiation period of 240 minutes in water. This indicates that BYI08330 is not stable against direct phototransformation 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 from the duplicates.

The estimates based on two different arithmetic models (GC-SOLAR and Frank & Kloepffer) by means of the resulting quantum yield and the light absorption in a range of wavelengths relevant for the environment were well comparable when considering identical marginal conditions. "Environmental direct phototransformation half-lives" of BYI08330 of 0.5 days to about one week during the period of main use (late spring to summer) can be assessed. Thus, direct phototransformation in water does contribute to elimination of BYI08330 in the environment. This assessment does not consider any indirect mechanisms, which may enhance the photodegradation in natural water.

Metabolite BYI08330-enol

Report:	**KIIA 7.6/04, [redacted], 2004 (MEF-04/438)**
Title:	**BYI08330-enol: Determination of the Quantum Yield and Assessment of the Environmental Half-life of the Direct Photodegradation in Water**
Report No & Document No	**MEF-04/080 M-243787-01-2**
Guidelines:	European Chemical Industry Ecology and Toxicology Centre (ECETOC) Technical Report No. 3 (1981) and Technical Report No. 12 (1984) Federal Agency of Environment (UBA) of Germany: Test Guideline Phototransformation of Chemicals in Water, Part A. (December 1992) US EPA Guideline No. 161-2 SUPP
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer CropScience AG, Metabolism and Environmental Fate, D-[redacted], GER, conducted the study during the period from Sep. to Oct. 2004. Study completion date: 2005-01-10

EXECUTIVE SUMMARY

The quantum yield for direct photodegradation of BYI08330-enol in aqueous solution was determined in a merry-go-round apparatus and a polychromatic mercury arc lamp. Light with a wavelength of less than 290 nm was filtered off. BYI08330 was dissolved in pure water at a concentration of 5.03 mg/L corresponding to 1.67 10^{-5} mol/L. The content of ACN was less 0.44% (v/v). 3-mL quartz glass cells of an optical pathway of 1 cm containing the solution of the test substance were irradiated at 25°C for various time intervals up the 500 min. The remaining concentration of the test substance was determined by quantitative HPLC-UV. The intensity of the incident light was determined by uranyl oxalate actinometer method. In addition, absorption data (molar extinctions coefficients) for the interesting wavelength range of 290 to 490 nm were taken from an UV/VIS absorption spectrum.

A degradation of BYI08330-enol of approx. 20% was measured during the maximum irradiation period of 500 minutes in water. Using the UV absorption data and the degradation kinetics of both the experiments a mean quantum yield of $\Phi = 2.522 \times 10^{-4}$ was calculated.
Based on this intrinsic value of the test substance environmental half lives for direct photolysis in surface water could be calculated employing modeling according to Zepp and Cline (using the program GCSOLAR) or to Frank and Kloepffer. Zepp and Cline assumed a cloudless sky for spring, summer, fall and winter at 30, 40, 50 and 60° northern latitude. Frank and Kloepffer assumed a low, medium and high cloudiness for the twelve months of the year and 50°N. The estimates were well comparable when using the identical marginal conditions. "Environmental direct phototransformation half-lives" of BYI08330-enol of about 9 to 12 days during the period of main use of BYI08330 (late spring to summer) can be assessed. Thus, direct phototransformation in water does contribute to elimination of BYI08330-enol in the environment. This assessment does not consider any indirect mechanisms, which may enhance the photodegradation in natural water.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BYI08330-enol: (ID: AZ10531, Batch NLL6383-16-18). Identity of test item in the application solutions was checked. Chemical purity was 99.4%.

2. Test System: This aqueous photolysis study was conducted using highly pure water (taken from a

Milli-Q-unit, Millipore Co.): conductivity = 18.2 mΩ cm; TOC = 9 ppb, hardness = 0 °dH. UV-VIS spectra were measured in aqueous test solution as well as in 0.01 mole/L aqueous buffer solutions: acetate pH 4, phosphate pH 7 and borate pH 9 were used in the study.

B. STUDY DESIGN / TEST METHOD

The quantum yield for direct phototransformation of BYI08330-enol in aqueous solution was determined in a merry-go-round apparatus (Type 13/150 Mangels Co.) that was equipped with a mercury arc lamp (Type TQ 150 Original Hanau Co.) in a Duran® 50 filter and cooling finger. The filter absorbed light with a wavelength below 290 nm and let pass the polychromatic light above this cut-off wavelength. The test substance was dissolved in pure water at test concentration of 5.03 mg/L corresponding to 0.0167 mmol/L. After the treatment only 0.44 % of acetonitrile were contained in the test solution.

Each 3 mL of this test solution was filled into quartz glass cells with an optical pathway of 1 cm. Ten quartz glass cells were placed at different positions in the merry-go-round after an equilibration time of approx. 30 min. The experiment was conducted in duplicate. The cells were irradiated at 25 ± 1°C for different time intervals up to 500 min while the cells in the merry-go-round turned around the centered lamp. The concentration of the test substance in cells was determined by HPLC (RP18 column and a gradient of water plus 0.1 % phosphoric acid and acetonitrile, UV detector operated at 250 nm, direct injection of 100 µL sample solution). The calibration curve of the UV detector signal versus concentration showed an excellent linearity (correlation coefficient R^2 = 0.99996) between a concentration range of 0.49 mg/L (= LOQ) and 15.16 mg/L. Peak area was evaluated against external standard, t_R = 16.9 min (RSD: Area = 0.47%, t_R = 0.03%; tested at 5.01 mg/L).

For all further information see study KIIA 7.6/03 before.

II. RESULTS AND DISCUSSION

A. DATA

From the actinometer measurements a photon flux of 7.94 x 10^{16} and 6.57 x10^{16} photons s^{-1} 3-mL^{-1} was derived for the wavelength range of 295 to 490 nm in two parallel experiments. Irradiation of 3 mL test solution of BYI08330-enol at this light intensity resulted in photodegradation as shown in Table IIA 7.6-13.

Table IIA 7.6-13: Photodegradation of BYI08330-enol in aqueous solution (MEF-04/438)

Duration of Irradiation [min]	Experiment #1 Concentration [mg/L]	Experiment #2 Concentration [mg/L]
0	5.03	5.03
50	4.91	4.85
100	4.66	4.62
150	4.68	4.62
200	4.57	4.46
250	4.64	4.34
300	4.36	4.33
350	4.51	4.33
400	4.69	4.24
450	4.29	4.15
500	4.16	3.85

Regression analysis of the degradation data resulted in a time interval needed for 10-% degradation of $t_{10\%}$ (DT10) time of 364 and 244 min in the two parallel experiments with a correlation coefficient of

-0.859 and -0.964. The corresponding DT50 amounted to 2393 and 1606 min. By Using the DT10 values and the sum of absorbed photons (calculated from incident light and the molar extinction coefficients) the quantum yield for direct photodegradation of BYI08330-enol in aqueous solution could be calculated as presented in Table IIA 7.6-14.

Table IIA 7.6-14: Quantum yield (Φ) for direct photolysis of BYI08330 in aqueous solution (MEF-04/080)

Experiment #1	Experiment #2	Mean
1.8010×10^{-4}	3.2435×10^{-4}	2.522×10^{-4}

Using the mean quantum yield for direct photolysis environmental half lives of BYI08330-enol were calculated using two methods, Zepp and Cline modeling and Frank and Kloepffer modeling. The Zepp and Cline model does not consider any clouds at the sky. In contrast to Zepp and Cline, the Frank and Kloepffer model takes into consideration the condition of cloudiness in Central Europe. Nevertheless, both models resulted in a similar half life for the spring and summer season at 50°N and minimum DT50 in the Frank and Kloepffer model (means low cloudiness). The results of both models are presented in Table IIA 7.6-15 and Table IIA 7.6-16.

Table IIA 7.6-15: Environmental half lives (given in days) for direct photolysis of BYI08330 according to Zepp and Cline modeling (MEF-04/080)

Season/Latitude	30°N	40°N	50°N	60°N
Spring	9.6	10	12	14
Summer	8.6	8.7	9.0	9.6
Fall	13	17	25	47
Winter	18	28	55	173

Marginal conditions: pure surface water at 0-5 cm depth, 10th degree eastern longitude, clear sky, typical ozone concentrations in the atmosphere, half-lives integrated over the entire day. The column of the 50th degree of northern latitude is more or less relevant to the conditions of Central Europe.

III CONCLUSIONS

A degradation of BYI08330-enol of approx. 20% was measured by HPLC-UV during the maximum irradiation period of 500 minutes in water. This indicates that BYI08330-enol is degradable by direct phototransformation in aqueous solution. Using the UV absorption data and the degradation kinetics of both the experiments a mean quantum yield of $\Phi = 2.522 \times 10^{-4}$ was calculated from the duplicates.

The estimates based on two different arithmetic models (GC-SOLAR and Frank & Kloepffer) by means of the resulting quantum yield and the light absorption in a range of wavelengths relevant for the environment were well comparable when considering identical marginal conditions. "Environmental direct phototransformation half-lives" of BYI08330-enol of about 9 to 12 days during the period of main use of BYI08330 (late spring to summer) can be assessed. Thus, direct phototransformation in water does contribute to elimination of BYI08330-enol in the environment. This assessment does not consider any indirect mechanisms, which may enhance the photodegradation in natural water.

Table IIA 7.6-16: Environmental half lives (given in days) for direct photolysis of BYI08330 according to Kloepffer modeling (MEF-04/080)

Month	Photolysis Constant [1/sec]	Minimum (d)	Mean (d)	Maximum (d)

January	0.589 x 10^{-7}	65	140	620
February	0.123 x 10^{-6}	31	65	280
March	0.242 x 10^{-6}	17	33	140
April	0.410 x 10^{-6}	11	20	78
May	0.525 x 10^{-6}	9.5	15	61
June	0.590 x 10^{-6}	9.1	14	54
July	0.526 x 10^{-6}	10	15	51
August	0.513 x 10^{-6}	10	16	52
September	0.296 x 10^{-6}	16	27	100
October	0.161 x 10^{-6}	26	50	230
November	0.709 x 10^{-7}	49	110	570
December	0.371 x 10^{-7}	98	220	1100

Marginal conditions: pure stagnant surface water at 0-5 cm depth, geographic and climatic conditions of Germany (50th degree northern latitude), no contribution of another mono- or bimolecular elimination process.

IIA 7.7 Ready biodegrability of the active substance

Report:	**KIIA 7.6/04, [redacted], 2005 (2005/0077/01)**
Title:	**BYI08330: Biodegradation**
Report No & Document No	**2005/0077/01 M-263287-01-1**
Guidelines:	Council Directive 92/69/EEC Method C.4-D "Manometric Respirometry Test". This test method is in all essential parts identical with OECD Guideline 301 F.
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and Dates	Bayer Industry Services GmbH & Co. OHG, BIS-SUA-Analytics, D-[redacted], GER, conducted the study during the period from Sep. to Nov. 2005. Study completion date: 2005-11-16

EXECUTIVE SUMMARY

This study was designed to assess the ready biodegradability of BYI08330. A solution of BYI08330 in a mineral medium was inoculated and incubated for 28 d under aerobic conditions. During this period the ready biodegradability is determined according to OECD Guideline 301 F. By this kind of test BYI08330 showed 1% degradation after 28 days, only, while the reference compound showed 89% degradation after 14 days. Therefore, BYI 08330 is considered to be "Not Readily Biodegradable".

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BYI08330, Article number: 0005892430, MIX-Batch 08045/0014. Identity of test item in the application solutions was checked. Chemical purity was 97.4%.

2. Test System: A mixed population of aquatic micro-organisms (activated sludge) originating from an aeration tank of a waste water plant treating predominantly domestic sewage (Wupper area water authority, STP Odenthal) was used without any pre-treatment. Date of collection was 2005-09-14. The concentration of the inoculums was 30 mg/L ss.

B. STUDY DESIGN / TEST METHOD

25 mg test item were weighed out on aluminum foil. This substance inclusive the aluminum foil was added to the test vessels to give a test concentration of 100 mg test item/L. Thus, the measured volume of inoculated mineral medium, containing the known concentration of the test chemical to give at least 50-100 mg ThOD/liter as the nominal sole source of organic carbon, is stirred in a closed flask at a constant temperature (22 ± 2°C) for up to 28 days. The consumption of oxygen is determined by measuring the quantity of oxygen (produced electrolytically) required to maintain constant gas volume in the respirometer flask. Evolved carbon dioxide is absorbed in a solution of potassium hydroxide. The amount of oxygen taken up by the test chemical (corrected for uptake by blank inoculums, run in parallel) is expressed as a percentage of theoretical oxygen demand (ThOD) or chemical oxygen demand (COD).

As reference compound sodium benzoate (Fluka-BioChemika), Batch-no. 450273/1 35103269 of 99% purity was used. The test volume was 250 mL, the test apparatus Voith Sapromat using 1 magnetic stirrer per test vessel for mixing. Chemical analysis was performed by Continuous Flow Analyzer SKALAR SAN Plus System (method: NO_3^{-N}/NO_2^{-N}): Determination of nitrite nitrogen and nitrate

nitrogen and the sum of both by flow analysis (CFA and FIA) and spectrometric detection according to standard EN ISO 13395.

II. RESULTS AND DISCUSSION

A. DATA

All the resulting data were shown in tables on page 13 to 16 of report 2005/0077/01. Within 28 days, a degradation of 1% was determined for BYI08330. All validity criteria of the test method were met. The reference compound had reached the level for ready biodegradability by 14 days. No toxicity of the test item has been observed in the toxicity control. The difference of extremes of replicate values of the removal of test chemical at the end of the test was less than 20%. The oxygen uptake of the inoculums blank was < 60 mg/1. The pH was between 6.0 and 8.5 in the test vessels at the end of the test (if degradation is less than 60%). Oxygen uptake by nitrification has been determined. The oxygen consumed by nitrification was 1 mg/1. This oxygen consumption by nitrification has been subtracted from the respective 28 days measurements of the test item.

III CONCLUSIONS

BYI 08330 is considered to be "Not Readily Biodegradable".

IIA 7.8 Degradation in aquatic systems

Degradation of BYI08330 in aquatic systems was investigated by studies on aerobic and anaerobic biodegradation under dark laboratory conditions using natural water-sediment systems (KIIA 7.8.3/01 and KIIA 7.8.3/02). Results also relevant for natural surface water, i.e. those of the abiotic aqueous photolysis study in natural water, were shown already in section IIA 7.6 (see KIIA7.6/02) and considered for the overall pathway of BYI08330 in water shown Figure IIA 7.8.3-2.

IIA 7.8.1 Aerobic biodegradation in aquatic systems

This point is covered by section IIA 7.8.3.

IIA 7.8.2 Anaerobic biodegradation in aquatic systems

This point is covered by section IIA 7.8.3.

IIA 7.8.3 Water/sediment studies

Report:	**KIIA 7.8.3/01, [redacted], 2006 (MEF-04/511)**
Title:	**BYI08330: Aerobic Aquatic Metabolism**
Report No & Document No	**MEF-04/511 M-269307-01-2**
Guidelines:	EU Commission Directive, 95/36/EC, amending Council Directive 91/414/EEC, Annexes I+II, Fate and Behavior in the Environment; OECD Guideline for Testing of Chemicals, Guideline 308, Aerobic and Anaerobic Transformation in Aquatic Sediment Systems; OPP Guideline No. 162-4: Aerobic Aquatic
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and dates	Bayer CropScience AG, RD, Metabolism and Environmental Fate, D-[redacted], Germany, conducted the study during the period Oct. 2003 to March 2006. Study completion date: 2006-03-29

EXECUTIVE SUMMARY

Distribution, degradation and metabolism of BYI08330 in water/sediment systems was investigated in two systems of natural water and sediment [[redacted] (HW) and [redacted] (AW)] using [azaspirodecenyl-3-^{14}C] and [azaspirodecenyl-5-^{14}C]BYI08330 (labels #1 and #2) as test substances. Samples were incubated in the dark under aerobic conditions at 20 °C for a period of 120 days. The test system consisted of glass vessels attached with a trap for collection of $^{14}CO_2$ and volatile organic compounds. Entire vessels filled with sediment and supernatant water (ratio of 3:1) were applied with [^{14}C]BYI08330 at a rate of 15.7 and 15.9 µg test item per vessel (label #1 and label #2, resp.), equivalent to 105 and 107% of an overspray of the maximum field application rate of 288 g/ha.
The vessels were processed and investigated at 0 (approx. 15 min), 0.2, 1, 3, 7, 14, 30, 60, 91 and 120 days after treatment (DAT). The water was decanted and centrifuged. The sediment was extracted three times with a mixture of acetonitrile/water/formiate (50/50/0.5, v/v/v, combined to "organic extract"), twice with acetonitrile/1 M HCl (1/1, v/v) and once with pure acetonitrile (combined to "acid extract", all extractions at room temperature). The BYI08330 residues were analyzed by High Performance Liquid chromatography (HPLC) on reversed phase. For identification of the transformation products co-chromatography with reference standards was performed. The two major and three minor metabolites were identified in addition by LC-MS and LC-MS/MS spectrometry.

A complete material balance was found at all sampling intervals, demonstrating that no significant portions of radioactivity dissipated from the vessels or was lost during processing. The content of total RA in the supernatant water decreased with the incubation interval in both test systems. This decrease was greater in the loamy system than in sandy system.

In both water/sediment systems the test item BYI08330 was quickly eliminated from the water body mainly via degradation. Within 7 days after application the parent compound was dissipated to non detectable levels in both systems. In the course of the study several HPLC peaks were detected and quantified besides unaltered BYI08330. In addition to the two major metabolites BYI08330-enol and BYI08330-ketohydroxy, several minor metabolites were detected three of which were analyzed in more detail. The most prominent metabolite in the water phase of both systems was BYI08330-enol reaching maximum mean levels of 76.4 and 78.8 % of AR at DAT-7 in HW and AW, respectively, then declining in the water phase to non detectable levels in HW and to levels of 8.1 % AR in AW at the end of the study. The main degradation product of BYI08330-enol, the metabolite BYI08330-ketohydroxy,

reached maximum mean level of 12.7 % of AR in the water phase of HW at the end of the study. In the water phase of AW the metabolite BYI08330-MA-amide amounted to levels above 5 % AR at one point in time (DAT-60), declining to a mean of 1.0 % AR at end of study. Two further minor metabolites occurring only in the water phase of AW at the late sampling intervals were identified as BYI08330-oxo-enol isomer and as BYI08330-di-hydroxy.

The extractable RA in the sediment increased with incubation interval till a maximum of 42.5 % AR in HW and of about 28.8 % AR in AW was reached at DAT-60. Then, the values decreased to a mean of 39.4 and 27.6 % AR for HW and AW until the end of the study. In both water/sediment systems the portion of BYI08330 translocated to the sediment was only minor, and it was quickly degraded being non-detectable at DAT-7 in both systems, already. As in the water phase, the most prominent metabolite in the sediment was BYI08330-enol reaching maximum levels of 36.6 % AR at DAT-60 of HW and declining to 10.5 % AR until the end of the study. The levels of BYI08330-ketohydroxy in the sediment were low until DAT-91 in HW and until DAT 30 in AW and increased to the end of the study to 27.8 % AR in HW and to 21.4 % AR in AW. BYI08330-MA-amide and BYI08330-oxo-enol isomer reached minor levels only (single values below 3 % AR) in the sediments of both systems whereas the AW-specific metabolite BYI08330-di-hydroxy was detected transiently in the sediment at DAT-60 and DAT-90 reaching maximum levels of 5.5 % AR at DAT-91 (single value).

The amount of non-extractable radioactivity (NER or bound residues) was low in both water/sediment systems until DAT-14. At the later sampling intervals the NER of HW increased up to levels of about 36 to 40 % AR in label #2 and to 33 % AR only in label #1. In AW the values reached a plateau of about 30-32 % AR beginning with DAT-60 in both labels. Thus, a DAT-120 NER chemical characterization of the non-extractable residues performed by organic matter fractionation after disintegration under excessive alkaline conditions indicated the major portion (between 12.9 and 17.5 % AR) attributable to the soluble humic acid fraction.

Considering the entire water/sediment system BYI08330 declined quickly and was not any longer detectable after DAT-3 and DAT-7 in HW and AW, respectively. When taken the water phase and sediment together, in both entire systems BYI08330-enol and BYI08330-ketohydroxy clearly exceeded 10 % AR during the study and, thus, were regarded as major metabolites. Minor metabolites BYI08330-MA-amide, BYI08330-di-hydroxy and BYI08330-oxo-enol reached maximum levels of around 5% AR. All identified degradation products occurring in the two systems were common to both labels. A significant amount of $^{14}CO_2$ was formed in both water/sediment systems (max. 11.0 % AR in HW and 24.0 % AR in AW after 120 days). This indicated a high rate of mineralization of the test item. No other volatiles were formed at detectable levels.

In conclusion, the data gathered in the current laboratory investigation demonstrated that BYI08330 is quickly degraded in natural water/sediment systems. DT50 values of 1.00 and 1.02 days were calculated for the water phases, and 1.06 and 1.05 days for the entire systems, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat: Code = BYI08330
Identity and purity of test item in the application solutions were checked
Label #1: Label position = [azaspirodecenyl-3-^{14}C] (sample ID: BECH0955)
Specific activity = 3.67 MBq/mg (99.1 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

Label #2: Label position = [azaspirodecenyl-5-^{14}C] (sample ID: BECH0956)
Specific activity = 4.03 MBq/mg (108.8 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC and -TLC)
Chemical purity: >98% (HPLC, UV detection at 210 nm)

2. Test System: The study was carried out using the natural water/sediment systems [redacted] (HW, [redacted], Germany) and [redacted] (AW, [redacted], Germany). [redacted] is an artificially dammed pond in the course of the [redacted] forming [redacted]. Due to its inlet and outlet the pond (about 1000 m² in surface area) has strong water current. [redacted] is a reclaimed gravel pit, which is used for fishing only. The small lake is entirely enclosed by a fence. The chosen systems are well characterized, were used in many water/sediment studies, and meet the European Guidelines' requirements. Description, collection and storage of both water/sediment systems are given in Appendix 2 of report MEF-04/511. Characteristics of the sediments and the corresponding supernatant water are summarized in Table IIA 7.8.3-1 and Table IIA 7.8.3-2.

Table IIA 7.8.3-1: Physico-chemical characteristics of the sediments used (MEF-04/511)

Parameter	Results/Units	
	[redacted] (HW)	[redacted] (AW)
Geographic location	[redacted], Northrhine-Westfalia, Germany	[redacted], Northrhine-Westfalia, Germany
Latitude and longitude	[redacted]	[redacted]
Type of aquatic system	meso-/oligotrophic	oligotrophic
Taxonomic classification	loam	sand
Textural class [USDA]	loam	loamy sand
Sand (2000-50 µm): (%)	32.9	81.0
Silt (50-2 µm): (%)	49.8	11.3
Clay (< 2 µm): (%)	17.3	7.7
pH: Water / $CaCl_2$	6.1 / 5.6	7.3 / 6.8
Organic matter (%) / Organic carbon (%)	7.62 / 4.42	1.71 / 0.99
Microbial activity (mg CO_2/hr*kg DM)		
- Initial (at date of sampling)	36	10
- At study start (DAT-0)	43	10
- Final (at latest processing date, DAT-120)	25	8
Cation exchange capacity (meq Ba^{2+}/100 g sediment)	16.5	6.1
Total nitrogen (% N)	0.35	0.09
Total phosphorous (mg P/kg DM)	770	224
$CaCO_3$ (%)	0.3	< 0.1
Water content (%)	58.1	33.7
Redox potential (mV)	-159	-54

Table IIA 7.8.3-2: Physico-chemical characteristics of the water used (MEF-04/511)

Parameter	Results/Units	
	[redacted] (HW)	[redacted] (AW)
Temperature at sampling (°C)	5.8	9.7
pH at sampling	6.68	7.16
Hardness (Grad DH)	4.3	10.2
Electrical conductivity	n.d.	n.d.
Oxygen concentration (mg/L) - Initial (one day after sampling) - Final (at latest processing date)	 3.08 7.23	 6.60 7.77
Dissolved organic carbon, DOC (mg C/L)	4	< 2
Total organic carbon, TOC (mg C/L)	8	< 2
Total nitrogen (mg N/L)	5.2	5.6
Total phosphorous (mg P/L)	0.11	< 0.03
Redox potential Eh (mV) - Initial (at date of sampling) - Final (at latest processing date)	 455 378	 461 366

n.d.: not determined

Stones and plant debris were removed before the sediment was passed wet through a 2-mm sieve. The percentage of dry matter (DM) at 105 °C was determined ([redacted]: 58.1 % [redacted]: 33.7 %).

B. STUDY DESIGN

1. Experimental conditions: The tests were performed using individual static test systems held at aerobic conditions at 20 ±1 °C for a maximum period of 120 experimental days. A portion of sieved wet sediment equivalent to a filling height of 2 cm or 173 ml ([redacted]: 244.8 g [102.4 g DM]; [redacted]: 274.5 g [182.1 g DM]) was filled into each test vessel (see Figure 2 of the report MEF-04/511). Subsequently the corresponding supernatant (pond) water was added (519 mL corresponding to a height of 6.0 cm) resulting in a volume ratio of water to sediment of 3:1. Then the test systems were pre-incubated under the projected test conditions (i.e. at 20 °C +/- 2 °C in the dark) for one week in order to equilibrate and to allow the micro-flora to acclimatize. Without any further processing in each one vessel per sediment the biomass measurement based on the substrate-induced initial respiratory response was determined (i.e. at DAT-0 and DAT-120).

The amount of radiolabelled BYI08330 for the treatment of the test systems was based on the highest recommended single field use rate of the test item (288 g/ha, calculated to a water depth of 100 cm), implying a surface water contamination to be in the same order of magnitude. The actual application rate set as 100 % of applied radioactivity (100 % AR) was measured to be 57625 Bq/vessel (label #1) and 64188 Bq/vessel (label #2). This corresponded to 15.7 and 15.9 µg per vessel for label #1 and #2, respectively. These values were equivalent to 105 and 107% (for label #1 and label #2, respectively) of the intended value of the maximum field application rate of 14.9 µg per vessel. After treatment the content of organic solvent (acetonitrile) was approx. 0.015% in the water.

Trap attachments for $^{14}CO_2$ and volatile organics were used to close the test vessels from DAT-0 on.

2. Sampling: The vessels were processed and investigated at 0 (approx. 15 min), 0.2, 1, 3, 7, 14, 30, 60,

91 and 120 days after treatment (DAT). The water was decanted and centrifuged. The sediment was extracted three times with a mixture of acetonitrile/water/formiate (50/50/0.5, v/v/v, combined to "organic extract"), twice with acetonitrile/1 M HCl (1/1, v/v) and once with pure acetonitrile (combined to "acid extract", all extractions at room temperature). The trap attachments for $^{14}CO_2$ and volatile organics were stored at ambient temperature until processing for analysis.

3. Description of analytical procedures: The radioactivity of the test solutions was radio-assayed by LSC. Chromatographic analyses by the primary method (reversed phase HPLC equipped with a radioactivity detector) were performed within one day after sampling. Linear regression analysis was used to determine the radioactive detector response (Microsoft Excel® 97). Arithmetic means were used in case of all LS measurements and in case of determination of the degradation kinetics of BYI08330. or identification of the transformation products co-chromatography with reference standards was performed. Furthermore parent compound in the extract was verified by thin layer chromatography (TLC). The two major and three minor metabolites were identified in addition by LC-MS and LC-MS/MS spectrometry.

Analyzed samples were stored deep-frozen at approximately -15°C or below until further investigations. Amounts of the test items and the transformation products were calculated as percentage of the applied radioactivity. Values were presented as single values and as means if replicates (label #1 and #2) were possible. Quantification of test item was based on the radioactivity measured in the solutions. Calculations were performed using the computer software Microsoft Excel® 97. The portion of non-extractable radioactivity in sediment was determined by combustion of usually five 1-g aliquots of air-dried sediments being homogenized.

C. DETERMINATION OF DEGRADATION KINETICS

DT_{50} and DT_{90} values were determined for the degradation of BYI08330. The determination of the kinetic values followed the recommendations of FOCUS rules and was aimed to investigate potential exceedance of trigger values according to the FOCUS guidance document on degradation kinetics (EU registration. A detailed report on the calculation of kinetic values for modeling aspects was prepared separately (see KIIA 7.8.3/02 later).

DAT-0 values in Table IIA 7.8.3-3 and in Table IIA 7.8.3-4 actually corresponded to values determined approximately 15 min after DAT-0 but were kept as DAT-0 values for the modeling calculations. The first value below the LOD was set to ½ LOD (0.1 % AR).

Model input datasets for the entire water/sediment systems HW and AW were the mean abundances of residual BYI08330, taken from Table IIA 7.8.3-3 and in Table IIA 7.8.3-4. Model input datasets for the water phase were the single values of residual BYI08330 for the two labels A and B. All data points were weighed equally. For optimal goodness of fit, the initial value was also allowed to be estimated by the model. The kinetic evaluations and the statistical calculations for the quality checks were implemented in the numerical software package MatLab 7.0.4.365. The differential equations were integrated by a Runge-Kutta method, and the Levenberg-Marquardt algorithm was used for the non-linear parameter optimization. The following kinetic models were tested in order to determine the best-fit kinetic model:

Simple first order model (SFO):

$$M_P(t) = M_0 \exp^{(-kt)}$$

$M_P(t)$ = Total amount of chemical present at time t
M_0 = Total amount of chemical present at time t = 0

k = Rate constant [d^{-1}]

First order multi compartment model (FOMC):

$$M_p(t) = M_0\left(\frac{t}{b}+1\right)^{-a}$$

$M_P(t)$ = Total amount of chemical present at time t
M_0 = Total amount of chemical present at time t = 0
a = Shape parameter determined by CV of k values
b = Location parameter

Bi-exponential model (double first order in parallel, DFOP):

$$M_P(t) = M_1 \exp^{(-k_1 t)} + M_2 \exp^{(-k_2 t)}$$

$M_P(t)$ = Total amount of chemical present at time t
M_1 = Amount of chemical applied to compartment 1 at time t = 0
M_2 = Amount of chemical applied to compartment 2 at time t = 0
k_1 = Rate constant in compartment 1 [d^{-1}]
k_2 = Rate constant in compartment 2 [d^{-1}]

The best-fit kinetic model was selected on the basis of a visual assessment of the goodness of fit (diagrams of measured and calculated values vs. time, diagrams of residuals vs. time) and on the basis of the chi^2 scaled-error criterion (Chi^2Err value in the report). The dissipation times DT50 and DT90 (time until 50 or 90% of disappearance) were calculated by the software from the optimized kinetic parameters for the best-fit kinetic model.

II. RESULTS AND DISCUSSION

The anticipated test conditions were maintained by using an "open" test system (so-called bio-meter flasks permeable for air) incubated in a dark climatic chamber. The mean temperature maintained throughout the study was of 20.29 °C. The pH in the water phase of HW increased from about 6.8 (DAT-0) to about to 7.5 at study termination whereas in the sediment it stayed more or less unchanged at around 6.7 - 6.9 during the study. The pH in the water phase of AW increased slightly from about 7.9 (DAT-0) to about 8.2 at study termination. In the sediment the pH stayed more or less unchanged at around 7.0 - 7.5 during the study. From the redox potential and accompanying measurements of the oxygen in both systems it can be concluded that water and sediment stayed aerobic throughout the entire period of the study. The respiration rate in the test vessels indicated that the systems were biologically active during the entire period of the test. In HW as well as in AW system, a reduction of the microbial activity in the course of the experiment was observed. This is characteristic for a laboratory experiment. It is due to the gradual depletion of nutrients in the sediment and lacking supply of organic matter as a source of energy.

A. DATA

A compilation of results is shown in the following Table IIA 7.8.3-3 for the system [redacted] and in Table IIA 7.8.3-4 for the system [redacted].

Table IIA 7.8.3-3: Distribution of radioactivity after application of [^{14}C]BYI08330 to water/sediment and aerobic incubation at 20°C; if applicable mean of both radiolabels #1 and #2, in % of applied radioactivity (MEF-04/511)

			Sampling time (days after application)

Compound	Source		0	0.2	1	3	7	14	30	60	91	120
[redacted] (HW)												
BYI08330	Water	Mean	79.7	71.8	43.0	6.4						
		SD	±2.7	±1.6	±1.6	±0.1						
	Sediment	Mean	1.8	3.2	3.1	1.7						
		SD	±0.4	±0.1	±0.2	±0.2						
-Enol	Water	Mean	16.6	20.7	42.7	62.1	76.4	68.7	21.5	13.1	6.7	
		SD	±0.9	±0.7	±2.3	±0.8	±1.1	±0.5	±1.8	±6.7	±6.7	
	Sediment	Mean		1.6	6.8	12.8	21.1	28.5	35.2	36.6	22.3	10.5
		SD		±0.1	±0.2	±0.7	±0.7	±1.3	±1.1	±4.6	±6.8	±10.5
-Keto-hydroxy	Water	Mean			0.8	13.5		0.5	3.4	4.7	3.6	12.7
		SD			±0.1	±0.0		±0.5	±0.3	±1.4	±0.3	±4.7
	Sediment	Mean							3.2	5.0	13.2	27.8
		SD							±0.3	±2.7	±0.3	±15.0
-MA-amide	Water	Mean								1.0	1.7	
		SD								±1.0	±0.3	
	Sediment	Mean									0.5	
		SD									±0.5	
Unidentified	Water	#1	0.7	0.9	0.5	0.9	1.1	0.9	0.4	4.1	4.0	0.3
		#2	0.4	1.0	0.6	2.7	0.8	0.5	0.9	0.5	2.3	0.2
	Sediment	#1	0.5	0.8	1.7	0.6	0.4	0.1	1.3	0.7	4.1	1.0
		#2	0.2	0.9	1.5	0.4	0.5	0.1	1.6	1.1	0.8	1.2
RA in sediment	Extracted	#1	2.7	5.4	12.0	15.9	20.9	27.3	38.2	35.0	33.5	34.8
		#2	1.6	5.9	11.0	14.1	22.3	29.9	41.4	50.0	43.4	44.0
	NER	#1	0.1	0.2	0.8	0.8	0.7	1.2	30.0	29.4	32.0	32.9
		#2		0.2	0.5	0.6	1.1	0.8	31.7	36.7	40.7	36.3
$^{14}CO_2$	Entire system	#1	n.a.				0.1	0.2	2.1	3.7	8.0	11.0
		#2	n.a.				0.1	0.1	0.8	2.2	4.2	5.9
Org. volatiles	entire system	#1	n.a.									
		#2	n.a.									
Total recovery of radioactivity	Water	#1	98.8	92.5	86.4	83.5	78.6	69.2	26.9	29.2	22.7	17.8
		#2	94.8	94.4	87.7	84.0	76.0	70.7	24.3	12.9	7.6	8.2
		Mean	96.8	93.5	87.0	83.8	77.3	69.9	25.6	21.1	15.2	13.0
	Sediment	#1	2.8	5.6	12.8	16.7	21.6	28.5	68.2	64.4	65.5	67.7
		#2	1.6	6.2	11.6	14.7	23.4	30.7	73.1	86.7	84.1	80.3
		Mean	2.2	5.9	12.2	15.7	22.5	29.6	70.7	75.6	74.8	74.0
	Entire system	#1	101.6	98.2	99.2	100.2	100.3	97.8	97.3	97.3	96.2	96.4
		#2	96.4	100.6	99.2	98.7	99.5	101.5	98.1	101.8	95.9	94.4
		Mean	99.0	99.4	99.2	99.5	99.9	99.7	97.7	99.5	96.0	95.4
		SD	±2.5	±1.2	±0.0	±0.8	±0.4	±1.8	±0.4	±2.2	±0.1	±1.0

Blank = < LOQ
n.a. = not analyzed
NER = not extractable radioactivity

Table IIA 7.8.3-4: Distribution of radioactivity after application of [^{14}C]BYI08330 to water/sediment and aerobic incubation at 20°C; if applicable mean of both radiolabels #1 and #2, in % of applied radioactivity (MEF-04/511)

Compound	Source		Sampling time (days after application)									
			0	0.2	1	3	7	14	30	60	91	120

[redacted] (AW)												
BYI08330	Water	Mean SD	70.7 ±0.5	62.8 ±2.1	39.4 ±0.5	5.6 ±0.2						
	Sediment	Mean SD	1.6 ±0.2	2.5 ±0.3	2.0 ±0.0	0.6 ±0.0						
-Enol	Water	Mean SD	25.0 ±0.7	30.6 ±1.4	48.3 ±2.3	78.6 ±0.0	78.8 ±0.9	72.0 ±0.5	59.8 ±0.1	15.0 ±0.1	7.1 ±0.8	8.1 ±0.4
	Sediment	Mean SD		1.6 ±0.4	5.6 ±0.3	7.2 ±0.1	12.7 ±1.5	15.1 ±0.6	14.5 ±1.1	3.1 ±0.9	2.4 ±2.4	3.2 ±3.2
-Keto-hydroxy	Water	Mean SD			1.4 ±0.6	2.2 ±0.2	2.8 ±0.6	2.9 ±0.3	6.4 ±0.3	6.2 ±0.5	2.0 ±0.2	4.0 ±1.6
	Sediment	Mean SD			0.3 ±0.3	0.5 ±00	0.5 ±0.5	2.3 ±0.5	10.3 ±1.7	20.9 ±2.1	18.9 ±5.0	21.4 ±10.1
-MA-amide	Water	Mean SD						1.6 ±0.0	2.2 ±0.1	6.9 ±0.8	4.9 ±1.3	1.0 ±1.0
	Sediment	Mean SD								1.7 ±0.4	1.0 ±1.0	
-Oxo-enol isomer	Water	Mean SD									3.7 ±0.1	3.0 ±3.0
	Sediment	Mean SD										1.2 ±1.2
-Di-hydroxy	Water	Mean SD								2.1 ±2.1	2.1 ±2.1	
	Sediment	Mean SD								1.8 ±0.7	3.8 ±1.7	
Unidentified	Water	#1 #2	1.0 0.6	0.6 0.7	0.1 1.5	1.2 1.2	0.3 0.8	2.5 1.0	0.7 3.1	4.7 0.2	4.9 6.6	0.4 4.8
	Sediment	#1 #2	0.2 0.2	0.5 0.4	1.4 1.5	2.2 1.9	2.7 1.6	0.5 0.6	1.5 3.6	0.7 1.5	2.0 0.6	3.1 0.5
RA in sediment	Extracted	#1 #2	1.6 2.0	4.5 4.7	9.3 9.4	10.6 10.1	12.0 15.8	18.0 18.1	26.9 27.7	24.2 33.3	21.4 33.2	23.1 32.0
	NER	#1 #2		0.1 0.2	1.0 1.2	2.0 2.2	3.8 3.6	5.0 4.8	22.1 18.4	29.3 30.5	30.4 33.2	32.3 33.9
$^{14}CO_2$	Entire system	#1 #2	n.a. n.a.			0.1 0.1	0.1 0.1	0.4 0.2	3.0 1.2	10.2 3.7	19.7 8.2	24.0 13.5
Org. volatiles	Entire system	#1 #2	n.a. n.a.									
Total recovery of radioactivity	Water	#1 #2 Mean	95.5 97.5 96.5	93.3 94.8 94.0	90.4 89.4 89.9	88.2 87.3 87.7	83.3 80.9 82.1	79.2 77.3 78.2	49.3 51.6 50.4	34.2 31.2 32.7	27.3 24.0 25.7	20.2 17.3 18.7
	Sediment	#1 #2 Mean	1.6 2.0 1.8	4.6 4.8 4.7	10.3 10.6 10.4	12.5 12.2 12.4	18.8 19.4 19.1	22.9 22.8 22.9	49.0 46.1 47.5	53.5 63.8 58.7	51.8 66.4 59.1	55.4 65.9 60.6
	Entire system	#1 #2 Mean SD	97.1 99.5 98.3 ±1.2	97.9 99.6 98.8 ±0.9	100.7 100.0 100.3 ±0.3	100.9 99.6 100.2 ±0.6	102.2 100.4 101.3 ±0.9	102.6 100.3 101.4 ±1.2	101.3 98.3 100.1 ±1.2	98.0 98.7 98.3 ±0.3	98.8 98.6 98.7 ±0.1	99.6 96.7 98.1 ±1.4

Blank = < LOQ n.a. = not analyzed
NER = not extractable radioactivity

B. MASS BALANCE

For this study the applied radioactivity (AR = 100%) was defined as the mean of the LSC in the application solution. The mass balances are presented in Table IIA 7.8.3-3 for the system [redacted] and in Table IIA 7.8.3-4 for the system [redacted].

During the study the total recovery of radioactivity (RA) in individual test vessels of HW ranged from 94.4 % to 101.8 % (mean 98.5 %; ±2.1 %). The RA in individual test vessels of the AW system ranged from 96.7 % to 102.6 % (mean 99.6 %; ±1.6 %). The complete material balance found at all sampling intervals demonstrated that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

C. RESIDUES IN WATER, BOUND AND EXTRACTABLE RESIDUES IN SEDIMENT

The portions of radioactivity measured in the supernatant water are presented in Table IIA 7.8.3-3 for the system [redacted] and in Table IIA 7.8.3-4 for the system [redacted]. As usual for aquatic water/sediment studies (i.e. application of the test item to the supernatant water) the content of total RA in the water phase decreased with incubation interval in both test systems. The decrease was stronger in the loamy system HW than in the less biologically active loamy sand system AW. At the end of the study (120 days), the portion had decreased to 17.8 and 8.2 % in the water of HW and to 20.2 and 17.3% in the water of AW, respectively.

The portions of radioactivity measured in the sediment are presented in Table IIA 7.8.3-3 for the system [redacted] and in Table IIA 7.8.3-4 for the system [redacted]. As usual for aquatic water/sediment studies (i.e. application of the test item to the supernatant water) the content of total RA in the sediment increased with incubation interval in both test systems, reaching a maximum of 38.2% (#1) at DAT-30) and 50.0% (#2) at DAT-60 for HW, and 26.9% (#1) at DAT-30) and 33.3% (#2) at DAT-60 for AW, respectively.

In both water/sediment systems the amounts of non-extractable radioactivity (NER; bound residue) determined in the sediments of early samplings were low, i.e. less or equal to 1.2 and 5.0 % of AR in HW and AW until DAT-14, respectively. Then, they quickly increased between DAT-14 and DAT-30 to levels greater than 30 % of AR. At the later sampling intervals the NER fraction slightly increased to maximum levels of 40.7 % of AR (HW, #2, DAT-91) and indicated a decline to the end of the study for both labels and test systems. A chemical characterization of the NER performed by organic matter fractionation after disintegration under excessive alkaline conditions (using the acid extracted soil residues of DAT-120) indicated that the major portion of radioactivity (between 12.9 and 17.5 % of AR) was attributable to the soluble humic acid fraction.

D. VOLATILIZATION

The portions of $^{14}CO_2$ are presented in Table IIA 7.8.3-3 for the system [redacted] and in Table IIA 7.8.3-4 for the system [redacted]. The formation of $^{14}CO_2$ was detectable earliest 7 days after application in both systems. Later, the amount of $^{14}CO_2$ steadily increased to values of up to 11.0 % AR in HW (label #1) and 24.0 % AR in AW (label #1) at termination of the study. From these data it can be concluded that BYI08330 and its metabolites are steadily mineralized in water/sediment systems. The radioactivity found in the PU traps amounted to <0.1 % AR for both systems indicating that no other volatiles were formed in detectable levels.

E. TRANSFORMATION OF PARENT COMPOUND

The portions of BYI08330 and its metabolites determined in water and sediment extracts are presented in Table IIA 7.8.3-3 for the system [redacted], and in Table IIA 7.8.3-4 for the system [redacted]. The results were considered for the proposed overall pathway of BYI08330 degradation in water (see Figure IIA 7.8.3-2).

In both water/sediment systems BYI08330 was eliminated very quickly from the water body within

about a week mainly via degradation (for calculation of DT_{50} and DT_{90} values of BYI08330 in the supernatant water, see later). Already 7 days after application BYI08330 was declined to non detectable levels in both systems.

In the course of the study several HPLC peaks were detected and quantified besides unaltered BYI08330. Under unidentified radioactivity, all minor peaks (<5 % of AR and occurring only once or twice in the entire study) plus diffuse radioactivity were summed up. The most prominent metabolite in the water phase of both systems was BYI08330-enol reaching maximum levels of 76.4 and 78.8 % of AR (mean values of both labels) at DAT-7 in HW and AW, respectively, and declining in the water phase to non detectable levels in HW and to levels of 8.1 % of AR in AW at the end of the study. The main degradation product of BYI08330-enol, BYI08330-ketohydroxy, reached levels of 12.7% of AR (mean value) in the water phase of HW at the end of the study. In contrast in the water phase of AW, after reaching a peak level of 6.4 % of AR at DAT-30, BYI08330-ketohydroxy declined until the end of the study to a level of 4.0 % of AR.

The water phase of HW at DAT-3 contained unexpected high and not reasonable values of BYI08330-ketohydroxy (13.5 % of AR for both labels). These values most presumably were the result of a non-biological (non-inherent to the water/sediment system) oxidation of BYI08330-enol to BYI08330-ketohydroxy and thus are regarded as clear outliers for plausibility reasons. Therefore the 13.5 % of AR of BYI08330-ketohydroxy would have to be added to the BYI08330-enol values of 62.9 and 61.3 % of AR, respectively, resulting in about 75.6 % of AR of BYI08330-enol (mean value). This value would be very consistent with the respective values in AW at the same interval of the study (78.6 % of AR, both labels).

In the water phase of AW the BYI08330-MA-amide amounted to levels above 5 % of AR (DAT-60), declining to 1.0 % of AR at end of study (mean values). In addition two minor metabolites occurring only in AW, BYI08330-oxo-enol isomer and BYI08330-di-hydroxy, were determined. BYI08330-oxo-enol isomer occurred in the water phase of AW, but only at the last two sampling intervals (3.7 and 3.8 % of AR at DAT-91, #1 and #2; 6.0 % of AR at DAT-120, only #1). Metabolite BYI08330-di-hydroxy was detected transiently at intervals DAT-60 and DAT-91 with levels of up to 5.5 % of AR (single value for label #1 at DAT-91.

In both water/sediment systems the portion of BYI08330 translocated to the sediment was minor (up to 3.2 % of AR in HW and up to 2.5 % of AR in AW, each at DAT-0.2) and this portion was quickly degraded being non-detectable already from DAT-7 on. As in the water phase, the most prominent metabolite in the HW sediment was BYI08330-enol reaching maximum levels of 36.6 % of AR at DAT-60 and declining to 10.5 % of AR until the end of the study. In contrast, in the AW sediment BYI08330-enol amounted only to 15.1 % of AR at DAT-14 and declined slowly to approx. 3 % of AR until end of study (means of both labels). The levels of BYI08330-ketohydroxy in the sediment were low until DAT-91 in HW and until DAT 30 in AW, then increasing to the end of the study to 27.8 % of AR in HW and to 21.4 % of AR in AW. BYI08330-MA-amide and BYI08330-oxo-enol isomer amounted to only minor levels (below 3 % of AR, single values) in the sediments of both systems whereas the AW-specific metabolite BYI08330-di-hydroxy was detected transiently in the sediment at DAT-60 and DAT-90 reaching maximum levels of 5.5 % of AR at DAT-91 (single value).

The amounts of unidentified radioactivity in the water and the sediment extracts are listed in Table IIA 7.8.3-3 for the system [redacted], and in Table IIA 7.8.3-4 for the system [redacted].

Considering the entire water/sediment test system, dissipation of BYI08330 was very fast in both entire test systems HW and AW (for kinetics of dissipation, see below and Table IIA 7.8.3-5). The parent compound was not detectable anymore from DAT-7 onwards. In both entire systems metabolite BYI08330-enol reached its highest levels at DAT-7 and DAT 14 of up to over 90% AR and than

decreased until end of study. Consistently in both entire systems the levels of BYI08330-ketohydroxy reached higher levels time-delayed as compared to BYI08330-enol and thus its concentrations were still increasing at the end of the study. Only in the AW test system BYI08330-MA-amide and BYI08330-di-hydroxy were observed and reached levels of above 5% of AR, but remained clearly below 10% of AR. BYI08330-oxo-enol isomer reached levels of up to 4.2% of AR. All identified degradation products occurring in the two systems were common for both labels.

F. DISSIPATION KINETICS

The dissipation kinetics of BYI08330 in the water phase and in the entire system was calculated according to focus kinetics guidelines. In all calculations the kinetic model Single First Order (SFO) was the most suitable as indicated by the lowest $Chi^2Err\%$ value. The following tables summarize the results of the DT50 calculations. In addition, for comparison, the respective values (plots and reports not shown) for two other kinetic models (First Order Multi Compartment, FOMC and Double First Order Parallel, DFOP) are presented (for respective results see Table IIA 7.8.3-5).

BYI08330 was quickly degraded in both natural water sediment systems. DT50 values of 1.00 and 1.02 days were calculated for the water phases, and 1.06 and 1.05 days for the entire systems, respectively.

Table IIA 7.8.3-5: Kinetics of BYI08330 dissipation after application to water/sediment systems and aerobic incubation at 20°C (MEF-04/511)

System [redacted]	DT50 [days]	DT90 [days]	Chi^2Err [%]	Model applied
Water Phase	1.00	3.31	4.31	SFO
Entire System	1.06	3.52	4.49	SFO
Water Phase	0.99	3.32	5.02	FOMC
Entire System	1.06	3.53	5.20	FOMC
Water Phase	1.00	3.31	6.15	DFOP
Entire System	1.06	3.52	6.40	DFOP
System [redacted]				
Water Phase	1.02	3.40	4.97	SFO
Entire System	1.05	3.50	5.24	SFO
Water Phase	1.02	3.41	5.78	FOMC
Entire System	1.05	3.52	6.11	FOMC
Water Phase	1.02	3.40	7.08	DFOP
Entire System	1.05	3.50	7.48	DFOP

III. CONCLUSION

It is concluded that BYI08330 once entering natural surface water will be degraded rapidly and thoroughly, mainly via the metabolites BYI08330-enol and BYI08330-ketohydroxy well known from the studies in soil, already.

Report: **KIIA 7.8.3/02, [redacted] 2006 (MEF-06/279)**

Title: **Kinetic evaluation of the aerobic aquatic metabolism of BYI08330, BYI08330-enol and BYI08330-ketohydroxy in water sediment systems**

Report No & Document No	**MEF-06/279** **M-277415-01-1**
Guidelines:	N/A
GLP	Not applicable (calculation)
Testing Laboratory and dates	Bayer CropScience AG, RD, Metabolism and Environmental Fate, D-[redacted], Germany, conducted the estimation in August 2006. Study completion date: 2006-08-30

EXECUTIVE SUMMARY

The degradation behavior of BYI08330, BYI08330-enol and BYI08330-ketohydroxy in an aquatic environment was investigated by kinetic evaluation of an aerobic water/sediment study (see study KIIA 7.8.3/01 described before) conducted with the two test systems, [redacted] and [redacted] (loam and sand sediment, 20°C).

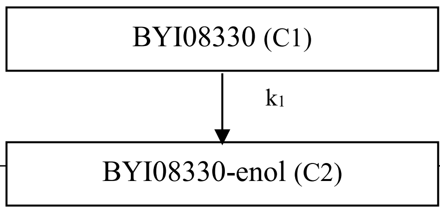
According to the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006) separate one compartment (Level I) dissipation half-lives of BYI08330, BYI08330-enol and BYI08330-ketohydroxy, for the water column compartment and the sediment compartment were derived. For use as persistence endpoints, the respective dissipation half-life derived from the best fit kinetic model, here single first order (SFO), should be employed (FOCUS, 2006). For use as modeling endpoints, SFO total system degradation half-lives were derived. Resulting dissipation water phase and total system degradation half-lives are summarized in Table IIA 7.8.3-7.

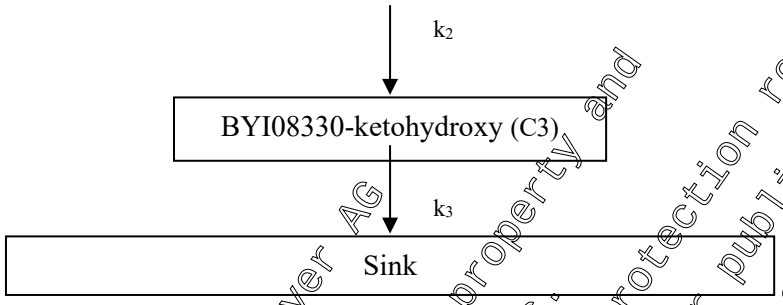
I. METHODS

Aquatic risk assessment requires the determination of the exposure to a chemical in a typical surface water environment. For this purpose, the distribution of BYI08330 and its metabolites between water and sediment phase, and their degradation in each of the two phases is measured in water-sediment systems under laboratory conditions. In a first step, the dissipation of the substance from the water- and sediment phase respectively, can be estimated with single first order (SFO) kinetics. Secondly, the distribution in the total system, considering the sum of substance mass in water and sediment, can be described by a compartment model assuming SFO kinetics for each reaction.

The substance properties of BYI08330-enol, BYI08330-kethohydroxy were summarized in chapter 3 of report MEF-06/279. In chapter 4 of report MEF-06/279 a summary of the degradation behavior of BYI08330, BYI08330-enol and BYI08330-ketohydroxy in an aquatic environment investigated by an aerobic water/sediment study (see study KIIA 7.8.3/01 described before) and conducted with the two test systems, [redacted] and [redacted] (loam and sand sediment, 20°C) is given. The kinetic evaluation approach is explained as follows.

Dissipation half-lives of the parent and metabolites in the water and sediment phase of water-sediment systems were derived assuming single first-order (SFO) decay. Additionally, overall degradation rates of each substance from the total water-sediment system were derived from an overall, compartment modeling approach. The proposed degradation scheme of BYI08330 was translated into a compartment model with compartments for each compound. They were associated with the sum of amounts measured in water and in sediment phase (see following figure; no values associated to the sink compartment). Between these compartments only one-way transformation reactions are assumed.





Free fitting the initial amount of the parent and fixing the initial amount to 0 for metabolites is used as default, here. Equal weighting of the data was performed in the kinetic analysis, which corresponds to an absolute error model. The computer program MatLab 7.0.4.365 (MatLab, 2005) was used for the kinetic modeling of the total water/sediment test systems.

Different kinetic model approaches were tested for BYI08330. SFO are to be always assessed first, as SFO is the standard kinetic approach in most environmental exposure models. Only if SFO kinetics does not meet the quality checks, more complex kinetics should be tested. The details as well as differential equations of these models, which need to be solved for parameter optimization, are listed chapter 5 of report MEF-06/279.

II. RESULTS

According to the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006) separate one compartment (Level I) dissipation half-lives of BYI08330, BYI08330-enol, BYI08330-ketohydroxy for the water column compartment and the sediment compartment were derived. For use as persistence endpoints, the respective dissipation half-life derived from the best fit kinetic model should be employed (FOCUS, 2006). For use as modeling endpoints, single first order (SFO) total system degradation half-lives were derived to be used in environmental fate models for both compartments at FOCUS surface water STEP 2 level and in conjunction with the default worst case DT50 = 1000 d for the respective other (e.g. sediment) compartment at STEP 3 level.

This default worst case option is suggested by the FOCUS working group on kinetics (FOCUS, 2006), if no statistically sound separate water and sediment half-lives can be derived from a two compartmental approach (Level II). This was the case for BYI08330 and BYI08330-enol in both water sediment systems. No valid water and sediment half-lives could be derived as the scaled errors ε for all degradation rates were clearly above 15% and with the exception of the degradation rate BYI08330 to BYI08330-enol in the water column the t-test yielded unacceptable probabilities ranging from 0.2-0.5 for all other degradation and transfer rates of the two compartmental systems.

The calculated dissipation half-lives of BYI08330, BYI08330-enol and BYI08330-ketohydroxy in the water phase of water-sediment systems [redacted] and [redacted] are presented in Table IIA 7.8.3-6a.

The SFO fits to the BYI08330, BYI08330-enol and BYI08330-ketohydroxy measured data was visually acceptable (with random distribution of the residuals) and yielded scaled errors ε of 4.3% ([redacted]) and 5% ([redacted]) for BYI08330, 15.3% ([redacted]) and 7.5% ([redacted]) for BYI08330-enol and 21.5% ([redacted]) for BYI08330-ketohydroxy. No statistically valid half-life could be derived for BYI08330-ketohydroxy from the [redacted] data. The FOMC model was tested, but did not lead to improved visual fits and lower scaled errors ε.

Table IIA 7.8.3-6a: Estimated parameters for dissipation from the water phase (MEF-06/279)

Test system	C_0 %	$DT_{50,\ water}$ d	$DT_{90,\ water}$ d	ε %	r^2	t-probability
			BYI08330			
[redacted], SFO	81.3	1.0	3.3	4.3	0.99	<0.001
[redacted], FOMC				5.0		
[redacted], SFO	71.8	1.0	3.4	5.0	0.99	<0.001
[redacted], FOMC				5.8		
Geom. mean		1.0				
			BYI08330-enol			
[redacted], SFO	80.0	16.7	55.4	15.3	0.94	<0.001
[redacted] FOMC				16.7		
[redacted], SFO	81.8	23.8	79.1	7.5	0.99	<0.001
[redacted], FOMC				8.2		
Geom. mean		19.9				
			BYI08330-ketohydroxy			
[redacted], SFO		n/a				
[redacted], FOMC		n/a				
[redacted], SFO	6.7	77.1	256.0	21.5	0.46	0.037
[redacted], FOMC				26.7		
Geom. mean		-				

The calculated dissipation half-lives of BYI08330 and BYI08330-enol and BYI08330-ketohydroxy in the sediment phase of water-sediment systems [redacted] and [redacted] are presented in Table IIA 7.8.3-6b.

The SFO fits to the BYI08330 and BYI08330-enol measured data was visually acceptable (with random distribution of the residuals) and yielded slightly higher scaled errors ε of 26.9% ([redacted])

and 24.0% ([redacted]) for BYI08330, 4.8% ([redacted]) and 21.4% ([redacted]) for BYI08330-enol. No statistically valid half-lives could be derived for BYI08330-ketohydroxy from the data of both test systems. The FOMC model was tested, but did not lead to improved visual fits and lower scaled errors ε.

Table IIA 7.8.3-6b: Estimated parameters for dissipation from the sediment phase (MEF-06/279)

Test system	C_0 %	$DT_{50,\ sediment}$ d	$DT_{90,\ sediment}$ d	ε %	r^2	t-probability
			BYI08330			
[redacted], SFO	2.9	3.45	11.46	26.9	0.64	0.027
[redacted], FOMC				31.4		
[redacted], SFO	2.2	2.21	7.36	24.0	0.76	0.003
[redacted], FOMC				27.9		
Geom. mean		2.8				
			BYI08330-enol			
[redacted], SFO	37.0	36.83	122.33	4.8	0.65	0.04
[redacted], FOMC				n/a		
[redacted], SFO	16.4	30.86	102.52	21.4	0.79	0.01
[redacted], FOMC				25.0		
Geom. mean		**33.7**				
			BYI08330-ketohydroxy			
[redacted], SFO		n/a				
[redacted], SFO		n/a				
Geom. mean		-				

The calculated degradation half-lives of BYI08330 and BYI08330-enol and BYI08330-ketohydroxy for the <u>total water-sediment systems</u> [redacted] and [redacted] are presented in Table IIA 7.8.3-6c.

The SFO fits to the BYI08330 and BYI08330-enol measured data was visually acceptable (with random distribution of the residuals) and yielded scaled errors ε of 6.2% ([redacted]) and 11.9% ([redacted]) for BYI08330, 13.4% ([redacted]) and 15.6% ([redacted]) for BYI08330-enol, 39.9 % ([redacted]) and 99.2 % ([redacted]) for BYI08330-ketohydroxy. Due to the high scaled errors ε and low correlation (especially in case of the [redacted] system) an unsatisfactory visual fit no statistically valid half-lives could be derived for BYI08330-ketohydroxy. The FOMC model was tested for the parent, but did not lead to improved visual fits and lower scaled errors ε.

Table IIA 7.8.3-6c: Estimated parameters for degradation in entire system (MEF-06/279)

Test system	C_0 %	$DT_{50, total}$ d	$DT_{90, total}$ d	ε %	r^2	t-probability
BYI08330						
[redacted], SFO	96.1	0.86	2.85	6.2	0.99	<0.001
[redacted], FOMC				7.1		
[redacted], SFO	94.3	0.70	2.34	11.9	0.97	<0.001
[redacted], FOMC				12.4		
Geom. mean		0.78				
BYI08330-enol						
[redacted], SFO	0	59.0	196.1	13.4	0.93	<0.001
[redacted], SFO	0	37.9	126.0	15.6	0.93	<0.001
Geom. mean		47.3				
BYI08330-ketohydroxy						
[redacted], SFO	0	30.79	102.3	99.2	0.32	<0.001
[redacted], SFO	0	27.71	92.1	39.9	0.60	<0.001
Geom. mean		-				

Table IIA 7.8.3-7: Estimated parameters for dissipation in water and degradation in the total water-sediment system of BYI08330 and BYI08330-enol (MEF-06/279)

Compound	System	$DT_{50, water\ dissipation}$ (d)	$DT_{50, total\ degradation}$ (d)
BYI08330	[redacted], SFO	1.0	0.86
	[redacted], SFO	1.0	0.70
	Geomean	1.0	0.78
BYI08330-enol	[redacted], SFO	16.7	59.0
	[redacted], SFO	23.8	37.9
	Geomean	19.9	47.3
BYI08330-ketohydroxy	[redacted], SFO	-	-
	[redacted], SFO	77.1	-

II. CONCLUSIONS

For use as persistence endpoints conservative one compartment (Level I) SFO dissipation half-lives of BYI08330, BYI08330-enol and BYI08330-ketohydroxy were derived for the water and the sediment compartment. The biphasic model FOMC model was tested, though did not improve the outcome for any of the compounds. SFO total system degradation half-lives were derived to be used as modeling endpoints for both compartments at FOCUS surface water STEP 2 level and STEP 3 level.

Report:	**KIIA 7.8.3/03, [redacted], [redacted], and [redacted], 2005 (MEFNX015)**
Title:	**BYI08330[azaspirodecenyl-3-^{14}C]: Anaerobic Aquatic Metabolism**
Report No & Document No	**MEFNX015 M-261943-01-1**
Guidelines:	US EPA, Subdivision N, Section 162-3 Canada PMRA DACO Number 8.2.3.5.6 OECD Guideline for Testing of Chemicals, Guideline 308
GLP	Fully GLP compliant - laboratory certified
Testing Laboratory and dates	Bayer CropScience AG, Environmental Research, [redacted], [redacted], [redacted], conducted the study during the period from October 2003 to August 2005. Study completion date: 2005-12-06

EXECUTIVE SUMMARY

The anaerobic biotransformation of radiolabeled BYI08330, was studied in a pond water/sediment system (water: pH 7.5, dissolved organic carbon = 8.4 ppm, sediment: texture = clay loam, pH 7.4, organic carbon = 0.7%) from [redacted], [redacted] (USA), for 120 days in the dark at 20 ± 1 °C. [Azaspirodecenyl-3-^{14}C]BYI08330 was applied at a rate of 14 ug/L, which closely approximates the single maximum field-use rate (288 g a.i./ha) applied by direct overspray of a non-target aquatic system to a depth of 200 cm.. The sediment/water ratio used was 1:3 (w/w). The test system consisted of a 250-mL Erlenmeyer flask with a double-valve sealable top containing sediment and water. Samples were analyzed at 0, 2, 7, 9, 14, 21, 28, 63, 90, and 120 days of incubation. The sediment samples were extracted with 90:10 ACN:water (0.1% formic acid) using a shaking method followed by an ASE extraction (accelerated solvent extraction) using the same solvents. The water samples and sediment extracts were concentrated by rotary evaporation. BYI08330 residues were analyzed by a HPLC coupled to a ^{14}C detector. Identification of the parent compound and metabolites were achieved by co-chromatography and liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS). The total material balance in the water/sediment system was 99.6 ± 3.6 % (mean ± SD) of the applied amount. The mean percent of applied radioactivity recovered at day 120 in water and extractable from sediment was 73.3% (± 2.3), and 26.7% (± 2.4), respectively. Extractable [^{14}C] residues in sediment increased from 8.8% at day 0, to 28.0% at day 63, and decreased to 26.7% by the end of the study. Non-extractable residues remained at < 0.6% of the applied radioactivity during the study. At the end of the study, 0.8% and 0.0% of the applied radioactivity was present as $^{14}CO_2$ and volatile organic compounds, respectively.

The concentration of [^{14}C]BYI08330 in water decreased to 57.2% at day 2, already, and continued to decrease to 0.0% by day 14. The concentration of BYI08330 in the sediment decreased from 8.8% at day 0 to 0.0% at day 14. The major transformation product detected in water was BYI08330-enol with a maximum concentration of 80.2% of the applied radioactivity on day 14 of the study. The corresponding concentration in water at the end of the study was 73.3% of the applied radioactivity. Again, the major transformation product detected in the sediment was BYI08330-enol with a maximum concentration of 28.0% of the applied on day 63 of the study. The corresponding concentration in sediment at the end of the study was 26.7% of the applied amount. No minor transformation products were detected during the study.

The half-lives using first-order nonlinear degradation kinetics for BYI08330 in anaerobic water, sediment, and in the entire system were 2.8 days (k= 0.25 day^{-1}; r^2 = 0.99), 3.1 days (k= 0.23 day^{-1}; r^2 = 0.95) and 2.8 days (k = 0.25 day^{-1}; r^2 = 0.99), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Spirotetramat: Code = BYI08330
Identity and purity of test item in the application solution were checked
Label position = [azaspirodecenyl-3-^{14}C] (sample ID: BECH0938)
Specific activity = 3.67 MBq/mg (37 uCi/µMol; 500 µCi/mL; 99.2 µCi/mg)
Radiochemical purity: 99% (acc. to radio-HPLC)

2. Test System: The study was carried out using a natural water/sediment system collected from a pond in [redacted] (Jefferson County), [redacted], USA. The matrix used in this study is representative of an agricultural area. The sediment was collected from the top 6 inches and flooded with corresponding water from the same pond. The sediment and water were transported, with minimal storage time, from the site of collection to the laboratory in 5-gallon buckets and stored at ambient temperature. The temperature, pH, redox potential and dissolved oxygen content were determined at the site of collection. The sediment was sieved through a 2-mm sieve under water, and moisture was determined before use. The physicochemical properties of the water and sediment are listed in
The microbial biomass was determined at the beginning and end of the study. The moisture content of the sediment was determined by heating 6 aliquots (approx. 10 g each) of sediment in a microwave repeatedly until their sequential weights were constant.
Characteristics of the sediments and the corresponding supernatant water are summarized in Table IIA 7.8.3-8 and Table IIA 7.8.3-9.

Table IIA 7.8.3-8: Physico-chemical characteristics of the sediment used (MEFNX015)

Parameter	Results/Units
Geographic location	[redacted], [redacted], KS, Jefferson County
Taxonomic classification	N/A
Soil Series	N/A
Mapping Unit	Latitude: [redacted] Longitude: [redacted]
Textural class [USDA]	Clay loam
Sand (%) Silt (%) Clay (%)	21 40 39
pH: 1:1 soil : water saturated paste 0.01 M $CaCl_2$	 7.6 7.4 7.1
Organic matter (%) / Organic carbon (%)	1.2 / 0.7
Sediment Biomass (cells/g sediment) Initial: Final:	 2.00 x 10^8 1 .07 x 10^8
Cation exchange capacity (meq/100 g sediment)	32.7
Field Moisture Capacity at 0.33 bar (%)	35.8
Bulk Density (g/cm³)	1.11

Table IIA 7.8.3-9: Physico-chemical characteristics of the water used (MEFNX015)

Parameter	Results/Units
Geographic location	[redacted], [redacted], KS, Jefferson County
Mapping Unit	Latitude: [redacted] Longitude: [redacted]
Temperature (°C)	23.3
pH	7.5
Hardness ($CaCO_3$) (ppm)	139
Electrical conductivity	n.d.
Oxygen concentration (mg/L)	
- Initial (one day after sampling)	0.0
- Final (at latest processing date)	0.0
Dissolved organic carbon, DOC (mg C/L)	8.4
Total organic carbon, TOC (mg C/L)	9.3
Redox potential Eh (mV)	
- Initial	55.2
- Final	-20
Biomass (cells/mL water)	
- Initial	3.48×10^6
- Final	2.55×10^6

n.d.: not determined

B. STUDY DESIGN

1. Experimental conditions: The tests were performed using individual test flasks held at anaerobic conditions at 20 ±1 °C for a maximum period of 120 experimental days. The experimental design is described in Table 2, the apparatus used to establish anaerobic conditions is shown in Figure 2, and the apparatus used for anaerobic incubation is shown in Figure 3 of the report MEFNX015.
The treated test system consisted of a 250-mL side-arm Pyrex® Erlenmeyer flask topped with a sealed double-valve top. Each flask was covered with aluminum foil to prevent exposure to light, and the test systems were kept in a temperature-controlled incubator with a nitrogen atmosphere.

Test systems were prepared containing 50 g sediment (dry weight) and 150 mL pond water. Prior to treatment of the test systems, a pre-incubation period of approximately 27 days was used to establish anaerobic conditions. The test systems were equipped with mineral oil traps, flushed with nitrogen, and acclimated in a nitrogen filled incubator. Two replicates were analyzed per interval (kinetic test).

Test systems were closed, and volatiles were trapped at each sampling interval. Volatile traps were comprised of six traps consisting of a blank, 30 mL of 2.0 M KOH (potassium hydroxide), another 30 ml of 2.0 M KOH, 30 mL of ethylene glycol, 30 mL of 1 M sulfuric acid, and a blank.

The amount of radiolabelled BYI08330 for the treatment of the test systems was based on the highest recommended single field use rate of the test item (288 g/ha, calculated to a water depth of 200 cm), implying a surface water contamination to be in the same order of magnitude. Thirty-three test systems were each treated with 100 µL of kinetic application solution. The final concentration of BYI08330 in each test system was 0.014 µg/mL, the final concentration of ACN was < 0.1% in the test flasks.
The application solution was uniformly applied to the surface of the water using a 100-uL Hamilton gas tight syringe to mimic the introduction of the active ingredient to a pond via direct overspray. The sample was not disturbed during the application of the solution. After treatment, the double-valve tops were put in place, and the test systems were flushed with nitrogen for approximately 10 minutes prior to being sealed and transferred to an incubator.

2. Sampling: Ten sampling intervals were conducted over a period of 120 days at 0, 2, 7, 9, 14, 21, 28, 63, 90, and 120 days post treatment. Samples were purged with oxygen-stripped nitrogen at 40 mL/min for 15 minutes into volatile trapping solutions. While continuing to purge with oxygen free nitrogen, the pH and dissolved oxygen content of the water were measured as well as the redox potential of the water and sediment for each test system. The water was then separated from the sediment by decanting and filtering. Samples were processed and sediment was extracted on the day of sampling. The maximum storage time of a water sample or sediment extract before analysis was 6 days. The majority of water samples and extracts were analyzed within an average of 3 days. The water samples and extracts were stored in a laboratory freezer below -15 °C until analysis.
Before sample processing, two replicate test systems were connected to volatile bubbler traps following removal from the incubator. Nitrogen was passed through the double valve top of the Erlenmeyer flask and through the volatile traps at approximately 40 mL/min for 15 minutes. The contents of the volatile traps were radio assayed in 1-mL aliquots by LSC in triplicate.

3. Description of analytical procedures: The aqueous portion of the sample was decanted and filtered into a 250-mL graduated cylinder. The sample was acidified with formic acid (100 µL) to stabilize the parent compound in the sample. The sample was radio assayed, and an aliquot (30 to 40 mL) was concentrated for HPLC injection. The volume concentrated typically allowed for detection of 1% of the applied radioactivity and 2 injections for each sample. Each aliquot was rotary evaporated under vacuum at approximately 30 to 35 °C to near dryness and typically reconstituted in a final volume of 5 mL (ACN:0.1% formic acid 2:8 v:v). Samples were then centrifuged for 10 minutes at 2100 g, and approximately 2 to 2.5 mL was analyzed by HPLC.
The sediment portion of the sample was transferred to a 250-mL Teflon® bottle with approximately 100 mL of 9:1 ACN:water (v:v, 0.1% formic acid) and extracted for 20 min by shaking on a bench-top shaker. The solvent and sediment were filtered through 12 g of hydro matrix and a Whatman 541 filter. The hydro matrix facilitated filtering, prevented plugging of the filter and compaction of the sediment during the ASE extraction. The sample was then transferred to a 100-g ASE cell for extraction using the a method at a cell temperature of 80 °C, a pressure of 1500psi, 2 cycles using the solvent ACN:Water (9:1 v:v, 0.1% formic acid). The sediment extracts were combined, acidified with approximately 1 ml formic acid, and triplicate 1-mL aliquots were radio assayed. An appropriate portion concentrated analyzed by HPLC. Representative samples were analyzed by LSC for concentration recovery.
The extracted sediment samples were air dried, weighed and homogenized thoroughly. Triplicate aliquots of the sediment were analyzed by combustion.
The parent compound and the major metabolites were identified by HPLC co-chromatography and by LC-MS and LC-MS/MS spectrometry.

C. Determination of Degradation Kinetics

First order degradation rates were determined by the software program, GraphPad™ PRISM® (GraphPad Software, San Diego) using a nonlinear optimization method. The percentage of applied radioactivity was plotted as BYI08330 in the water, sediment, and total system against time. A first-order degradation relationship can be written as $c(t) = c_0 e^{-kt}$,
where c_0 and c_t are the BYI08330 concentrations at time 0 and t (days), respectively. In PRISM®, c_t is represented as y, c_0 is represented as SPAN and t is represented as x. The nonlinear optimization method uses an iterative procedure to determine the most appropriate value for the variables being optimized (c_0 and k). The model begins with estimated values based on the mode parameters and data set and then generates a curve defined by the initial values. The program then calculates the sum of squares of errors and recalculates the variables to improve the fit of the curve. The readjustment continues until any

further adjustments make no significant difference in the sum of squares. The program then reports the values calculated for each variable and reports the best-fit. Based on the above relationship the half-life ($T_{1/2}$) and DT90 ($T_{1/10}$) values, in days, were calculated as follows:

$$T_{1/2} = \ln(0.5)/-k$$
$$T_{1/10} = \ln(0.1)/-k$$

A linear regression analysis was used to determine the detector response (Microsoft Excel 2000). The Q-test (rejection quotient) was used to determine if data could be rejected as statistical outliers, at a 90% confidence limit.

II. RESULTS AND DISCUSSION

The anticipated anaerobic test conditions were maintained during the incubation in a dark climatic chamber. The mean temperature maintained throughout the study was of 19.2 °C. At the start of the study, the redox potential (Eh) of the water and sediment and dissolved oxygen content of the water were an average of 55.2 mV (water), 68.5 mV (sediment), and 0.0 mg/L, respectively, thus indicating an anaerobic environment had been established prior to treatment (Eh < 200mV is regarded as an anaerobic environment). Throughout the study, the average redox potential (Eh) (25 mV in water, 14.5 mV in sediment) and the average dissolved oxygen concentrations remained low (< 0.1 mg/L) showing that an anaerobic reducing environment had been maintained. The average pH of the water ranged from 6.7 to 7.3. The determination of microbial biomass (see Table IIA 7.8.3-8 and Table IIA 7.8.3-9) indicated that the test systems remained microbial viable throughout the study.

A. DATA

A compilation of results is shown in the following Table IIA 7.8.3-10.

B. MASS BALANCE

For this study the applied radioactivity (AR = 100%) was defined as the mean of the LSC in the application solution. By Table IIA 7.8.3-8 it is shown that the material balance (average of replicates) for the study ranged from 90.1 to 102.2% of the applied radioactivity. The mean material balance for the study was 99.6 ± 3.6%. The complete material balance demonstrated that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

Table IIA 7.8.3-10: Distribution of radioactivity after application of [^{14}C]BYI08330 to water/sediment and anaerobic incubation at 20°C; values in % of applied radioactivity (MEFNX015)

Compound	Source		Sampling time (days after application) 0	2	7	9	14	21	28	63	90	120
BYI08330	Water	Mean	81.3	57.2	9.9	7.8						
		SD	±2.8	±5.9	±1.6	±1.9						
	Sediment	Mean	8.8	7.1	1.1	1.1						
		SD	±0.8	±0.1	±1.5	±1.5						
	Subtotal	Mean	90.1	64.4	11.0	8.9						
		SD	±3.6	±5.8	±3.1	±3.5						
-Enol	Water	Mean		22.6	66.7	69.4	80.2	75.7	76.4	72.3	76.4	73.3
		SD		±5.7	±3.5	±2.0	±0.5	±3.6	±0.8	±0.2	±0.2	±2.3
	Sediment	Mean		10.6	20.1	21.2	21.0	25.5	24.1	28.0	24.0	26.7
		SD		±0.5	±1.4	±2.7	±0.2	±4.2	±1.3	±0.2	±0.1	±2.4
	Subtotal	Mean		33.1	86.8	90.6	101.2	101.2	100.5	100.3	100.4	100.0
		SD		±5.2	±4.9	±4.7	±0.3	±0.6	±0.5	±0.0	±0.2	±0.1
Total extractable RA	Water	Mean	81.3	79.8	76.6	77.2	80.2	75.7	76.4	72.3	76.4	73.3
		SD	±2.8	±0.1	±1.9	±0.1	±0.5	±3.6	±0.8	±0.2	±0.2	±2.3
	Sediment	Mean	8.8	17.7	21.2	22.3	21.4	25.5	24.1	28.0	24.0	26.7
		SD	±0.8	±0.4	±0.2	±1.1	±0.4	±4.2	±1.3	±0.2	±0.1	±2.4
	Subtotal	Mean	90.1	97.5	97.8	99.5	101.6	101.2	100.5	100.3	100.4	100.0
		SD	±3.6	±0.6	±1.7	±1.2	±0.9	±0.6	±0.5	±0.0	±0.2	±0.1
$^{14}CO_2$ Volatile	Entire system	Mean		0.6	0.5	0.6	0.6	0.6	0.7	0.7	0.7	0.8
		SD		±0.1	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.1	±0.0
Org. Volatiles	Entire system	Mean										
		SD										
Total Volatiles	Entire system	Mean		0.6	0.5	0.6	0.6	0.6	0.7	0.7	0.7	0.8
		SD		±0.1	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.1	±0.0
NER	Sediment	Mean									0.5	0.6
		SD									±0.1	±0.1
Total recovery *)	Entire system	Mean	90.1	98.1	98.3	100.1	102.2	101.8	101.3	101.0	101.5	101.4
		SD	±3.6	±0.5	±1.7	±1.2	±0.8	±0.6	±0.5	±0.0	±0.0	±0.2

Blank = < LOQ
NER = not extractable radioactivity (bound residues)
*): Mean material balance = 99.6 ±3.6 %

C. RESIDUES IN WATER, BOUND AND EXTRACTABLE RESIDUES IN SEDIMENT

The distribution of radioactive residues (average of replicates) during the course of the study is summarized in Table IIA 7.8.3-10. The radioactive residues in the water phase decreased from 81.3% at day 0 to 73.3% at day 120. The observation in the sediment phase was reversed, with the radioactive residues increasing from 8.8% on day 0 to 26.7% at day 120.

Non-extractable residues in the sediment were very low and did not exceed 0.6% of the applied radioactivity.

D. VOLATILIZATION

Volatile compounds remained low with maximum $^{14}CO_2$ levels of 0.8%, and volatile organics of <0.1% of the total applied radioactivity.

E. TRANSFORMATION OF PARENT COMPOUND

In the aqueous phase, BYI08330 decreased from 81.3% at day 0 to 57.2% at day 2 and continued to decrease to <0.1% by day 14. In the sediment phase, BYI08330 decreased from 8.8% at day 0 to <0.1% by day 14. In the total sediment/water system, BYI08330 decreased from 90.1 % at day 0 to 64.4% at day 2 and 0.0% by day 14. The major transformation product detected in water was BYI08330-enol. The formation of BYI08330-enol made up 22.6% of the applied radioactivity by Day 2 in the aqueous phase, increased to a maximum of 80.2% on Day 14, and decreased to 73.3% of the applied radioactivity by the end of the study. The formation of BYI08330-enol made up 10.6% of the applied radioactivity by Day 2 in the sediment, increased to a maximum of 28.0% by Day 63, and decreased to 26.7% of the applied radioactivity by the end of the study. There was no unidentified radioactivity during the study.

The results of this study were considered for the proposed overall pathway of BYI08330 degradation in water (see Figure IIA 7.8.3-2).

F. DISSIPATION KINETICS

The following Table IIA 7.8.3-11 summarizes the results of the kinetics calculations. BYI08330 was quickly degraded in the anaerobic natural water/sediment system. The first-order degradation rate calculated for BYI08330 in anaerobic water, sediment, and in the entire system resulted in half-lives of 2.8, 3.1, and 2.8 days, respectively.

Table IIA 7.8.3-11: Kinetics of BYI08330 dissipation after application a water/sediment system and anaerobic incubation at 20°C (MEFNX015)

Matrix	First Order		DT50 (days)	DT90 (days)
	$c(t) = c_0 \times e^{-kt}$	R^2		
Water	$c(t) = 84.14 \times e^{-.025t}$	0.99	2.8	9.2
Sediment	$c(t) = 9.3 \times e^{-0.23t}$	0.95	3.1	10.2
Entire System	$c(t) = 93.44 \times e^{-0.25t}$	0.99	2.8	9.3

III. CONCLUSION

It is concluded that BYI08330 once entering an anaerobic natural water/sediment system will be degraded rapidly, mainly to the metabolite BYI08330-enol well known from the studies in aerobic soil and water/sediment systems, already.

Summary: Metabolic pathway and degradation rate of spirotetramat in aquatic systems

The fate and behavior of spirotetramat in aquatic systems was investigated under standardized laboratory conditions, using radiolabeled as well as unlabeled test substance. Under dark conditions spirotetramat was found to be degradable by abiotic degradation processes. Hydrolysis is regarded as relevant for the degradation of BYI08330 in the environment, especially under neutral and alkaline conditions. The hydrolytic half-life at pH 7 and 25°C (20°C) is expected to be in the range of 8.6 days (13 days). In the total pH range tested (pH 4 to 9) the formation of BYI08330-enol as the only common hydrolysis product was observed. From a separate study investigating the hydrolysis of the major degradate it was concluded that hydrolysis is not relevant for the degradation of BYI08330-enol in the environment, since

the hydrolytic half-life at pH 4, 7 and 9 at 25°C is expected to be > 1 year.
BYI08330-ketohydroxy is hydrolytically stabile under acidic conditions but labile at a pH above 7. The experimental half-lives of BYI08330-ketohydroxy at pH 9 were 4.9 days (25°C) and 15.6 days (20°C). Thereby, one major degradation product is formed. The formation of BYI08330-MA-amide as a common hydrolysis product was observed at pH 7 and pH 9. That metabolite is considered as hydrolytically stable since its concentration increased towards the end of the incubation period at pH 7 and pH 9.

Based on the experimental DT50 of 2.7 days for BYI08330 in sterile pure buffered water and related predicted environmental DT50 (e.g. of 12.9 solar summer days at [redacted], AZ, USA or 19.9 summer days at [redacted], Greece) it was concluded that photo-transformation of BYI08330 in aqueous systems is a significant route for the elimination of this compound. However, that basic tests are to be performed under sterile conditions in highly purified buffer of pH 5, in order to help distinguish between hydrolytic or/and biotic and direct photolytic reactions. Thus, it was expected that the behavior will be different in natural aqueous systems, since in the case of BYI08330 biodegradation will happen quickly, hydrolysis will be faster with increasing pH, as well as indirect reactions might compete with the re-arrangement reactions observed in the prevailing study. This expectations were confirmed by an investigation of the phototransformation of [^{14}C]BYI08330 (labels #1 and #2) in sterile natural water by a supportive study. Based on the experimental DT50 of 0.2 days for BYI08330 and related predicted environmental DT50 (e.g. of 0.6 solar summer days at [redacted], AZ, USA or 1.0 summer days at [redacted], Greece) it is concluded that photo-transformation of BYI08330 in aqueous systems is a significant route for the elimination of this compound in natural water. This test clearly showed that competition of hydrolysis and indirect photo-reactions does not allow the light-induced re-arrangement reactions of parent compound observed in highly purified buffer of pH 5. Together with the well-known fast biodegradation this was the justification to consider the products formed in the natural water study as relevant for the overall pathway of spirotetramat degradation in water (see Figure IIA 7.8.3-2), but not the re-arrangement photo-products found in the highly artificial laboratory study performed in sterile pure buffer.

From a laboratory study investigating the route and rate of degradation in two natural water/sediment systems under aerobic laboratory conditions in the dark at 20 °C it is concluded that BYI08330 once entering aqueous systems will be degraded rapidly and thoroughly, mainly via the metabolites BYI08330-enol and BYI08330-ketohydroxy well known from the studies in soil, already. DT50 values of 1.00 and 1.02 days were calculated for BYI08330 in the water phase, and 1.06 and 1.05 days in case of the entire systems, respectively.

The results of a laboratory study investigating the route and rate of degradation in a completely anaerobic water/sediment system in the dark at 20 °C showed that BYI08330 once entering an anaerobic natural aqueous environment will also be degraded rapidly, mainly to the metabolite BYI08330-enol well known from the studies in aerobic soil and water/sediment systems, already. The first-order degradation rate calculated for BYI08330 in anaerobic water, sediment, and in the entire system resulted in half-lives of 2.8, 3.1, and 2.8 days, respectively. For use as persistence endpoints conservative one compartment (Level I) SFO dissipation half-lives of BYI08330, BYI08330-enol and BYI08330-ketohydroxy were derived for the water and the sediment compartment in a special modeling study. The biphasic model FOMC model was tested, though did not improve the outcome for any of the compounds. SFO total system degradation half-lives were derived to be used as modeling endpoints for both compartments at FOCUS surface water STEP 2 level and STEP 3 level.

The proposed overall metabolic pathway summarizing findings from different studies on aquatic degradation is shown in Figure IIA 7.8.3-2.

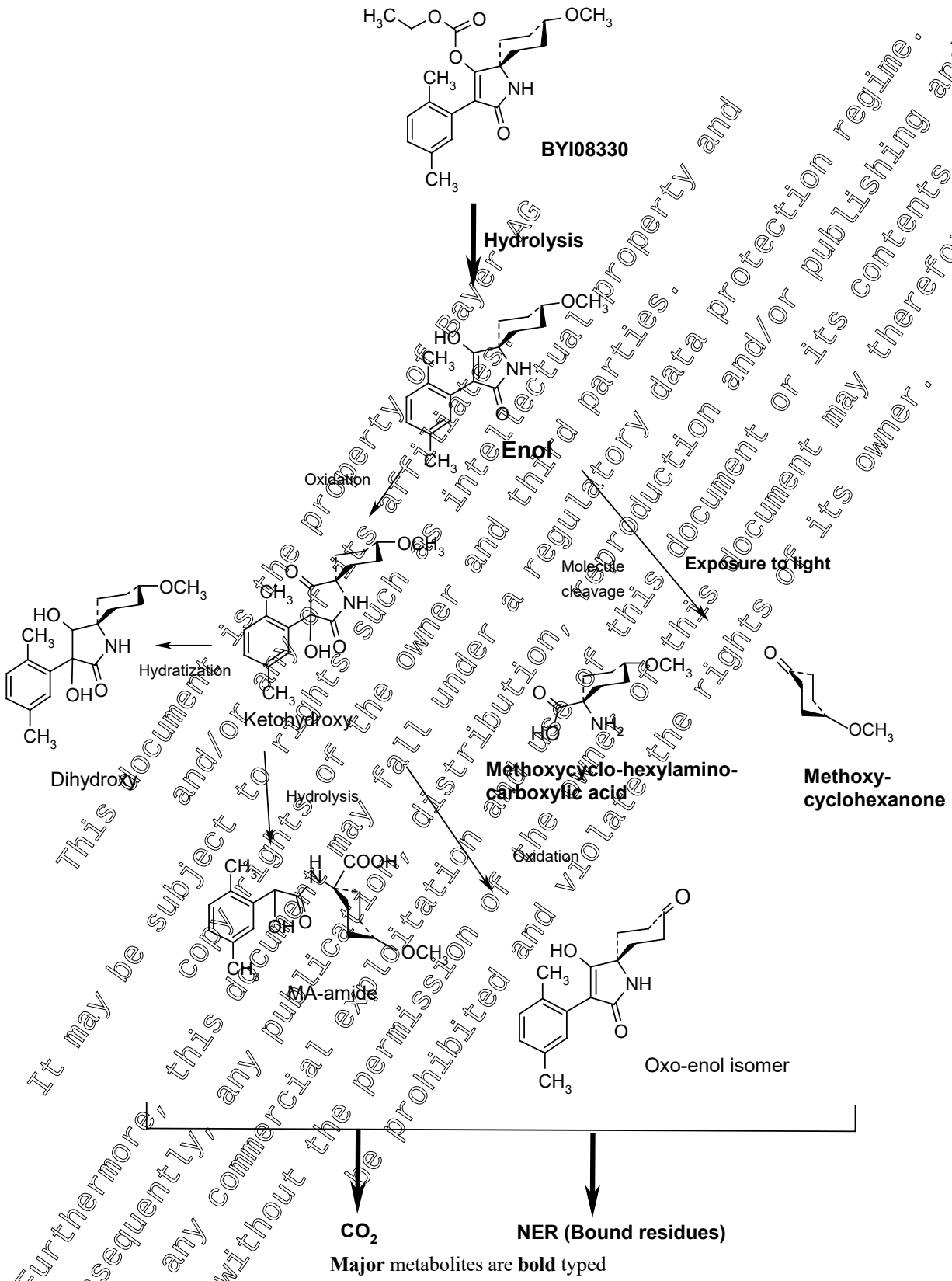


Figure HA 7.8.3-2: Proposed overall pathway of spirotetramat in aquatic systems

IIA 7.9 Degradation in the saturated zone

The degradation behavior of spirotetramat in the saturated zone has not been investigated in specific studies since it is not expected to reach such zones after its use according to good agricultural practices. If at all needed it can be estimated from laboratory soil degradation studies as well as from the laboratory water/sediment studies performed under dark aerobic and anaerobic conditions. In these studies degradation of spirotetramat and its residues was comparatively fast.

IIA 7.10 Rate and route of degradation in air

Based on the estimation method according to structure-activity relationship (SAR) methods developed by Roger Atkinson and co-workers the chemical lifetime of the BYI08330 and its major metabolite BYI08330-enol in the air was assessed by the program AOPWIN (version 1.91).

Report:	**KIIA 7.10/01, [redacted]; 2004 (MEF-04/400)**
Title:	**Calculation of the chemical lifetime of BYI08330 in the troposphere**
Report No & Document No	**MEF-04/400 M-092840-01-1**
Guidelines:	EU Commission Directive, 95/36/EC, amending Council Directive 91/414/EEC: Annexes I+II, Fate and Behavior in the Environment, 7171/VI/94-EN, 7.2.2.3 Photolysis in Air; Federal Biological Institute for Agriculture and Forestry (BBA), Germany: Guidelines for the Testing of Plant Protection Products in Registration Procedure, Part IV, 6-1 (July 1990) entitled "Determination of the volatilization and the fate of plant protectants in the air". BBA-Guidelines for Testing of Plant Protectants in the Registration Process. Part IV, 6-1, Determination of the volatilization and the fate of plant protectants in the air, 1990
GLP	Not applicable (calculation)
Testing Laboratory and dates	Bayer CropScience AG, RD, Metabolism and Environmental Fate, D-[redacted], Germany, conducted the assessment in September 2004. Completion date: 2004-09-14

Executive Summary

Based on the estimation method according to structure-activity relationship (SAR) methods developed by Roger Atkinson and co-workers the chemical lifetime of the BYI08330 in the air was assessed by the program AOPWIN (version 1.91). A value (τ) of at the most 3 hours corresponding to a half-life of approx. 2 hours resulted, 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 BYI08330.

Reactions with OH radicals and ozone contribute to the degradation of BYI08330 in the air to a high extent. The chemical stability of BYI 08330 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.

I. METHODS

There are different reaction mechanisms that may result in degradation of organic trace substances in the air being gaseous or bound to particles. According to the present knowledge mainly reactions with photochemical produced hydroxyl radicals, with nitrate radicals and ozone as well as direct photolysis are possibilities. The abiotic degradability and/or the reversal of that, the persistence of a substance X, can be predicted if the reaction rate k_i and the concentrations $c(y_i)$ of the potential reaction partners are known.

On account of the molecular structure of BYI08330 it can be taken for granted with great certainty that mainly reactions with photochemical produced hydroxyl radicals and with ozone determine its degradation rate (K_{total}) and chemical lifetime (τ) in the air.

A value of 0.5 x 10^{+6} OH radicals/cm^3 is generally regarded nowadays as the mean OH radical concentration in the troposphere (global 24-hrs-mean). A value of 1.5 x 10^{+6} OH radicals/cm^3 is regarded as the global 12-hrs-day-time concentration (excluding the night). Consequently the following assumptions can be made for compounds that are emitted into the air during the day and that are degradable within a period of a few hours:

$$K_{total} \geq k_{OH} + K_{ozone} \text{ [cm}^3\text{/molecules-sec]} \qquad \text{(I)}$$

$$\text{Chemical lifetime OH } (\tau) \leq \frac{1}{(k_{OH} \times 0.5 \times 10^6)} \text{ [sec]} \qquad \text{(II)}$$

$$\text{Half-life OH } (t_{1/2}) \leq 0.69 \times (\tau) \text{ [sec]} \qquad \text{(III)}$$

The measurement of k_{OH} is experimentally very laborious and can hardly be carried out in the gas phase for larger molecules with lower vapor pressures. Therefore, procedures have been developed to calculate this important tropospheric parameter. They are based on the molecular structure of a compound.

Starting out from a comprehensive set of experimental data Roger Atkinson developed such a calculation procedure by means of quantitative structure reactivity relations (QSAR). The transformation of the calculation procedure into a personal computer program (Atmospheric Oxidation Program, AOP) was carried out by W. Meylan & P. Howard. The AOPWIN Program version 1.91 (U.S. EPA 2003) was used for the calculations here.

Estimation methods based on structure-activity relationship (SAR) methods developed by Roger Atkinson and co-workers proved to be a good approach for the evaluation of the reaction with hydroxyl radicals in the air. This estimation method adds up the partial reaction rates of the hydroxyl reaction with subgroups of the test molecule (increments) resulting in the overall reaction rate. The following hydroxyl reactions are considered:

k_{add} : addition of HO˙ to olefin bonds

k_{ar} : addition of HO˙ to aromatic rings

k_{abs} : abstraction of hydrogen

k_{NSP} : reaction with N, S or P atoms

The listed increments (group rate constants for hydrogen abstraction, addition to double and triple bonds and reaction with hetero atoms) are compiled in a data base which additionally uses algorithms for consideration of adjacent groups (substituent factors) and the position of the attack to substituted aromatic rings (electrophilic substituent factors).

Generally, a value of 7 x 10^{+11} mol/cm^3 is regarded nowadays as mean ozone concentration in the troposphere. This was the assumption made for the ozone calculations.

$$\text{Chemical lifetime ozone } (\tau) = \frac{1}{(k_{Ozone} \times 7 \times 10^{11})} \text{ [sec]} \qquad \text{(IV)}$$

$$\text{Half-life ozone } (t_{1/2}) = 0.69 \times (\tau) \text{ [sec]} \qquad \text{(V)}$$

II. Results

Calculating BYI08330 by AOPWIN (version 1.91) by using the standard data of Atkinson's list the resulting overall OH reaction rate of 76.1856 10^{-12} cm^3/molecules-sec was mainly obtained by various hydrogen abstractions (k_{abs}) and OH addition to the olefinic bond (k_{add}). Based on the before-mentioned calculated overall OH rate constant and using a 12-hrs-day with 1.5 x 10^{+6} OH radicals/cm^3 the following was assessed:

half-life of BYI08330 in air = 1.685 hrs
chemical lifetime (τ) of BYI08330 in air = 2.4 hrs.

Further considering the reactions with ozone (the k_{ozone} was estimated to be 2.1 x 10^{-17} cm^3/molecules-sec), by which a half-life of 13.1 hrs results, and some uncertainties noted as ** in the estimation, the maximum overall chemical lifetime (τ) of BYI08330 in air is assessed to be not more than 3 hours.

The before-mentioned estimations do not consider any contribution of an attack by other radicals (*i.e.* by nitrate radicals). Whenever the active substance is applied during early afternoon (in opposite to early morning or late afternoon), it is to be expected that the chemical lifetime is shorter at that moment, as during the day the OH-radical concentration in the troposphere may increase unto 5 x 10^{+6} radicals/cm^3. On the other hand, the OH-radical concentration in the night decreases to zero.

III. Conclusion

Reactions with OH radicals and ozone contribute to the degradation of BYI08330 in the air to a high extent. The chemical stability of BYI08330 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.
On account of an estimated chemical lifetime of BYI08330 in the air of at the most 3 hours it is to be expected that the active ingredient can not be transported in gaseous phase over large distances and can not accumulate in the air. Thus, no difference in the behavior between BYI08330 and other organic substances emitted into the air from natural sources (e.g. from plants, soil) is indicated.

Report:	**KIIA 7.10/02, [redacted]; 2004 (MEF-04/401)**
Title:	**Calculation of the chemical lifetime of BYI08330-enol in the troposphere**
Report No &	**MEF-04/401**
Document No	**M-092841-01-1**
Guidelines:	EU Commission Directive, 95/36/EC, amending Council Directive 91/414/EEC, Annexes I+II, Fate and Behavior in the Environment, 7171/VI/94-EN, 7.2.2.3 Photolysis in Air; Federal Biological Institute for Agriculture and Forestry (BBA), Germany, Guidelines for the Testing of Plant Protection Products in Registration Procedure, Part IV, 6-1 (July 1990) entitled "Determination of the volatilization and the fate of plant protectants in the air" BBA-Guidelines for Testing of Plant Protectants in the Registration Process. Part IV, 6-1, Determination of the volatilization and the fate of plant protectants in the air, 1990
GLP	Not applicable (calculation)
Testing Laboratory and dates	Bayer CropScience AG, RD, Metabolism and Environmental Fate, D-[redacted], Germany, conducted the assessment in September 2004. Completion date: 2004-09-21

Executive Summary

Based on the estimation method according to structure-activity relationship (SAR) methods developed by Roger Atkinson and co-workers the chemical lifetime of the BYI08330-enol in the air was assessed by the program AOPWIN (version 1.91). A value (τ) of at the most 3 hours corresponding to a half-life of approx. 2 hours resulted, 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 BYI08330-enol.

Reactions with OH radicals and ozone contribute to the degradation of BYI08330-enol in the air to a high extent. The chemical stability of BYI08330-enol 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.

I. METHODS

See for report KIIA 7.10/01 described before.

II. Results

Calculating BYI08330-enol by AOPWIN (version 1.91) by using the standard data of Atkinson's list the resulting overall OH reaction rate of 74.6640 10^{-12} cm^3/molecules-sec was mainly obtained by various hydrogen abstractions (k_{abs}) and OH addition to the olefinic bond (k_{add}). Based on the before-mentioned calculated overall OH rate constant and using a 12-hrs-day with 1.5 x 10^{+6} OH radicals/cm^3 the following was assessed:

half-life of BYI08330 in air = 1.719 hrs

chemical lifetime (τ) of BYI08330 in air = 2.5 hrs.

Further considering the reactions with ozone (the k_{ozone} was estimated to be 2.1 x 10^{-17} cm^3/molecules-sec), by which a half-life of 13.1 hrs results, and some uncertainties noted as ** in the estimation, the maximum overall chemical lifetime (τ) of BYI08330-enol in air is assessed to be not more than 3 hours.

The before-mentioned estimations do not consider any contribution of an attack by other radicals (*i.e.* by nitrate radicals). Whenever the active substance is applied during early afternoon (in opposite to early morning or late afternoon), it is to be expected that the chemical lifetime is shorter at that moment, as during the day the OH radical concentration in the troposphere may increase unto 5 x 10^{+6} radicals/cm^3. On the other hand, the OH radical concentration in the night decreases to zero.

III. Conclusion

Reactions with OH radicals and ozone contribute to the degradation of BYI08330-enol in the air to a high extent. The chemical stability of BYI08330-enol 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.
On account of an estimated chemical lifetime of BYI08330-enol in the air of at the most 3 hours it is to be expected that the active ingredient can not be transported in gaseous phase over large distances and can not accumulate in the air. Thus, no difference in the behavior between BYI08330-enol and other organic substances emitted into the air from natural sources (e.g. from plants, soil) is indicated.

IIA 7.11 Definition of the residue

By all the studies performed under laboratory conditions a comparatively short half-life of BYI08330 was determined. The major metabolite detected was the BYI08330-enol which further degrades well via BYI08330-ketohydroxy and many others thoroughly.

Thus, the proposed definition of residue in soil, in water and in air for enforcement purposes is BYI08330 and BYI08330-enol, only.

IIA 7.12 Monitoring data concerning fate and behavior

Plant protection products containing BYI08330 are not yet authorized for use. Accordingly, monitoring data concerning fate, behavior and concentration in the environment are not available so far.

IIA 7.13 Other/special studies

Other environmental fate studies than those presented in the earlier chapters have not been performed or are not applicable to this submission. However, some further studies in order to get a basic physical-chemical data set for major metabolites (e.g. for modeling purposes) were performed as follows.

Physical-chemical data of BYI08330-enol

Report:	**KIIA 7.13/01, [redacted] and [redacted], 2006 (PA06/035)**
Title:	**Water Solubility of BYI08330-enol (AE 1302944) at pH 5, pH 7 and pH 8 (Flask Method)**
Report No & Document No	**PA06/035** **M-275829-01-2**
Guidelines:	Guideline 92/69/EEC, appendix A.6; OECD-Guideline 105
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and dates	Bayer CropScience GmbH, Product Technology Analytics [redacted], D-[redacted], Germany, conducted the study in April 2006. Completion date: 2006-08-11

Summary

The water solubility C_S of the test item at pH 5, pH 7 and pH 8 has been determined according to the "flask method" described in guideline 92/69/EEC, appendix A.6 and OECD-guideline 105.

The results of the solubility measurements are given in the following table:

Table IIA 7.13-1: Water solubility of BYI08330-enol (PA06/035)

Solubility in	pH range	C_S Solubility at 20°C	SD [g/L]	RSD [%]
buffer pH 5	4.98 [1)]	0.09 g/L	0.01	14.3
buffer pH 7	6.97 – 6.99 [1)]	2.7 g/L	0.003	0.1
buffer pH 8 [2)]	7.82 – 7.83 [1)]	28 g/L	0.3	1.0

[1)]: pH- range of 3 experiments

[2)]: Due to the strong acidic character of the test item the buffer capacity of the used buffer pH 9 was not sufficient. Therefore, the pH value of the saturated solution declined to pH 8.

Report:	**KIIA 7.13/02, [redacted] and [redacted], 2006 (PA06/036)**
Title:	**Partition Coefficients 1-Octanol / Water of BYI08330-enol (AE 1302944) at pH 5, pH 7 and pH 9 (Shake flask method)**
Report No & Document No	**PA06/036 M-276091-01-2**
Guidelines:	EEC Test Guideline 92/69, A.8.; OECD Test Guideline 107
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and dates	Bayer CropScience GmbH, Product Technology-Analytics [redacted], D-[redacted] [redacted], Germany, conducted the study in May 2006. Completion date: 2006-08-17

Summary

The partition coefficients 1-octanol / water of the test item BYI08330-enol (AE 1302944) at pH 5, pH 7 and pH 9 at room temperature have been determined according to the "flask-shaking" method described in EEC Test Guideline 92/69, A.8. and OECD Test Guideline 107

The partition coefficients (1-octanol / water) of the test item were:

Table IIA 7.13-2: Partition coefficients (1-octanol / water) of BYI08330-enol (PA06/036)

	pH 5	pH 7	pH 9
log Pow	2.0	0.3	-1.3
Pow	109	2.1	0.05

Report:	**KIIA 7.13/03, [redacted], [redacted] and [redacted]., M. 2006 (AF06/031)**
Title:	**BYI08330-enol (AE 1302944): Determination of the Dissociation Constant (Spectrophotometric Screening Method)**
Report No & Document No	**AF06/031 M-274084-01-2**
Guidelines:	Based on OECD Test Guideline 112
GLP	None GLP screening test
Testing Laboratory and dates	Bayer CropScience GmbH, Product Technology-Analytics [redacted], D-[redacted] [redacted], Germany, conducted the study in the period from April to May 2006. Completion date: 2006-07-05

Summary

The dissociation constant of BYI08330-enol was determined using a spectrophotometric method, based on the OECD-Guideline 112. The experimental work has been performed by Dr. M. [redacted] (PT - Analytics [redacted]). An instrument for pKa screening measurements from Sirius Analytical Instruments Ltd (UK) was used (type GLpKa with D-Pas, Dip Probe Absorption Spectroscopy).

The dissociation constant (pKa) of BYI08330-enol (AE 1302944) was 5.2.

Physical-chemical data of BYI08330-ketohydroxy

Report:	**KIIA 7.13/04, [redacted] and [redacted], 2006 (PA05/099)**
Title:	**Water Solubility of BYI 08330-cis-ketohydroxy in Distilled Water (Flask Method)**
Report No & Document No	**PA05/099 M-268425-01-2**
Guidelines:	Guideline 92/69/EEC, appendix A.6; OECD-Guideline 105
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and dates	Bayer CropScience GmbH, Product Technology-Analytics [redacted], D-[redacted], Germany, conducted the study in September 2005. Completion date: 2006-03-17

Summary

The water solubility C_S of the test item has been determined in distilled water according to the "flask method" described in guideline 92/69/EEC, appendix A.6 and OECD-guideline 105.
The results of the solubility measurements are given in the following table:

Table IIA 7.13-3: Water solubility of BYI08330-ketohydroxy in distilled water (PA05/099)

Cs Solubility at 20 °C	SD [g/L]	RSD [%]
0.228 g/L	0.0042 g/L	1.8 %

Report:	**KIIA 7.13/05, [redacted] and [redacted], 2006 (PA05/098)**
Title:	**Partition Coefficient 1-Octanol/Water of BYI08330-cis-ketohydroxy (HPLC Method)**
Report No & Document No	**PA05/098 M-268428-01-2**
Guidelines:	92/69/EEC A.8, OECD TG 117, OPPTS 830.7570
GLP	Fully GLP compliant - laboratory certified by German "Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen".
Testing Laboratory and dates	Bayer CropScience GmbH, Product Technology-Analytics [redacted], D-[redacted], Germany, conducted the study in September 2005. Completion date: 2006-03-17

Summary

The partition coefficient (1-octanol/water) of BYI08330-ketohydroxy was determined according to OECD test guideline 117, EEC-guideline 92/69/EEC A.8 and EPA guideline OPPTS 830.7570, HPLC-method. Nine calibration substances were injected into a HPLC-system under the same analytical conditions (column temperature 40°C) as the test item. Calibration curves were created by using the measured retention times (log k'-values) and the known log P_{OW}-values of the calibration substances for linear regression.

The measured log k'-values of the test item were within the calibrated log k'-range. From the resulted calibration curves and their equations the log P_{OW} of the substance has been interpolated. The partition coefficients (1-octanol/water) of the test item were:

Table IIA 7.13-4: Partition coefficients (1-octanol/water) of BYI08330-ketohydroxy (PA05/098)

	pH 7
log Pow	1.3
Pow	20

Report:	**KIIA 7.13/06, [redacted], [redacted] and [redacted], M., 2006 (AF05/098)**
Title:	**BYI08330-cis-ketohydroxy (AE 1422479): Determination of the Dissociation Constant (Spectrophotometric Screening Method)**
Report No & Document No	**AF05/098** **M-263603-01-2**
Guidelines:	Based on OECD Test Guideline 112
GLP	None GLP screening test
Testing Laboratory and dates	Bayer CropScience GmbH, Product Technology-Analytics [redacted], D-[redacted] Germany, conducted the study in October 2005. Completion date: 2005-12-16

Summary

The dissociation constant of BYI08330-ketohydroxy was determined by using a spectrophotometric method based on the OECD Test Guideline 112. The experimental work has been performed by Dr. M. [redacted] (PT - Analytics [redacted]). An instrument for pKa screening measurements from Sirius Analytical Instruments, Ltd (UK) was used (type GLpKa with D-Pas, Dip Probe Absorption Spectroscopy).

The dissociation constant (pKa) of BYI08330-ketohydroxy (AE 1422479) was 11.0.

Overall summary: Fate and behavior in the environment

Fate and behavior in soil

The basic soil metabolism study showed that parent compound BYI08330 is quickly degraded. Already after 1-2 days more than 90% of the test item dissipated and declined. At study termination, evolved $^{14}CO_2$ (no volatile organics occurred) accounted for up to 19.4% of AR at DAT-50 (EU soils), and accounted for 15.3% of AR for the US soil after 360 days. During the course of the study a number of degradates was observed in all four soils. Besides the two main soil metabolites BYI08330-enol (max. 24.3% of AR at DAT-3) and BYI08330-ketohydroxy (max. 16.3%, DAT-1), two enol-dimers (more or less artificially formed) and BYI08330-MA-amide (max. 6.4%, DAT-179; were identified. In addition, two minor degradates were identified as BYI08330-desmethyl-enol and BYI08330-oxo-enol amounting to maximum 3.7% and 1.2% of AR, respectively.

Furthermore, the biotransformation of spirotetramat was investigated in two soils using [azaspirodecenyl-3-^{14}C]BYI08330 for 127 days under outdoor climatic conditions realistic for the intended use. Thereby BYI08330 formulated as an OD 100 (pH 5) was applied at 94.6% of the highest recommended single use rate for field application (288 g/ha). The parent compound was quickly and thoroughly degraded, and already one day after application, only 53.6 and 72.2% of the applied test item were detectable in both soils. During the course of the study a number of degradates was observed in all four soils. Only two major degradates were detected, BYI08330-ketohydroxy (max. 25.3% AR, DAT-14) and BYI08330-enol (max. 7.8% AR, DAT-7). Three minor metabolites were identified as glyoxylic amide, benzoic acid and ketohydroxy-carboxy. The results obtained confirmed and completed the pathway already established in the guideline aerobic soil metabolism laboratory studies.
In the BYI08330 studies the soil processing procedure was optimized to get >90% extraction efficiency and >90% recovery of the test item at time zero. However, under the acidic extraction conditions needed for spirotetramat, the major metabolite BYI08330-enol was found to be partly unstable. It degrades under the formation of BYI08330-ketohydroxy and others. Therefore, the degradation and metabolism of the BYI08330-enol in soil was investigated in a separate study (see below), and those results needed to be included in the proposed overall metabolic pathway of spirotetramat in soil (see Figure IIA 7.1-1). This fact was also the reason to base the degradation kinetics of the major spirotetramat metabolites on the BYI08330-enol study, but not on the parent study.

Thus, the biotransformation of [azaspirodecenyl-3-^{14}C] and [azaspiro-decenyl-5-^{14}C]BYI08330-enol was studied in three EU soils and one US soil for 119 days under aerobic conditions in the dark at 20 ±1 °C and at approx. 80% of 1/3 bar moisture (US soil) or 60% WHC_{max} (EU soils). BYI08330-enol dissipated following pronounced biphasic kinetics, with an extremely quick first phase. Within a second slower degradation phase, the test item declined to 2.7 to 6.1% of AR in the four soils at the end of the study at DAT-119. During the course of the study a number of degradates was observed in all four soils. Label-specific degradates were not observed throughout the entire study, and the degradation pathway found in before-mentioned study on spirotetramat was confirmed. In addition, a metabolite previously not found, the BYI08330-oxo-ketohydroxy was identified. However, it was a very minor contaminant and was not quantifiable. In all four soils, BYI08330-ketohydroxy was analyzed at levels > 10%. BYI08330-enol-dimer 1 amounted once to 5.0% (DAT-60) in one soil, and BYI08330-enol-dimer 2 to a maximum of 3.6% (DAT-14). BYI08330-MA-amide amounted once >5% (5.2% at DAT-4), and BYI08330-desmethyl-enol was max. 1.8% at DAT-4. All degradates were transient during the study and did not increase towards the end of the study.
Based on the degradate profiles obtained within an anaerobic soil metabolism study, a degradation pathway was proposed which is almost identical to the degradation pathway obtained in aerobic soil. It is concluded that BYI08330 applied to soil will be degraded rapidly in a subsequently flooded anaerobic

soil situation, and will not form degradates different from those observed in soil under aerobic conditions, and/or known from abiotic hydrolysis experiments (see section later).

The parent compound was well degraded on irradiated soil samples of phototransformation study on soil surface. However, the biotransformation in the dark controls was approx. four times faster and, considering real environmental conditions (e.g. in June at [redacted], Greece) even approx. 20 times faster compared to soil samples irradiated by natural sunlight. This kinetics results together with the findings that the pathway of degradation was similar indicate that a distinct phototransformation product is not to be expected in soil after the use of spirotetramat under outdoor conditions.

A study using sterile soil surface confirmed the before-mentioned findings that a major photo-transformation product is not to be expected. Furthermore, contrary to the non-sterile soil study not any dimers were found, and higher portions of bound residues were not formed. It can be concluded that bound residues of Spirotetramat were formed exclusively by microbial activity and thus indirectly indicating their irreversible nature.

Referring to the behavior in the environment it can be concluded that the active substance spirotetramat (BYI08330) predominantly degrades to the metabolite BYI08330-enol which is further oxidized to BYI08330-ketohydroxy. Subsequently BYI08330-ketohydroxy is hydrolytically opened to BYI8330-MA-amide, as it is included in the proposed overall metabolic scheme outlined in Figure IIA 7.1-1. All components are subject to further degradation to form non-extractable residues (NER) and CO_2.

The found normalized geometric mean BYI08330 DT_{50} value of 0.13 days is a suitable input parameter for environmental fate models. Further, the kinetics of biotransformation of spirotetramat was investigated in two soils using ^{14}C-BYI08330 for 127 days under outdoor climatic conditions realistic for the intended use. Thereby ^{14}C-BYI08330 formulated as an OD 100 (pH 5) was applied at 94.6% of the highest recommended single use rate for field application (288 g/ha). The parent compound was quickly and thoroughly degraded, and a mean DT_{50} of approx. 2 days was estimated.

The investigation of BYI08330-enol as test item showed that it dissipates following a biphasic kinetics, with an extremely quick first phase. This portion is regarded as a strong bound fraction of BYI08330-enol in soil. The respective kinetic modeling of test item by using MatLab® (application KinGUI) indicated that the best fit DT_{50} (days) of test item resulted by using the bi-exponential model DFOP (double first order in parallel). This model yielded a mean BYI08330-enol DT_{50} value of 0.08 days (chi^2 statistics mean value of 7.7). Thus it can be concluded that BYI08330-enol is a fast degrading major metabolite of spirotetramat in soil.
A more detailed kinetics modeling investigation based on the results of BYI08330-enol studies yielded geometric mean normalized $DT_{50\text{-ref}}$ values of 0.03 days for BYI08330-enol, 3.8 days for BYI08330-ketohydroxy and 1.0 days for BYI08330-MA-amide. They are suitable input parameters for environmental fate models. It must be noted, that the extremely short half life time of 0.03 days for BYI08330-enol may only be used for modeling purposes and in conjunction with the SFORB model or a kinetic sorption model as implemented in PEARL.

Based on the results obtained within a further laboratory soil degradation study using three aerobic soils it was shown that the metabolite 4-methoxy cyclohexanone is fast and steadily degrading in soil (DT_{50} < 1 day) and that there is no potential for accumulation of 4-methoxy cyclohexanone residues in viable soils. The observed higher level of 4-methoxy cyclohexanone residues in the laboratory study on phototransformation of BYI08330 on soil surface might have been caused by a decreasing viability of test soil during the strong irradiation in such a laboratory test system.

From an anaerobic soil metabolism study it is concluded that BYI08330 applied to soil will be degraded rapidly in a flooded anaerobic soil situation, and will not form degradates different from those observed

in soil under aerobic conditions, and/or known from abiotic hydrolysis experiments. Compared to the before mentioned fast biotransformation in dark soils (DT_{50} < 1 day) phototransformation of BYI08330 on soil surface is not regarded as a relevant degradation process under environmental sunlight irradiation conditions. These findings were confirmed by a phototransformation study carried out with sterile soil.

From all the laboratory studies and a radiolabeled outdoor study it can be concluded that spirotetramat is a very fast degrading compound in soil, and all metabolites generated from BYI08330-enol, the predominant first metabolite, are further degraded and are expected not to accumulate in the environment. The soil dissipation testing in a range of representative soils and locations in the USA confirmed that findings.

In order to determine the residues during US terrestrial field dissipation trials an analytical method (EN-002-S05-02) for the determination of BYI08330 and its metabolites BYI08330-enol, BYI08330-ketohydroxy and BYI08330-MA-amide in soil and sediment by LC/MS/MS was developed and successfully validated for the determination of residues in soil and sediment. The method was evaluated by determining the average recoveries and relative standard deviation at the LOQ of 5 ng/g and at 5x LOQ (25 ng/g).
BYI08330 dissipated rapidly in soil under field conditions. The dissipation rates of BYI08330 calculated for four sites in the US, resulted in half-life (DT_{50}) values from 0.9 to 1.0 days and the periods required for 90% dissipation (DT_{90}) ranged 1.1 to 3.5 days with no apparent obvious correlation with soil properties or the management (bare ground vs. cropped). The DT_{50} values of the combined residues of BYI08330 (i.e. BYI08330, BYI08330-enol, and BYI08330-ketohydroxy) ranged from 5.0 to 23.4 days and the DT_{90} values ranged from 16.7 to 77.8 days.
Residues of BYI08330 did not move below the surface layer (0 to 15 cm) in all the sites, except in Florida where residues of BYI08330-enol and BYI08330-ketohydroxy were detected above the LOQ at 15 to 30 cm layer between 1 day and 7 days after application. After that the residues completed degradation to less than LOQ and MDL. It should be noted that the Florida site represents a worst case condition with heavy rainfall and very light soil (95% sand in the surface layer) with very low organic matter (0.5 %). Therefore, leaching and groundwater contamination is not likely with BYI08330.
BYI08330 degraded to less than the MDL levels (0.5 µg/kg) within 14 days after application. The soil concentration the metabolites of BYI08330 were below the LOQ within 28 to 365 days after application. Based on these results, the carry over potential of soil residues from one year to another is very low.

Considering the results from laboratory soil metabolism studies and terrestrial field dissipation studies the major route(s) of dissipation for BYI08330 are degradation to BYI08330-enol and BYI08330-ketohydroxy, subsequent biodegradation to non-extractable soil residues and mineralization to CO_2.

BYI08330 and its metabolites BYI08330-ketohydroxy and BYI08330-MA-amide showed no evidence of any degradation in the four soils during a maximum storage interval of 334 days in frozen storage and there was little variation in the results for the four soils. BYI08330-enol recoveries declined during storage, with the majority of the loss occurring during the first 30 days of storage. The primary causes of these low recoveries were degradation of BYI08330-enol to BYI08330-ketohydroxy and binding of the analyte to soil.

Mobility

Freundlich adsorption and desorption constants K_F and K_{OC} of spirotetramat have been determined in batch equilibrium experiments with five different soils using radiolabeled test substance ([azaspirodecenyl-3-^{14}C]BYI08330). Since significant degradation of test item was observed in a pre-

test, the main test was performed with sterilized soil. K_{OC} values for the different soils were in the range of 159 to 435 mL/g with a mean K_{OC} of 281 mL/g (1/n = 0.941). Based on this value, spirotetramat can be classified as low mobile in soil.

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-enol, the major metabolite in soil, was attempted in batch equilibrium experiments with five different soils using radiolabeled test substance ([azaspirodecenyl-3-^{14}C]BYI08330-enol. However, the study showed that the sorption characteristics of BYI08330-enol to soil cannot be determined by a batch equilibrium test according to OECD Guideline 106. In order to asses the environmental behavior of the test item more suitable test methods had to be employed. Another option, i.e. a so-called time-dependent sorption study, demonstrated that sorption and binding of BYI08330-enol to soil is extremely fast and increases very rapidly with aging time in soil. The portion not tightly bound to soil, i.e. the portion that is releasable by aqueous solution from soil (weakly sorbed), is degraded within a few hours. From these results it can be concluded that BYI08330-enol is absent from the soil pore water (either degraded or tightly bound to soil) within a very short period of time This study confirmed that the sorption characteristics of the test item BYI08330-enol to soil cannot be determined accurately by a batch equilibrium test according to OECD TG 106. Since only partial degradation of BYI08330-enol occurred during the course of a soil column leaching study performed with four soils the test system allowed the calculation of adsorption constants for the test item in soils. For the strongly bound BYI08330-enol fraction K_{OC} values between 828 and 1711 mL/g were calculated, resulting in a mean value of 1187 mL/g over four soil types. For the weakly bound BYI08330-enol fraction K_{OC} values between 27 and ca. 99 mL/g were calculated, resulting in a mean value of 55 mL/g over four soil types. Based on the classification of soil mobility potential according to Briggs, the strongly sorbed BYI08330-enol fraction is classified as immobile, and the weakly bound BYI08330-enol fraction has an intermediate potential to leach through soil.

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-ketohydroxy, a major metabolite in soil, have been determined in batch equilibrium experiments with five different soils using [azaspirodecenyl-3-^{14}C]BYI08330-ketohydroxy. Since significant degradation of BYI08330-ketohydroxy was observed in a pre-test, the equilibration solution used was 0.01 M aqueous $CaCl_2$ solution spiked with 50 mg $HgCl_2$ as biocide. $K_{OC(ads)}$ values for the different soils were in the range of 41.0 to 99.1 mL/g with a mean K_{OC} of 63.7 mL/g (1/n = 0.922). Based on this value, BYI08330-ketohydroxy can be classified as intermediate to mobile in soil.

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI08330-MA-amide have been determined in batch equilibrium experiments with five different soils using [hydroxy-^{14}C]BYI08330-MA-amide. The calculated adsorption constants K_F of the Freundlich isotherms for the four test soils ranged from 0.06 to 0.18, and the $K_{OC(ads)}$ values were in the range of 4.4 to 25.5 mL/g with a mean $K_{OC(ads)}$ of 9.3 mL/g (mean 1/n = 0.948). Based on this value, BYI08330-MA-amide can be classified as high mobile in soil. The desorption K_{des} values were 0.13 to 0.37 and higher than those obtained for K_F in the adsorption phase indicating a little stronger binding once adsorbed to soil.

BYI08330-enol dimers 1 and 2 were minor metabolites in the relevant metabolism studies. Nevertheless, their adsorption coefficients on soil were estimated by using the HPLC method according to OECD TG No. 121. Based on the resulting calibration equation for pH 6, the soil adsorption coefficients of BYI08330-enol dimer 1 were estimated to be log K_{OC} = 3.23 and K_{OC} = 1708. For the BYI08330-enol dimer 2 a log K_{OC} = 3.46 and a K_{OC} = 2896 were estimated. According to the Briggs' classification BYI08330-enol dimer 1 and BYI08330-enol dimer 2 would be categorized as immobile.

From all the before mentioned laboratory studies it is concluded that the mobility of spirotetramat residues in soil is sufficiently understood. Since a long-term leaching simulations indicated that the PEC_{GW} values are generally far below 0.1 µg/L in all application scenarios relevant for the intended uses of spirotetramat on citrus, oranges, mandarins, lemons, limes, etc. in EU-South, and on lettuce in EU-

North and EU-South, no concern with regard to groundwater contamination of parent compound and its metabolites is indicated, and a safe use is given in the EU.

Volatility

Based on the vapor pressure of BYI08330 of 5.6 x 10^{-9} Pa and BYI08330-enol[5] of 1.2 x 10^{-10} Pa, considerable volatilization of both substances when applied to soil surfaces or leaves is not to be expected. This evaluation is also confirmed by the rating of trigger values for volatilization as described in the model EVA 2.0 developed from the FOCUS Air group. There, for compounds with a vapor pressure < 10^{-4} Pa at 20 °C volatilization from soil surfaces or with a vapor pressure < 10^{-5} Pa at 20 °C volatilization from plant surfaces is not considered relevant.

Fate and behavior in water

The fate and behavior of spirotetramat in aquatic systems was investigated under standardized laboratory conditions, using radiolabeled as well as unlabeled test substance. Under dark conditions spirotetramat was found to be degradable by abiotic degradation processes. Hydrolysis is regarded as relevant for the degradation of BYI08330 in the environment, especially under neutral and alkaline conditions. The hydrolytic half-life at pH 7 and 25°C (20°C) is expected to be in the range of 8.6 days (13 days). In the total pH range tested (pH 4 to 9) the formation of BYI08330-enol as the only common hydrolysis product was observed. From a separate study investigating the hydrolysis of the major degradate it was concluded that hydrolysis is not relevant for the degradation of BYI08330-enol in the environment, since the hydrolytic half-life at pH 4, 7 and 9 at 25°C is expected to be > 1 year.
BYI08330-ketohydroxy is hydrolytically stabile under acidic conditions but labile at a pH above 7. The experimental half-lives of BYI08330-ketohydroxy at pH 9 were 4.9 days (25°C) and 15.6 days (20°C). Thereby, one major degradation product is formed. The formation of BYI08330-MA-amide as a common hydrolysis product was observed at pH 7 and pH 9. That metabolite is considered as hydrolytically stable since its concentration increased towards the end of the incubation period at pH 7 and pH 9.

Based on the experimental DT50 of 2.7 days for BYI08330 in sterile pure buffered water and related predicted environmental DT50 (e.g. of 12.9 solar summer days at [redacted], AZ, USA or 19.9 summer days at [redacted], Greece) it was concluded that photo-transformation of BYI08330 in aqueous systems is a significant route for the elimination of this compound. However, that basic tests are to be performed under sterile conditions in highly purified buffer of pH 5, in order to help distinguish between hydrolytic or/and biotic and direct photolytic reactions. Thus, it was expected that the behavior will be different in natural aqueous systems, since in the case of BYI08330 biodegradation will happen quickly, hydrolysis will be faster with increasing pH, as well as indirect reactions might compete with the re-arrangement reactions observed in the prevailing study. This expectations were confirmed by an investigation of the phototransformation of [^{14}C]BYI08330 (labels #1 and #2) in sterile natural water by a supportive study. Based on the experimental DT50 of 0.2 days for BYI08330 and related predicted environmental DT50 (e.g. of 0.6 solar summer days at [redacted], AZ, USA or 1.0 summer days at [redacted], Greece) it is concluded that photo-transformation of BYI08330 in aqueous systems is a significant route for the elimination of this compound in natural water. This test clearly showed that competition of hydrolysis and indirect photo-reactions does not allow the light-induced re-arrangement reactions of parent compound observed in highly purified buffer of pH 5. Together with the well-known fast biodegradation

[5] : The vapor pressure was estimated by using MPBPWIN v1.41 (MPBPWIN^{TM} is owned by the U.S. E PA): acc. to Modified Grain Method a vapor pressure of 0.912 x 10E-12 mm Hg at 20 °C resulted, then transferred to Pa by a factor of 133.3.

this was the justification to consider the products formed in the natural water study as relevant for the overall pathway of spirotetramat degradation in water (see Figure IIA 7.8.3-2), but not the re-arrangement photo-products found in the highly artificial laboratory study performed in sterile pure buffer.

From a laboratory study investigating the route and rate of degradation in two natural water/sediment systems under aerobic laboratory conditions in the dark at 20 °C it is concluded that BYI08330 once entering aqueous systems will be degraded rapidly and thoroughly, mainly via the metabolites BYI08330-enol and BYI08330-ketohydroxy. DT50 values of 1.00 and 1.02 days were calculated for BYI08330 in the water phase, and 1.06 and 1.05 days in case of the entire systems, respectively. For use as persistence endpoints conservative one compartment (Level I) SFO dissipation half-lives of BYI08330, BYI08330-enol and BYI08330-ketohydroxy were derived for the water and the sediment compartment in a special modeling study. The biphasic model FOMC model was tested, though did not improve the outcome for any of the compounds. SFO total system degradation half-lives were derived to be used as modeling endpoints for both compartments at FOCUS surface water STEP 2 level and STEP 3 level.
The results of a laboratory study investigating the route and rate of degradation in a completely anaerobic water/sediment system in the dark at 20 °C, showed that BYI08330 once entering an anaerobic natural aqueous environment will also be degraded rapidly, mainly to the metabolite BYI08330-enol well known from the studies in aerobic soil and water/sediment systems, already. The first-order degradation rate calculated for BYI08330 in anaerobic water, sediment, and in the entire system resulted in half-lives of 2.8, 3.1, and 2.8 days, respectively.

Fate and behavior in air

Reactions with OH radicals and ozone contribute to the degradation of BYI08330 and BYI08330-enol in the air to a high extent. The chemical stabilities in air are 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.
On account of an estimated chemical lifetime of both compounds in the air of at the most 3 hours it is to be expected that they can not be transported in gaseous phase over large distances and can not accumulate in the air.