Document Title

Tier 2 Summary of Fate and Behaviour in the Environment for Flupyradifurone (BYI 02960)

Data Requirements

Regulation (EC) No 1107/2009

Regulatory Directive 2003-01/Canada/PMRA

OPPTS guidelines/US/EPA

Annex IIA
Section 5, Point 7
Document M

According to OECD format guidance for industry data submissions on plant protection products and their active substances

Date

2012-04-20

Author(s)

[redacted]

Bayer CropScience

OWNERSHIP STATEMENT

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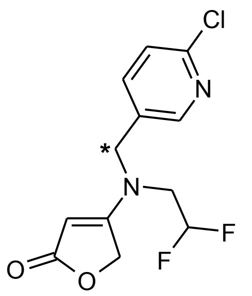
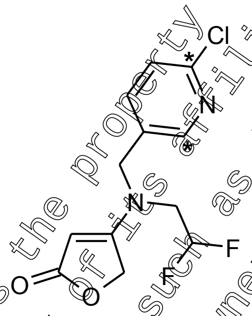
IIA 7 Fate and Behaviour in the Environment

Information is provided in this chapter with respect to the fate and the behavior in soil, water and air of flupyradifurone (BYI 02960). This active substance is an insecticide which is active against various pests such as aphids, white flies and hoppers in many target crops such as fruits, vegetables, plantations, cereals and soybean.

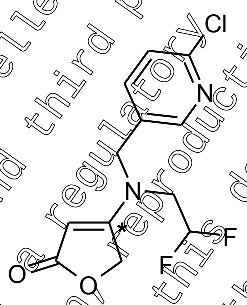
The studies concerning the fate and behavior of BYI 02960 in the environment were conducted using different radiolabelled forms, [pyrindinyl-methyl-^{14}C], [pyridine-2,6-^{14}C], [furanone-4-^{14}C] and [ethyl-1-^{14}C]BYI 02960 as well as the non-labeled parent compound. These label positions are sufficient to define the degradation pathway. In the Tier II summaries that follow, the different radiolabels are referred to as PYM (= pyrindinyl-methyl-14C-label), PYR (= pyridine-2,6-^{14}C-label), FUR (= furanone-4-^{14}C-label) and ETH (= ethyl-1-^{14}C-label). The structure of BYI 02960 and the positions of the different radiolabels are as follows:



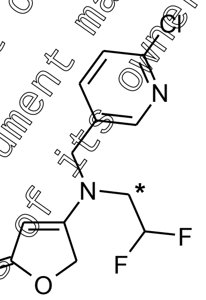
[Pyridinyl-methyl-14C]
BYI 02960
(PYM-^{14}C)



[Pyridine-2,6-^{14}C]
BYI 02960
(PYR-^{14}C)



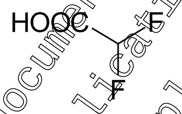
[Furanone-4-^{14}C]
BYI 02960
(FUR-^{14}C)



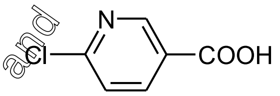
[Ethyl-1-^{14}C]
BYI 02960
(ETH-^{14}C)

* indicates position of radiolabel

The results of the studies are summarized in the following chapters. The proposed metabolic pathways in soil and water are given in Figure 7.1.2- 1 and Figure 7.8-1. In addition, studies were performed with the following metabolites (radiolabelled or non-radiolabelled):



Difluoroacetic acid
(DFA)



6-Chloronicotinic acid
(CNA)

In original reports study authors may have used different names or codes for degradation products of BYI 02960. In this summary, a single name and a single code number is used for each metabolite, details are given at the end of the section . In Document N of this dossier a full list of metabolites contains the structural formula, various names, short forms and code numbers attributed to the metabolites.

IIA 7.1 Route of Degradation in Soil - Laboratory Studies

IIA 7.1.1 Aerobic Degradation

Report:	**KIIA 7.1.1/01, [redacted], 2011**
Title:	[Pyridinylmethyl-14C]BYI 02960: Aerobic soil metabolism/degradation and time-dependent sorption in soils
Report No & Document No	MEF-07/334 M-414615-01-2
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil** **US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008,** **OECD: Guideline 106: Adsorption/Desorption, 2001 (only in parts)**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The biotransformation and time dependent sorption of [pyridinylmethyl-^{14}C]BYI 02960 was studied in four European soils: [redacted] [redacted] (AX), [redacted] am [redacted] (HF), [redacted] Plot 611 (HN), and [redacted] II (DD) for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 0.53 mg/kg soil, which is equivalent to 200 g/ha field application rate.

At each sampling date the soil samples were shaken for 24 hours with 400 mL $CaCl_2$-solution in order to measure the time-dependent desorption of the test item. Subsequently they were extracted by shaking at ambient temperature and in a microwave at 70 °C with acetonitrile/water mixtures, and the BYI 02960 residues were analyzed and quantified by TLC with HPLC as the confirmatory method.

Material balances were complete throughout the study, and the test item declined from 97.1, 96.1, 96.5 and 93.1% AR at DAT-0 to 37.1, 24.5, 50.2 and 28.7% in soils AX, HF, HN and DD, respectively, at the end of the study. Applying double first-order kinetics a half-life (geometric mean) of 68.8 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

The mineralization of [PYM-^{14}C]BYI 02960 in this study was high. At the end of the study (DAT-120) up to 45.3 (AX), 58.6 (HF), 29.4 (HN) and 57.3% AR (DD) of $^{14}CO_2$ were generated. Volatile organic compounds were negligible (≤ 0.1% AR). With the exception of carbon dioxide only very minor transformation products (all were below 3% AR) were detected. Non-extractable ^{14}C-residues (NER) increased from 1.0, 1.5, 1.9 and 4.1% AR at DAT-0 to 12.6, 13.2, 16.8 and 12.5% AR at the end of the study period.

The part of the study related to time dependent sorption of [PYM-^{14}C] BYI 02960 is summarized in the mobility chapter (see KIIA 7.4.1/03).

I. MATERIALS AND METHODS

A. Materials

1. Test Item: Flupyradifurone: Code = BYI 02960;
Label PYM = [Pyridinyl-methyl-^{14}C]BYI 02960 (sample ID: BECH 2123)
Specific activity 4.37 MBq/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 confirmed.

2. Soil: The biotransformation of [PYM-^{14}C]BYI 2960 was studied in four different soils. These soils are representative for agricultural use areas as required by the guidelines and cover a representative range of physico-chemical properties. All soils were taken on 2007-03-06 fresh from the fields. Two days later, i.e. four days before starting the test the air dried soils were sieved through a 2 mm sieve. Three days before application aliquots equivalent to 100 g dry matter were weighed into individual 300 mL Erlenmeyer flasks and fitted with trap attachments. The soils were pre-equilibrated at 20 °C in the dark.

Table 7.1.1- 1: Soil physicochemical properties

Parameter	Results/Units			
Soil Batch ID	[redacted] (AX) 20070306	[redacted] am [redacted] (HF) 20070306	[redacted] (HN) 20070306	[redacted] II (DD) 20070307
Location	[redacted] Germany	[redacted] Germany	[redacted] Germany	[redacted], Germany
Soil Taxonomic Classification (USDA)	Sandy floodplain deposits of the lower terrace of the Rhine river, material from the Pleistocene Ice Age	Loess or loess colluvium (Pleistocene, Holocene)	Not available	Not available
Soil Series	Sandy, mixed, mesic Typic Cambudolls	Loamy, mixed, mesic Typic Argudalfs	Not available	Not available
Texture Class (USDA)	Sandy Loam	Silt Loam	Loam	Clay loam
Sand Silt Clay	71 % 16 % 13 %	19 % 62 % 19 %	43 % 39 % 18 %	37 % 34 % 29 %
pH in Water pH in $CaCl_2$ pH in KCl	6.8 6.4 6.2	7.0 6.5 6.2	5.9 5.4 5.0	7.7 7.4 7.1
Organic Matter	2.1 %	3.1 %	4.0 %	7.9 %
Organic Carbon	1.2 %	1.8 %	2.3 %	4.6 %
Soil Microbial Biomass (mg microbial carbon per kg of soil)				
0 days	437	664	495	1908
59 days	283	468	371	1683
120 days	220	418	308	1375
Cation Exchange Capacity (CEC)	7.5 meq/100 g	11.9 meq/100 g	9.7 meq/100 g	20.5 meq/100 g
WHCmax	42.2 %	53.9 %	57.1 %	83.5 %
Moisture at 1/3 bar				

B. Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 19.8 ±0.26 °C. The test system consisted of Erlenmeyer flasks (300 mL) attached with a trap attachment (permeable to 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 (each 21 flasks/soil). For all soils replicates were set up for each sampling (9 sampling dates including time 0). Three flasks for each soil were used for determination of the microbial biomass. The final soil moisture was adjusted to 55% of WHCmax by adding pure water.

2. Test Item Stock Solution: The entire amount of [PYM-^{14}C]BYI 02960 delivered was dissolved in 5 mL acetonitrile / water 1:1 (v/v).

3. Test Item Application Solution: An application solution (total volume 120 mL) was made by diluting 2082 µL of the stock solution with 117.92 mL of distilled water.

4. Mode of Application: On 2007-03-12 aliquots of 991 µL of the application solution were applied in droplets onto each of the 100 g pre-incubated sub-samples from each soil. BYI 02960 was applied to the test soils at a treatment rate of 50.22 µg (219.442 kBq) per vessel. This value is equivalent to 94% of the nominal value of the application rate of 53.33 µg per vessel (calculated for a single application of 200 g BYI 02960 per hectare).

5. Sampling: Microbial biomass was determined prior to commencement of the test (soils sampled at the day of application), after 59 days, and at the end of the study, 120 Days After Treatment (DAT-120). Entire test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-3, DAT-7, DAT-14, DAT-21, DAT-30, DAT-59, and DAT-120.
Prior to opening the incubation flasks (for moistening or sampling of soil), volatile (radioactive) compounds, possibly still present in the flasks, were transferred into the trap attachment by subjecting the flasks to vacuum in an excicator. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

6. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. First, the test soils were shaken for 24 hours with 400 mL $CaCl_2$-solution to measure the time-dependent desorption of the test item. Subsequently they were extracted 4 times by shaking at ambient temperature and once in a microwave at 70 °C with acetonitrile/water mixtures, the BYI 02960 residues were analyzed and quantified by LSC and normal phase Si-60 TLC with HPLC as the confirmatory method. Solid samples (i.e. soil and paper filters) were combusted and ^{14}C levels were measured using LSC. The identity of individual residues was established initially by spectroscopic methods. Within the course of the study compound identities were confirmed by co-chromatography using non-labeled reference substances.

II. RESULTS AND DISCUSSION

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study.

A. Data

The respective data for the four soils are shown in Table 7.1.1- 2 to Table 7.1.1- 5.
The DAT-0 extraction efficiency of total radioactivity was 94.4 to 97.8% AR (mean 96.6% AR; sum of extracts only). The stability of the test item was verified by DAT 0 values of 93.1 to 97.1% AR for the soil extracts (mean 95.2% AR). These results indicated that the extraction method was appropriate for extraction of the applied [^{14}C]-labeled test item from the soil matrix.

Table 7.1.1- 2: Biotransformation of [PYM-^{14}C]BYI 02960 in sandy loam soil AX under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)								
	0	1	3	7	14	21	30	59	120
BYI 02960	97.1 ±0.4	95.9 ±0.1	90.1 ±0.2	86.7 ±0.4	76.5 ±0.3	71.4 ±0.2	63.1 ±0.5	49.9 ±0.0	37.1 ±0.7
ROI 1	n.d.	0.2 ±0.0	1.5 ±0.1	1.4 ±0.0	1.5 ±0.1	0.6 ±0.1	0.5 ±0.0	0.1 ±0.0	0.1 ±0.0
ROI 2	n.d.	0.2 ±0.0	0.2 ±0.0	0.5 ±0.0	1.1 ±0.3	1.3 ±0.1	2.0 ±0.1	2.6 ±0.0	1.9 ±0.6
ROI 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ±0.0
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-characterized radioactivity	0.7 ±0.0	0.2 ±0.1	0.5 ±0.0	0.4 ±0.1	1.0 ±0.1	0.4 ±0.0	0.6 ±0.1	0.4 ±0.0	2.0 ±1.4
Total extractable residues	97.8 ±0.4	96.5 ±0.0	92.3 ±0.4	88.9 ±0.3	80.0 ±0.1	73.8 ±0.0	66.2 ±0.8	53.0 ±0.0	41.3 ±0.2
$^{14}CO_2$	n.a.	0.3 ±0.0	1.4 ±0.0	4.7 ±0.0	10.8 ±0.2	15.4 ±0.3	20.2 ±0.2	32.3 ±0.9	45.3 ±2.8
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.0 ±0.0	<0.1
Non-extractable residues (NER)	1.0 ±0.0	1.9 ±0.0	4.1 ±0.0	4.9 ±0.1	6.7 ±0.0	8.2 ±0.1	9.7 ±0.0	11.7 ±0.3	12.6 ±0.1
Total recovery	98.8 ±0.4	98.7 ±0.0	97.8 ±0.4	98.5 ±0.2	97.6 ±0.2	97.5 ±0.2	96.1 ±0.1	97.1 ±0.6	99.2 ±3.1

n.d. = not detected; n.a. = not analyzed

Table 7.1.1- 3: Biotransformation of [PYM-^{14}C]BYI 02960 in sandy loam soil HF under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)								
	0	1	3	7	14	21	30	59	120
BYI 02960	96.1 ±0.0	95.9 ±1.0	92.1 ±0.3	87.1 ±0.5	77.1 ±0.9	71.4 ±0.6	63.2 ±0.3	45.2 ±1.0	24.5 ±0.2
ROI 1	n.d.	0.3 ±0.0	1.6 ±0.1	1.2 ±0.0	1.1 ±0.1	0.4 ±0.0	0.5 ±0.0	0.2 ±0.0	0.5 ±0.0
ROI 2	n.d.	0.1 ±0.0	n.d.	0.2 ±0.0	0.5 ±0.0	0.3 ±0.0	0.6 ±0.1	0.9 ±0.1	0.6 ±0.0
ROI 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ±0.0	0.1 ±0.0
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-characterized radioactivity	0.8 ±0.2	0.3 ±0.0	0.6 ±0.1	0.4 ±0.1	0.6 ±0.0	0.5 ±0.1	0.6 ±0.2	0.5 ±0.0	3.6 ±0.2
Total extractable residues	97.0 ±0.2	96.6 ±1.0	94.4 ±0.2	89.0 ±0.6	79.3 ±0.9	72.5 ±0.7	64.9 ±0.4	47.0 ±1.0	29.3 ±0.4
$^{14}CO^2$	n.a.	0.3 ±0.0	1.7 ±0.1	5.3 ±0.1	11.8 ±0.2	17.0 ±0.5	23.0 ±0.4	37.1 ±1.0	58.6 ±0.2
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	1.5 ±0.1	3.1 ±0.9	3.1 ±0.1	4.8 ±0.1	6.6 ±0.1	8.0 ±0.0	9.3 ±0.1	12.0 ±0.0	13.2 ±0.1
Total recovery	98.4 ±0.1	100.0 ±0.1	99.2 ±0.2	99.1 ±0.7	97.7 ±0.6	97.4 ±0.3	97.2 ±0.7	96.1 ±0.1	101.1 ±0.6

n.d. = not detected; n.a. = not analyzed

Table 7.1.1- 4: Biotransformation of [PYM-14C]BYI 02960 in sandy loam soil HN under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)								
	0	1	3	7	14	21	30	59	120
BYI 02960	96.5 ±0.1	98.8 ±0.1	92.0 ±0.2	88.6 ±0.2	82.3 ±0.0	79.5 ±0.3	74.2 ±0.1	63.2 ±0.2	50.2 ±0.3
ROI 1	n.d.	0.2 ±0.0	0.8 ±0.0	0.5 ±0.1	<LOQ	n.d.	n.d.	0.2 ±0.0	n.d.
ROI 2	n.d.	n.d.	<LOQ	0.5 ±0.0	0.7 ±0.0	0.6 ±0.0	1.1 ±0.1	1.6 ±0.1	1.0 ±0.2
ROI 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ±0.0
ROI 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-characterized radioactivity	0.8 ±0.0	0.1 ±0.1	0.5 ±0.1	0.5 ±0.0	0.4 ±0.1	0.4 ±0.0	0.4 ±0.1	0.4 ±0.0	2.0 ±1.0
Total extractable residues	97.2 ±0.0	99.1 ±0.0	93.3 ±0.2	90.0 ±0.3	83.5 ±0.1	80.3 ±0.3	75.7 ±0.2	65.3 ±0.3	53.4 ±0.5
$^{14}CO^2$	n.a.	0.3 ±0.0	1.2 ±0.0	3.2 ±0.0	3.9 ±2.5	9.1 ±0.1	11.8 ±0.0	19.7 ±0.2	29.4 ±0.1
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	1.9 ±0.0	3.0 ±0.0	4.7 ±0.1	6.3 ±0.1	8.2 ±0.1	9.6 ±0.1	11.2 ±0.1	14.0 ±0.3	16.8 ±0.3
Total recovery	99.2 ±0.0	102.4 ±0.0	99.2 ±0.1	99.6 ±0.2	95.6 ±2.5	99.2 ±0.3	98.6 ±0.1	99.1 ±0.2	99.5 ±0.1

n.d. = not detected; n.a. = not analyzed

Table 7.1.1- 5: Biotransformation of [PYM-^{14}C]BYI 02960 in sandy loam soil DD under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)								
	0	1	3	7	14	21	30	59	120
BYI 02960	93.1 ±0.4	91.9 ±1.7	89.8 ±0.5	86.0 ±0.0	75.9 ±0.3	70.8 ±0.0	63.1 ±0.0	46.1 ±0.5	28.7 ±0.2
ROI 1	<LOQ	0.2 ±0.0	1.2 ±0.1	0.6 ±0.1	0.3 ±0.0	n.d.	0.2 ±0.0	n.d.	0.3 ±0.3
ROI 2	n.d.	n.d.	<LOQ	<LOQ	<LOQ	n.d.	0.2 ±0.0	0.4 ±0.0	0.2 ±0.0
ROI 3	n.d.	n.d.	0.1 ±0.0	n.d.	n.d.	n.d.	n.d.	0.4 ±0.0	0.1 ±0.0
ROI 4	0.6 ±0.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ROI 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
ROI 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ±0.1
Non-characterized radioactivity	0.7 ±0.1	0.2 ±0.0	0.5 ±0.0	0.4 ±0.0	0.6 ±0.1	0.4 ±0.1	0.6 ±0.0	0.4 ±0.0	2.0 ±0.3
Total extractable residues	94.4 ±0.4	92.2 ±1.7	91.6 ±0.4	87.1 ±0.1	76.9 ±0.4	71.3 ±0.0	64.3 ±0.1	47.4 ±0.5	31.5 ±0.3
$^{14}CO_2$	n.a.	0.3 ±0.0	1.8 ±0.0	5.7 ±0.1	12.1 ±0.0	17.1 ±0.2	23.5 ±0.2	39.5 ±0.3	57.3 ±0.3
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	0.0 ±0.0	<0.1	<0.1
Non-extractable residues (NER)	4.1 ±0.7	5.5 ±1.6	6.2 ±0.7	6.3 ±0.3	8.9 ±0.1	8.9 ±0.1	9.5 ±0.1	11.8 ±0.2	12.5 ±0.1
Total recovery	98.5 ±0.2	98.1 ±0.1	99.6 ±0.3	99.2 ±0.1	97.8 ±0.5	97.2 ±0.1	97.3 ±0.2	98.7 ±0.1	101.3 ±0.0

n.d. = not detected; n.a. = not analyzed

B. Mass Balance

The material balances were 96.1 to 99.2% (soil AX), 96.1 to 101.1% (soil HF), 95.6 to 102.4% (soil HN), and 97.1 to 101.3% (soil DD) of the applied radioactivity (% AR; mean values). There was no decrease over the incubation time.

C. Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 97.8, 97.0, 97.2, and 94.4% AR at DAT-0 to 41.3, 29.3, 53.4 and 31.5% AR at study end (DAT-120) in soils AX, HF, HN, and DD, respectively. Non-extractable ^{14}C-residues (NER) increased from 1.0, 1.5, 1.9 and 4.1% at DAT-0 to 12.6, 13.2, 16.8 and 12.5% of AR at the end of the study period. These portions of NER are comparatively low.

Table 7.1.1- 6: Summary of extractable and non-extractable residues

Soil		[redacted]	[redacted]	[redacted]	[redacted]
Extracted RA (%)	Day 0	97.8±0.4	97.0±0.2	97.2±0.0	97.4±0.4
	Day 120	41.3±0.2	29.3±0.4	53.4±0.5	31.5±0.3
Non-Extracted RA (%)	Day 0	1.0±0.0	1.5±0.1	1.9±0.0	4.1±0.7
	Day 120	12.6±0.1	13.2±0.1	16.8±0.3	12.5±0.1

D. Volatilization

The mineralization of [PYM-14C]BYI 02960 was high. At the end of the study (DAT-120) up to 45.3 (AX), 58.6 (HF), 29.4 (HN) and 57.3% AR (DD) of $^{14}CO_2$ were generated. Volatile organic compounds were very negligible (≤ 0.1% AR).

E. Transformation of Test Item

The test item declined from 97.1, 96.1, 96.5 and 93.1% AR at DAT-0 to 37.1, 24.5, 50.2 and 28.7% in soils AX, HF, HN and DD, respectively, at the end of the study.
Only very minor transformation products (all were below 3% AR) were detected. In all soils three very minor metabolites, designated ROI 1, ROI 2 and ROI 3 were quantified and characterized by their chromatographic behavior. ROI 2 reached maximum levels eat DAT-59 of 2.6 (in soil AX), 0.9 (HF), 1.6 (HN) and 0.4% AR (DD). In the different soil ROI 1 did not exceed 1.5 (AX), 1.6 (HF), 0.8 (HN) and 1.2% AR (DD). ROI 3 was a maximum of 0.4% AR in the four soils. In soil DD, three additional very minor peaks were detected with maximum levels of 0.6% AR.
The total of non-characterized extracted radioactivity did not exceed 3.1% AR.
The mentioned results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure 7.1.2- 1.

F. Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculations for the test item is given in **Error! Reference source not found.**.

Overall, the amount of BYI 02960 declined during the test period of 120 days. The GEOmean of the DT_{50} and the DT_{90} values for degradation of BYI 02960 in the tested soils under aerobic conditions at 20 °C were 68.8 and 327.3 days, respectively.

III CONCLUSIONS

A. Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that BYI 02960 is degraded in the four soils.
Three very minor metabolites were detected and quantified together with the test item. All further formed metabolites are regarded as transient, which is confirmed by the high mineralization rate of [PYM-^{14}C]BYI 02960 to $^{14}CO_2$ observed in this study, i.e. between 29.4 % (soil HN) and 58.6% of AR (soil HF) at the end of the study. Volatile organic compounds were very low (≤ 0.1% AR) at all sampling dates.

Table 7.1.1- 7: Synopsis of overall results

Soil	AX	HF	HN	DD
Total Recovery (%)	96.1 – 99.2	96.1 – 101.1	95.6 – 102.4	97.1 – 101.3
Extracted RA (%)	41.3 – 97.8	29.3 – 97.0	53.4 – 99.1	31.5 – 94.4
RA desorbed (%)	13.5 – 64.5	9.2 – 56.8	12.2 - 45.4	6.3 – 35.4
Max. CO2 (%)	45.3	58.6	29.4	57.3
Bound Residues (%)	1.0 – 12.6	1.5 – 13.2	1.9 – 16.8	4.1 – 12.5
Extraction Efficiency of Test Substance DAT-0 (%)	97.1	96.1	96.5	93.1
Major metabolites	-	-	-	-

B. Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that BYI 02960 is microbially degradable in soils under aerobic conditions. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant, metabolites formed did not accumulate in soil and can therefore be regarded as transient. Non-extractable residues were low, maximum 16.8%.

Table 7.1.1- 8: Synopsis of results of biotransformation of [PYM-^{14}C]BYI 02960 in soils incubated at 20 °C and 55 % of WHCmax under aerobic conditions

Soil	[redacted]	[redacted] am [redacted]	[redacted]	[redacted] II
Soil type	Sandy loam	Silt loam	Loam	Clay loam
Major transformation products *	CO_2 (max. 58.6%) NER (comparatively low, max 16.8%)			
Minor transformation products	N/A			

*): 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.

Report:	KHA 7.1.1/02, [redacted], [redacted], 2011
Title:	[Furanone-4-^{14}C]BYI 02960: Aerobic Soil Metabolism/Degradation
Report No & Document No	MEF-10/804 M-411625-01-2
Guidelines:	OECD TG 307, Aerobic and Anaerobic Transformation in Soil US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.
GLP:	Yes (fully GLP compliant and certified laboratory)

EXECUTIVE SUMMARY

The biotransformation of [furanone-4-^{14}C]BYI 02960 was studied in four European soils: [redacted] (AX), [redacted] am [redacted] (HF), [redacted] Plot 611 (HN), and [redacted] II (DD) for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 1.07 mg/kg dry weight of soil, which is equivalent to 400 g/ha field application rate.

At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave.

The BYI 02960 residues and transformation products were analyzed and quantified by HPLC. TLC was used as confirmation method.

Material balances were complete throughout the study, and the test item declined from 96.9, 95.9, 96.4 and 94.3% of AR at DAT-0 to 37.3, 20.2, 45.2 and 26.9% at the end of the study for soils AX, HF, HN and DD, respectively. Applying double first-order kinetics a half-life (geometric mean) of 56.2 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

The mineralization of [furanone-4-^{14}C] BYI 02960 in this study was high. At the end of the study (DAT-120) up to 27.6 (soil AX), 38.9 (HF), 18.0 (HN) and 32.0% AR (DD) of $^{14}CO_2$ were generated. Volatile organic compounds were negligible (≤ 0.1% AR). Except carbon dioxide only very minor transformation products (all were below 2% AR) were detected. Non-extractable ^{14}C-residues (NER) increased from 2.4, 3.8, 3.3 and 4.6% of AR at DAT-0 to 27.8, 33.6, 31.0 and 34.1% AR at the end of the study period for soils AX, HF, HN and DD, respectively. The major portions of NER radioactivity were found in the insoluble humin fraction.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: Flupyradifurone: Code = BYI 02960;
Label FUR = [Furanone-4-^{14}C]BYI 02960 (sample ID: KATH 6101)
Specific activity 3.94 MBq/mg
Radiochemical purity: >98% (acc. radio-HPLC), >99% (acc. radio-TLC)
Chemical purity: >98% (HPLC, UV detection at 210 nm)

Identity and purity of test item in the application solution were confirmed.

2. Soil: The biotransformation of [FUR-^{14}C]BYI 2960 was studied in four different soils. These soils are representative for agricultural use areas as required by the guidelines and cover a representative range of physico-chemical properties. All soils were taken on 2008-03-25 fresh from the fields. Three days later, i.e. three days before starting the test the air dried soils were sieved through a 2 mm sieve and aliquots equivalent to 100 g dry matter were weighed into individual 300 mL Erlenmeyer flasks and fitted with trap attachments. The soils were pre-equilibrated at 20 °C in the dark.

Table 7.1.1- 9: Soil physicochemical properties

Parameter	Results/Units			
Soil Batch ID	[redacted] 20080325	[redacted]4a 20080325	[redacted] 20080325	[redacted] 20080325
Location	[redacted] Germany	[redacted] Germany	[redacted] Germany	[redacted] Germany
Soil Taxonomic Classification (USDA)	Sandy floodplain deposits of the lower terrace of the Rhine river, material from the Pleistocene Ice Age	Loess or loess colluvium (Pleistocene, Holocene)	Not available	Not available
Soil Series	Sandy, mixed, mesic Typic Cambudolls	Loamy, mixed, mesic Typic Argudalfs	Not available	Not available
Texture Class (USDA)	Sandy Loam	Silt Loam	Silt Loam	Silty Clay
Sand Silt Clay	67% 19% 14%	21% 59% 20%	27% 53% 20%	17% 41% 42%
pH in Water pH in CaCl2 pH in KCl	6.3 6.1 5.9	6.6 6.5 6.2	5.1 4.8 4.4	7.2 7.1 6.8
Organic Matter	3.4%	4.3%	5.7%	7.1%
Organic Carbon	2.0%	2.5%	3.3%	4.1%
Soil Microbial Biomass	mg microbial carbon per kg of soil			
0 days	561	1140	751	2541
59 days	433	719	477	1999
120 days	367	635	336	1797
Cation Exchange Capacity (CEC)	9.6 meq/100 g	13.4 meq/100 g	10.5 meq/100 g	19.2 meq/100 g
WHCmax	51.6 g H_2O/ 100 g DM	66.3 g H_2O/ 100 g DM	75.2 g H_2O/ 100 g DM	77.6 g H_2O/ 100 g DM

B. Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 20.6 ± 0.3 °C. The test system consisted of Erlenmeyer flasks (300 mL) attached with a trap attachment (permeable to 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 (each 28 flasks/soil). For all soils replicates were set up for each sampling (10 sampling dates including time 0, 4 spare flasks, and 1 flask for metabolite identification purposes treated with a 10x rate). Three flasks from each soil were used for determination of the microbial biomass. The final soil moisture was adjusted to 55% of WHC_{max} by adding deionized water.

2. Test Item Stock Solution: The entire delivered amount of [FUR-^{14}C]BYI 02960 was dissolved in 4 mL methanol.

3. Test Item Application Solution: An application solution (total volume 60 mL) was made by diluting 2853 µL of the stock solution with 57.1 mL of purified water.

4. Mode of Application: On 2008-03-31, aliquots of 391 µL of the application solution were applied in droplets onto the 100 g pre-incubated subsamples of each soil. By the addition of the application

solution, the water content was finally adjusted to 55% of WHC_{max}. The test vessels for DAT-0 were immediately processed for analysis. All other test vessels, including the biomass flasks which were not spiked with application solution, were fitted with trap attachments and incubated in the dark at nominal 20 ± 1°C.

[FUR-^{14}C]BYI 02960 was applied at a rate of 416200 Bq per vessel. This corresponds to 105.63 µg per vessel which is equivalent to 99% of the application rate of 106.7 µg per vessel (calculated. a single application rate of 400 g BYI 02960 per hectare).

5. Sampling: Microbial biomass was determined prior to commencement of the test (soils sampled at the day of application), after 59 days, and at the end of the study 120 Days After Treatment (DAT-120). Entire test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-3, DAT-7, DAT-14, DAT-30, DAT-45, DAT-59, DAT-85, and DAT-120.

Prior to opening the incubation flasks (for moistening or sampling of soil), volatile (radioactive) compounds, possibly still present in the flasks, were transferred into the trap attachment by subjecting the flasks to vacuum in an excicator. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

6. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave.

The BYI 02960 residues and transformation products were analyzed and quantified by LSC and reversed phase radio-HPLC. Normal-phase Si-60 radio-TLC was used as a confirmatory method. The limit of quantification (LOQ) was derived from the LOD by the operation: LOQ = 3 x LOD resulting in a LOQ of about 0.9% of AR. However, values between LOD and LOQ were also used for quantification. The limit of the detection in ambient and aggressive organic extracts was in the range of 0.3% of AR. The limit of detection for a single TLC peak was estimated from the data sheets used for the comparison of the results obtained by HPLC and TLC. The lowest peaks quantified were assigned to DFA in the aggressive and account for 0.1% of AR.

The identity of the test item in stock solution and in extracts was confirmed by spectroscopic methods. In addition, spectroscopic methods were used to identify one metabolite.

II. RESULTS AND DISCUSSION

Results indicated that anticipated standardized conditions were maintained, and the soils were microbially active over the duration of the laboratory study.

A. Data

The respective data for the four soils are shown in Table 7.1.1- 10 to Table 7.1.1- 13.

The DAT-0 extraction efficiency was in the range of 94.6 to 97.3% of AR (mean 96.2% AR; sum of extracts only). The stability of the test item was verified by DAT 0 values of 94.3 to 96.9% AR for the soil extracts (mean 95.9% AR). These results indicate that the extraction method was appropriate to extract the applied [^{14}C]-labeled test item from the soil matrix.

Table 7.1.1- 10: Biotransformation of [FUR-^{14}C]BYI 02960 in sandy loam soil AX under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	59	85	120
BYI 02960	96.9 ±0.2	94.4 ±0.1	89.3 ±0.0	83.0 ±0.0	74.2 ±0.1	62.6 ±0.3	54.3 ±0.1	48.4 ±0.1	42.3 ±0.3	37.3 ±0.5
Reg 1	n.d.	n.d.	n.d.	0.4 ±0.0	0.8 ±0.2	n.d.	1.2 ±0.0	1.0 ±0.2	1.1 ±0.1	1.0 ±0.0
BYI 02960-chloro	n.d.	n.d.	n.d.	0.4 ±0.0	0.8 ±0.2	0.8 ±0.1	1.3 ±0.2	1.5 ±0.1	1.7 ±0.1	1.8 ±0.0
Reg 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ±0.1	0.4 ±0.2	0.3 ±0.0	0.4 ±0.1
Reg 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reg 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ±0.1	n.d.
Non-charact. radioactivity	0.4 ±0.0	0.4 ±0.1	0.3 ±0.1	0.4 ±0.0	0.3 ±0.1	0.3 ±0.0	0.3 ±0.1	0.2 ±0.0	0.3 ±0.0	0.2 ±0.0
Total extractable residues	97.3 ±0.2	94.8 ±0.0	89.7 ±0.1	84.1 ±0.0	76.0 ±0.3	63.7 ±0.1	57.3 ±0.5	51.6 ±0.0	45.7 ±0.1	40.4 ±0.6
$^{14}CO_2$	n.a.	0.5 ±0.0	1.6 ±0.0	3.7 ±0.0	6.8 ±0.1	13.2 ±0.1	16.9 ±0.8	20.4 ±0.2	24.5 ±0.1	27.6 ±0.2
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	2.4 ±0.1	4.5 ±0.1	6.9 ±0.0	11.0 ±0.1	16.1 ±0.0	21.2 ±0.1	23.7 ±0.1	25.2 ±0.2	27.1 ±0.3	27.8 ±0.1
Total recovery	99.7 ±0.1	99.7 ±0.1	98.1 ±0.1	98.8 ±0.2	99.0 ±0.4	98.0 ±0.3	98.1 ±0.2	97.2 ±0.1	97.3 ±0.3	95.8 ±0.3

n.d. = not detected; n.a. = not analyzed
Reg 2 was identified as compound BYI 02960-chloro

Table 7.1.1- 11: Biotransformation of [FUR-^{14}C]BYI 02960 in silt loam soil HF under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	59	85	120
BYI 02960	95.9 ±0.3	93.7 ±0.1	90.4 ±0.7	80.6 ±0.5	71.1 ±0.0	52.7 ±0.4	40.6 ±0.5	33.3 ±0.4	26.2 ±0.1	20.2 ±0.2
Reg 1	n.d.	n.d.	0.2 ±0.2	0.4 ±0.1	0.7 ±0.0	0.8 ±0.2	1.6 ±0.0	1.4 ±0.0	1.4 ±0.1	1.2 ±0.1
BYI 02960-chloro	n.d.	n.d.	n.d.	0.4 ±0.1	0.5 ±0.1	1.0 ±0.2	1.3 ±0.1	1.3 ±0.3	1.3 ±0.0	1.5 ±0.3
Reg 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ±0.3	0.5 ±0.1	n.d.	n.d.
Reg 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reg 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-charact. radioactivity	0.3 ±0.0	0.4 ±0.1	0.4 ±0.1	0.2 ±0.0	0.2 ±0.0	0.4 ±0.0	0.3 ±0.0	0.2 ±0.0	0.3 ±0.0	0.1 ±0.0
Total extract. residues	96.3 ±0.3	94.1 ±0.1	91.0 ±0.7	81.5 ±0.5	72.5 ±0.1	54.9 ±0.4	44.2 ±0.2	36.8 ±0.0	29.2 ±0.0	23.0 ±0.5
$^{14}CO_2$	n.a.	0.5 ±0.0	1.6 ±0.0	4.3 ±0.0	8.3 ±0.0	17.3 ±0.0	23.6 ±0.6	27.7 ±0.1	32.7 ±0.1	38.9 ±0.2
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extract. residues (NER)	3.8 ±0.1	5.3 ±0.0	7.9 ±0.2	12.5 ±0.1	17.7 ±0.1	25.5 ±0.1	29.7 ±0.2	31.7 ±0.1	33.6 ±0.2	33.0 ±0.4
Total recovery	100.1 ±0.4	99.9 ±0.2	100.5 ±0.9	98.4 ±0.6	98.6 ±0.1	97.7 ±0.0	97.5 ±0.2	96.1 ±0.0	95.5 ±0.3	94.9 ±0.2

n.d. = not detected; n.a. = not analyzed
Reg 2 was identified as compound BYI 02960-chloro

Table 7.1.1- 12: Biotransformation of [FUR-^{14}C]BYI 02960 in silt loam soil HN under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	59	85	120
BYI 02960	96.4 ±0.2	92.0 ±0.2	88.4 ±0.0	82.3 ±0.2	77.7 ±0.0	66.9 ±0.6	60.9 ±0.4	56.6 ±0.2	50.7 ±0.2	45.2 ±0.3
Reg 1	n.d.	n.d.	n.d.	0.4 ±0.0	0.8 ±0.0	1.3 ±0.1	1.7 ±0.0	1.6 ±0.0	1.2 ±0.0	1.1 ±0.1
BYI 02960-chloro	n.d.	n.d.	n.d.	0.4 ±0.0	0.6 ±0.1	1.2 ±0.1	1.2 ±0.2	1.2 ±0.0	1.3 ±0.1	1.3 ±0.1
Reg 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ±0.2	0.1 ±0.1	n.d.	n.d.
Reg 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reg 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-charact. radioactivity	0.4 ±0.0	0.5 ±0.0	0.3 ±0.1	0.4 ±0.0	0.4 ±0.1	0.4 ±0.1	0.3 ±0.0	0.2 ±0.0	0.2 ±0.0	0.3 ±0.1
Total extract. residues	96.7 ±0.2	92.5 ±0.2	88.7 ±0.1	83.6 ±0.2	79.5 ±0.2	69.9 ±0.3	64.4 ±0.4	59.7 ±0.1	53.4 ±0.1	48.0 ±0.4
$^{14}CO_2$	n.a.	0.4 ±0.0	1.1 ±0.0	2.3 ±0.0	3.9 ±0.0	7.0 ±0.0	9.2 ±0.1	11.0 ±0.2	14.0 ±0.1	18.0 ±0.1
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extract. residues (NER)	3.3 ±0.1	6.9 ±0.3	9.4 ±0.1	12.8 ±0.1	16.5 ±0.2	21.7 ±0.1	24.9 ±0.1	26.7 ±0.4	29.8 ±0.1	31.0 ±0.1
Total recovery	100.0 ±0.3	99.8 ±0.4	99.2 ±0.0	98.7 ±0.3	99.9 ±0.4	98.6 ±0.3	98.4 ±0.3	97.5 ±0.1	97.2 ±0.2	96.9 ±0.1

n.d. = not detected; n.a. = not analyzed
Reg 2 was identified as compound BYI 02960-chloro

Table 7.1.1- 13: Biotransformation of [FUR-^{14}C]BYI 02960 in silty clay soil DD under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	59	85	120
BYI 02960	94.3 ±0.7	93.3 ±0.2	89.5 ±0.0	84.5 ±0.3	75.5 ±0.1	63.5 ±0.9	50.9 ±1.4	41.4 ±0.0	34.0 ±0.5	26.9 ±0.3
Reg 1	n.d.	n.d.	n.d.	n.d.	0.8 ±0.2	0.9 ±0.1	1.6 ±0.2	1.7 ±0.1	1.6 ±0.1	1.6 ±0.0
BYI 02960-chloro	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ±0.2	0.4 ±0.1	0.4 ±0.0	n.d.
Reg 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5 ±0.0	n.d.	n.d.	n.d.
Reg 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ±0.1	n.d.	n.d.
Reg 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-charact. radioactivity	0.4 ±0.1	0.5 ±0.0	0.5 ±0.0	0.3 ±0.1	0.3 ±0.1	0.4 ±0.0	0.3 ±0.0	0.2 ±0.1	0.3 ±0.1	0.3 ±0.0
Total extract. residues	94.6 ±0.8	93.8 ±0.1	90.0 ±0.0	84.8 ±0.4	76.7 ±0.0	64.8 ±0.9	53.4 ±0.9	44.2 ±0.1	36.2 ±0.4	28.8 ±0.3
$^{14}CO_2$	n.a.	0.3 ±0.0	1.0 ±0.0	2.8 ±0.0	5.5 ±0.0	11.7 ±0.4	17.4 ±0.4	21.6 ±0.1	26.8 ±0.0	32.0 ±0.5
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extract. residues (NER)	4.6 ±0.6	5.5 ±0.0	8.2 ±0.4	11.0 ±0.0	15.8 ±0.3	21.6 ±0.7	24.9 ±0.9	30.2 ±0.1	32.4 ±0.1	34.1 ±0.1
Total recovery	99.3 ±0.1	99.6 ±0.1	99.2 ±0.3	98.6 ±0.5	98.0 ±0.3	98.1 ±0.2	95.8 ±0.4	96.0 ±0.2	95.4 ±0.3	94.9 ±0.2

n.d. = not detected; n.a. = not analyzed
Reg 2 was identified as compound BYI 02960-chloro
Reg 4 was tentatively identified as compound BYI 02960-des-difluoroethyl

B. Mass Balance

Material balances ranged from 95.8 to 99.7% (soil AX), 94.9 to 100.5% (soil HF), 96.9 to 100.0% (soil HN) and 94.9 to 99.6% (soil DD) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

C. Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 97.3, 96.3, 96.7, and 94.6% AR at DAT-0 to 40.4, 23.0, 48.0 and 28.8% AR at study end (DAT-120) in soils AX, HF, HN, and DD, respectively.
Non-extractable ^{14}C-residues increased from 2.4, 3.8, 3.3 and 4.6% of AR at DAT-0 to 27.8, 33.6, 31.0 and 34.1% AR at the end of the study period for soils AX, HF, HN and DD, respectively. The major portions of non-extractable radioactivity were found in the insoluble humin fraction.

Table 7.1.1- 14: Summary of extractable and non-extractable residues

Soil		AX	HF	HN	DD
Extracted RA (%)	Day 0	97.3±0.2	96.3±0.3	96.7±0.2	94.6±0.8
	Day 120	40.4±0.6	23.0±0.5	48.0±0.4	28.8±0.3
Non-Extracted RA (%)	Day 0	2.4±0.1	3.8±0.1	3.3±0.1	4.6±0.6
	Day 120	27.8±0.1	33.0±0.4	31.0±0.1	34.1±0.1

D. Volatilization

The mineralization of [FUR-^{14}C]BYI 02960 was high. At the end of the study (DAT-120), $^{14}CO_2$ accounted for 27.6 (soil AX), 38.9 (HF), 18.0 (HN) and 32.0% (DD) of AR.
Volatile organic compounds were not detected in significant amounts ($\leq$0.1% AR).

E. Transformation of Test Item

The test item declined from 96.9, 95.9, 96.4 and 94.3% of AR at DAT-0 to 37.3, 20.2, 45.2 and 26.9% at the end of the study for soils AX, HF, HN and DD, respectively.
Only very minor transformation products (all were below 2% AR) were detected in the extracts. Three metabolites, designated Reg 1, Reg 2 and Reg 3, were detected in all four soils and were characterized by their retention times. Reg 1 reached maximum levels of 1.2 (AX), 1.6 (HF), 1.7 (HN) and 1.7% (DD) of AR at DAT-45 or DAT-59. Reg 2 amounted to maximally 1.8 (AX), 1.5 (HF), 1.3 (HN) and 0.4% (DD) of AR with increasing amounts towards the end of the study. This metabolite was identified as BYI 02960-chloro by spectroscopic methods. Reg 3 accounted for up to 0.4 (AX), 0.5 (HF), 0.2 (HN) and 0.5% (DD) of AR, respectively. The transformation products assigned to Reg 4 and Reg 5 appeared only once with mean values of 0.4% (DD) and 0.1% (AX) of applied radioactivity, respectively. Reg 4 was tentatively identified as BYI 02960-des-difluoroethyl via co-chromatography. The total of non-characterized extracted radioactivity did not exceed 0.5% of AR.

The results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure 7.1.2- 1.

F. Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculations for the test item is given under paragraph 7.2.

III CONCLUSIONS

A. Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that BYI 02960 is degraded in the four soils; a mean DT_{50} value of 56.2 days (GEOmean, n = 4 soils) was calculated. A synopsis of results is shown in Table 7.1.1- 16.
A few very minor metabolites were detected and quantified together with the test item. All further formed metabolites are regarded as transient, which is confirmed by the high mineralization rate of [FUR-^{14}C]BYI 02960 to $^{14}CO_2$ observed in this study, i.e. between 18.0 % (soil HN) and 38.9% of AR (soil HF) until the end of the study. Volatile organic compounds were very low ($\leq$ 0.1% AR) at all sampling dates.

Table 7.1.1- 15: Synopsis of overall results

Soil	AX	HF	HN	DD
Total Recovery (%)	95.8 – 99.7	94.9 – 100.5	96.9 – 100.0	94.9 – 99.6
Extracted RA (%)	40.4 – 97.3	23.0 – 96.3	48.0 – 96.7	28.8 – 94.6
Max. CO2 (%)	27.6	38.9	18.0	32.0
Bound Residues (%)	2.4 – 27.8	3.8 – 33.6	3.3 – 31.0	4.6 – 34.1
Extraction Efficiency on DAT-0 (%)	97.3	96.3	96.7	94.6

B. Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant, however, other metabolites are not to be expected in soil, since – after their formation – they are well mineralized (see Table 7.1.1- 16).

Table 7.1.1- 16: Synopsis of results of biotransformation of [FUR-^{14}C]BYI 02960 in soils incubated at 20 °C and 55 % of WHCmax under aerobic conditions

Soil	[redacted]	[redacted]	[redacted]	[redacted]
Soil type	Sandy loam	Silt loam	Silt loam	Silty clay
Major transformation products *	$^{14}CO_2$ (max. 38.9% NER (max. 34.1%)			
Minor transformation products	BYI 02960-chloro (max. 1.8%) BYI 02960-des-difluoroethyl (max. 0.4%)			

*): 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.

Report:	**KIIA 7.1.1/03, [redacted], 2011**
Title:	[Furanone-4-^{14}C]BYI 02960: Aerobic Soil Metabolism in Two US Soils
Report No & Document No	MERVP037-2 M-405497-03-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002** **US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The biotransformation of [FUR-^{14}C] BYI 02960 was studied in two US soils: silt loam [redacted], NE, and sandy loam [redacted], CA, for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and soil moistures maintained between pF 2.0 to 2.5. Gamma irradiated samples were investigated along with microbial active test systems. BYI 02960 was applied at the rate of 1.1 µg a.i./g soil, equivalent to 410 g a.i./ha assuming a 2.5 cm soil depth.

Duplicate test systems were analyzed at 0, 3, 7, 14, 28, 60, 92, and 120 days of incubation. The 50-g soil samples were extracted by shaking with acetonitrile:water (70:30) and acetonitrile (100%), followed by microwave extraction at 70 °C(aggressive extract) using acetonitrile:water (70:30). Extract aliquots were concentrated and analyzed by HPLC. Identification of the transformation products was performed by LC/MS, co-chromatography, and/or retention time comparison with reference standards.

Material balances were complete throughout the study, and the test item declined from 98.0 and 99.4% and of the applied amount at day 0 to 67.3 % and30.8% of the applied at the end of the study. The first-order half-life of BYI 02960 in silt loam was 228 days. The first-order half-life in the sandy loam was 65.7 days.

BYI 02960 mineralizes relatively rapidly under aerobic condition to $^{14}CO_2$ (12.3% [redacted], 36.1% [redacted]) and becomes increasingly bound to soil (16.4% [redacted], 30.6% [redacted], CA) by study end. The amount of $^{14}CO_2$ and bound residue formed in gamma-irradiated soils was significantly less than in non-sterile soils indicating a biological component to degradation and the formation of non-extractable residues from BYI 02960. Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: Flupyradifurone: Code = BYI 02960;
Label FUR = [Furanone-4-14C]BYI 02960 (sample ID: KATH 6252)
Specific activity: 30.74 mCi/mMole
Supplied substance was repurified (vial C-1116A, 429.4 µCi/mL); the final radiochemical purity was 100%.
Identity and purity of test item in the application solution were confirmed.

2. Soil: The biotransformation of [FUR-^{14}C]BYI 2960 was studied in two different soils. These soils are representative for agricultural use areas as required by the guidelines and cover a representative range of physico-chemical properties.

NE and CA soils were taken on 2009-11-30 and 2010-01-07, fresh from the fields. Each soil was collected from the top 0 to 8 inches of the field and shipped to the testing facility subsequently. No pesticides were applied in the past 5 years to these sites where soils were collected. The NE soil was planted with alfalfa and maintained in a greenhouse prior to use (40 days). The CA soil was maintained in a walk-in refrigerator prior to use (7 days).

The soils were air dried and sieved through a 2-mm sieve. Subsequently, aliquots equivalent to 50 g dry matter were weighed into individual 250 mL glass flasks until starting the test.

Test systems were pre-equilibrated at 20 ± 1°C with soil moisture between pF 2.0 to 2.5 for six days prior to the treatment performed on 2010-01-25.

Table 7.1.1- 17: Soil physicochemical properties

Parameter	Results/Units	Results/Units
Geographic Location	[redacted], [redacted], USA	[redacted], [redacted], USA
	[redacted]	[redacted]
Soil Mapping Unit	[redacted]	[redacted]
Taxonomic Name:	Marshal fine-silty mixed superactive mesic typic Hapludolls	Hanford Fine Sandy Loam Gravely substrate
Texture Class (USDA)	Silt Loam	Sandy Loam
Sand Silt Clay	13.4% 63.8% 22.8%	67.8% 25.0% 7.2%
pH Saturated paste 1:1 soil:water 0.01 M CaCl2	 6.7 6.7 6.5	 7.4 7.3 7.0
Organic Matter	4.0%	0.97%
Organic Carbon[B]	2.3%	0.57%
Cation Exchange Capacity (CEC)	17.8 meq/100 g	6.3 meq/100 g
Maximum Water Holding Capacity	49.6 g/100 g	27.7 g/100 g
Soil Moisture at 0.1 bar (pF 2.0)	37.9	15.8
Soil Moisture at 0.33 bar (pF 2.5)	26.4	9.1
Bulk Density (disturbed)	1.04 g/m3	1.29 g/m3
Microbial Biomass:[C] Initial (day 6) Middle (day 71) Final (day 120)	(mg Microbial C/kg soil) 833 686 660	Microbial Biomass:[C] Initial (day 6) Middle (day 71) Final (day 120)

B. Methods

1. Experimental conditions: All test systems were incubated at 20 ± 1°C in the dark in a temperature-controlled environmental chamber. The test systems containing 50 g soil (dry-weight basis) consisted of a 250-mL glass flask (21 cm long and 7.2 cm internal diameter) connected to a flow-through system, containing an ethylene glycol trap for volatile organics followed by two 2 M potassium hydroxide traps for collecting CO_2 and a 1 M sulfuric acid trap for volatile acids. The headspace above the soil was continuously purged with humidified air throughout the study.

For both soils twenty test systems were treated at 1X for the kinetic study rate with each soil (1.1 µg/g; see next section). Three test systems were prepared for metabolite identification (MID) purposes, and these systems were treated at 10x the kinetic rate. MID test systems were not used for kinetic evaluation. Four control test systems were prepared as biomass test systems to demonstrate biological activity of the soil.

For the sterile portion of this study, soil was sent to Food Technology Service, Inc. (FTSI; Mulberry, FL) for gamma irradiation (actual delivered absorbed dose of 24.67 kGy). Gamma-irradiated soils were transferred to sterilized 250-mL glass flasks within a BioGuard Laminar Flow Hood using aseptic techniques. Soils were treated at the kinetic rate in the laminar flow hood using aseptic techniques, and then connected to the flow-through system. Sterility was tested at each sampling interval on each test system by plating soil dilutions on 3M Petrifilm Agar plates which were incubated at ~34°C for a minimum of 48 hours.

In total, 64 test systems were prepared. All the soils were adjusted to the appropriate moisture level for each soil, and the moisture was maintained throughout the course of the study.

2, Test Item Application Solution: The application (treatment) solution was prepared by diluting 955 µL of [FUR-^{14}C]BYI 02960 (C-1116A) with 36 mL of H_2O:MeOH (4:1) for a theoretical concentration of 0.11 µg/µL. The extensive radio-assays to determine the concentration of kinetic treatment solution resulted in 25,996,470 dpm/mL or 110 µg/mL. Radiochemical purity and identity were confirmed by HPLC and mass spectral analysis.

3. Mode and Rate of Application: On 2010-01-25, aliquots of 500-µL of the kinetic treatment solution were applied in droplets onto the 50 g pre-incubated subsamples of soils, using a 500-µL gas-tight syringe. This rate is equal to the field-use rate of 410 g a.i./ha at 2.5 cm depth and 1.5 g/cm^3 soil density. The final concentration of a.i. in each test system was 1.1 µg BYI 02960 per g soil (dry weight). By the addition the water contained the soil moisture was finally adjusted to approx. pF 2.0. Material balance for the study was based on the day 0 recovery of radioactivity (12,632,000 dpm [redacted], [redacted] and 12,557,000 dpm [redacted], CA).

4.Test System Maintenance and Sampling: Test systems were checked for air flow through the traps and water levels in the moisture flasks. The test systems were incubated at 20 ± 1°C in the dark in an environmental chamber. The moisture loss in each system was monitored periodically by comparing the weight of the test system with the weight on Day 0. The moisture of the test systems was measured at each interval. The moisture was maintained at pF 2.5 to pF 2.0 for each soil by the addition of HPLC grade water, as needed, at periodic intervals.
Duplicate test systems were extracted and analyzed on days 0, 3, 7, 14, 28, 60, 92 and 120 days after treatment. Gamma-irradiated test systems were processed at 0, 60, 61 and 122 days after treatment. For the sterile portion of this study, the goal was to compare the formation of bound residues with non-sterile test systems and therefore, soil extracts were radio-assayed but not analyzed by HPLC. Extracted soils were analyzed by combustion.
On the day of sampling, the test system and associated volatile traps were removed from the incubator for analysis. Prior to removing samples, the air flow was increased for approx. 15 minutes to ensure all headspace volatiles had been purged and trapped. The two KOH trap solutions were combined, volumes of the traps were recorded and triplicate aliquots were radio-assayed.
The soil was extracted and analyzed by LSC on the day of sampling. The soil extracts were analyzed by HPLC within 16 days after sample extraction. Concentrated extracts were stored in a laboratory freezer.

5. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge bottle and extracted for 30 minutes with approximately 40 mL of acetonitrile:water (70:30, v/v) using a Eberbach horizontal table top shaker, and centrifuged using an Eppendorf table-top centrifuge (5 minutes at 1850 g).
The supernatant was decanted, and the remaining soil was extracted two additional times with acetonitrile:water (70:30,v/v) followed by an extraction with approximately 40 mL of acetonitrile. The supernatants were pooled, and the volume was recorded. Extracts were radioassayed by LSC in triplicate (0.25 to 1.0 mL). An aliquot (approximately 4 mL) of the combined extracts was concentrated by a stream of nitrogen and analyzed by HPLC.

Soil remaining after the ambient acetonitrile/water and acetonitrile extractions was extracted by microwave extraction using 50 mL of acetonitrile:water (70:30, v/v) at 70°C for 10 minutes. The volume was recorded, and the extract was radioassayed by LSC in triplicate (0.25 to1.0 mL). An aliquot (approximately 4 mL) was concentrated by a stream of nitrogen and then analyzed by HPLC.

Extracted soils were air-dried under a fume hood and thoroughly homogenized using a Sunbeam Kitchen Assistant coffee grinder. Subsamples (0.3 to 1.0 g) of the non-extracted residue (NER) were quantified by combustion.

To characterize the NER, the extracted soils from the day 92 sandy loam samples were fractionated to quantify the amounts of radioactivity associated with humic acid, fulvic acid, and humin.

The BYI 02960 residues and transformation products were analyzed and quantified by LSC and radio-HPLC. Recovery of radioactivity from the HPLC column was determined by comparing radioactivity of the collected HPLC effluent (triplicate aliquots, radio-assayed) to the calculated radioactivity of the injected representative samples.

The LOD for HPLC was determined empirically by a series of injections of increasing radioactivity. The lowest amount of radioactivity detected resulted in minimum peak height of 3 times the background level in the chromatogram (520 dpm) and was considered to be the instrument LOD. The minimum amount of radioactivity injected for a sample was 3,274 dpm from the [redacted], CA day 14, replicate 2 microwave extract. This extract contained 2.8% of the applied activity which results in a LOQ of 0.4% of the applied radioactivity.

Liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) and liquid chromatography/electrospray ionization-mass spectrometry/mass spectrometry analyses of standards and isolated compounds was performed using a Finnigan-MAT Ultra-AM (Thermo Electron, San Jose, CA) triple quadrupole mass spectrometer interfaced to a Surveyer autosampler and quaternary HPLC system (Thermo Electron).

II. RESULTS AND DISCUSSION

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study.

A. Data

The respective data for the two soils are shown in Table 7.1.1- 18 and Table 7.1.1- 19.

The ambient and aggressive extracts were effective in extracting essentially all of the ^{14}C-residues in both soils at DAT-0, the beginning of the study (silt loam = 99.2%, sandy loam = 99.8%).

The stability of the test item was verified by DAT 0 values of 98.0 and 99.4% AR (mean of duplicates) for the soil extracts. These results indicate that the extraction method was appropriate for extraction of the applied [^{14}C]-labeled test item from the soil matrix.

Table 7.1.1- 18: Biotransformation of [FUR-^{14}C]BYI 02960 in silt loam [redacted] (NE) under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)							
	0 b)	3 b)	7	14	28 c)	60	92	120
BYI 02960	98.0 ±0.4	93.1 ±0.4	94.0 ±0.8	89.8 ± 0.7	87.1 ± n.a.	78.2 ±0.0	72.1 ±0.6	67.3 ±2.3
Non-characterized radioactivity a)	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ± n.a.	0.0 ±0.0	2.4 ±0.4	3.6 ±1.4
Total extractable residues	99.2 ±0.4	95.8 ±0.3	94.0 ±0.8	89.8 ±0.7	87.1 ± n.a.	78.2 ±0.0	74.5 ±0.2	70.9 ±0.9
$^{14}CO_2$	0.0 ±0.0	0.5 ±0.0	1.4 ±0.1	2.2 ±0.1	4.2 ± n.a.	7.8 ±0.1	9.8 ±0.7	12.3 ±1.1
Volatile organics	0.0 ±0.0	0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± n.a.	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0
Non-extractable residues (NER)	0.8 ±0.0	2.6 ±0.2	4.5 ±0.2	6.7 ±0.0	9.0 ± n.a.	13.4 ±0.3	14.4 ±0.2	16.4 ±0.3
Total recovery	100.0 ±0.4	99.0 ±0.5	99.9 ±1.2	98.9 ±0.6	100.4 ± n.a.	99.3 ±0.2	98.6 ±0.7	99.6 ±1.8

a) No individual peak accounted to more than 1.5%.
b) Aggressive extracts were not analyzed since the RA content was less than 3% of total applied.
c) SD not to be calculated because just a single replicate was considered: moisture level of one replicate was less than pF 2.5.
n.a. = not analyzed
Note: ppm analyte = analyte % of AR x (ppm parent applied/100%) x MW analyte/288.7)

Table 7.1.1- 19: Biotransformation of [FUR-^{14}C]BYI 02960 in sandy loam [redacted] (CA) under aerobic conditions; mean values and standard deviations expressed as % of AR

Compound	Days After Treatment (DAT)							
	0 b)	3 b)	7 b)	14	28	60	92	120
BYI 02960	99.4 ±0.3	95.2 ±0.8	86.5 ±0.4	79.8 ± 0.2	67.7 ± 0.6	48.3 ±0.9	37.7 ±1.4	30.8 ±0.0
Non-characterized radioactivity a)	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.6 ±0.6	2.8 ±0.2	2.8 ±0.3
Total extractable residues	99.8 ±0.3	96.2 ±0.6	89.0 ±0.4	79.8 ±0.2	67.7 ± 0.6	49.0 ±0.0	40.5 ±1.1	33.7 ±0.2
$^{14}CO_2$	0.0 ±0.0	0.7 ±0.0	2.7 ±0.1	6.2 ±0.0	11.7 ± 0.1	23.9 ±0.2	30.1 ±0.6	36.1 ±0.1
Volatile organics	0.0 ±0.0	0.0 ± 0.0	± 0.0 ±0.0	± 0.0 ± 0.0	± 0.0 ± n.a.	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0
Non-extractable residues (NER)	0.2 ±0.0	2.8 ±0.1	8.4 ±0.5	13.6 ±0.4	19.6 ± 0.5	27.9 ±0.2	28.7 ±0.7	30.6 ±0.0
Total recovery	100.0 ±0.2	99.6 ±0.8	100.1 ±0.8	99.7 ±0.2	99.1 ± 1.1	100.8 ±0.3	99.3 ±0.2	100.4 ±0.0

a) No individual peak accounted to more than 1.8%.
b) Aggressive extracts were not analyzed since the RA content was less than 3% of total applied.
Note: ppm analyte = analyte % of AR x (ppm parent applied/100%) x MW analyte/288.7)

B. Mass Balance

Material balances ranged from 98.6 to 100% (NE soil) and from 99.1 to 100.8% (CA soil) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

In gamma-irradiated silt loam and sandy loam test systems, the material balances were complete as well.

Table 7.1.1- 20: Summary of material balances of radioactivity on four soils

Soil	[redacted]	[redacted]
Total Recovery (%)	100-99.6	100-100.4
Mean (%)	99.5	99.4
Relative SD (%)	0.6	0.6

C. Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 99.2 and 99.8% of AR at DAT-0 to 70.9 and 33.7% of AR at study end (DAT-120) in NE and CA soil, respectively.
In gamma-irradiated test systems extractable ^{14}C-residues only decreased from 100.8 and 99.4% of AR at DAT-0 to 90.7 and 82.4 % of AR at study end (DAT-120) in NE and CA soil, respectively.
Non-extractable ^{14}C-residues increased from 0.8 and 0.2% of AR at DAT-0 to 16.4 and 30.6% of AR at study end (DAT-120) in NE and CA soil, respectively. The majority of the NER was associated with the humin. In the sandy loam after extraction with the strong acid and base, an average of 74% still remained with the solid fraction (humin). Thus, even with exposure to strong base a significant portion of non-extractable BYI 02960 residues still remained bound to the solid phase of the soil, supporting the observations of biologically-mediated bound residues.
In gamma-irradiated test systems NER only increased from 0.3 and 0.2% of AR at DAT-0 to 6.2 and 8.7% of AR at study end (DAT-120) in NE and CA soil, respectively. Thus, NER were significantly lower in gamma-irradiated compared to non-sterile soils, indicating a biological component to the formation of bound residue.

Table 7.1.1- 21: Summary of extractable and non-extractable residues

Soil		[redacted]	[redacted] gamma-irradiated	[redacted]	[redacted] gamma-irradiated
Extracted RA (%)	Day 0	99.2	100.8	99.8	99.4
	Day 120 (122[#])	71.9	90.7	33.8	82.4
Non-Extracted RA (%)	Day 0	0.8	0.3	0.2	0.2
	Day 120	16.4	6.2	30.6	8.7

[#] gamma-irradiated soils

D. Volatilization

The mineralization of [FUR-^{14}C]BYI 02960 was high. At the end of the study (DAT-120), $^{14}CO_2$ accounted for 12.3% (NE soil) and 36.1% (CA soil) of AR. Volatile organic compounds were not detected in significant amounts ($\leq$ 0.1% AR) throughout the study.
In gamma-irradiated test systems the mineralization of [FUR-^{14}C]BYI 02960 was low. At the end of the study (DAT-120), $^{14}CO_2$ accounted for 2.9% (NE soil) and 4.1% (CA soil) of AR, only. This indicates a biological component to the formation of $^{14}CO_2$.

E. Transformation of Test Item

The test item declined from 98.0 and 99.4% of AR at DAT-0 to 67.3 and 30.8% at the end of the study for soils NE and CA, respectively. Other than parent, only unidentified metabolites were measured and accounted for a maximum of 3.6 and 2.8% for soils NE and CA, respectively. The results are included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure 7.1.2- 1.

F. Kinetics of Test Item Degradation

The degradation of the parent compound during the study is given under point 7.2.1.

.

III CONCLUSIONS

A. Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that BYI 02960 is degraded microbially in the soils. One part becomes increasingly bound to soil (16.4% [redacted], 30.6% [redacted]) by study end. The other part is well mineralized to $^{14}CO_2$ (12.3% [redacted], 36.1% [redacted]).
A synopsis of results is shown in Table 7.1.1- 23.
All formed metabolites are regarded as transient. The mineralization rate of [FUR-^{14}C]BYI 02960 to $^{14}CO_2$ was high in this study. Volatile organic compounds were very low (≤ 0.1% AR) at all sampling dates.
The comparison of the behavior of the sterile and non-sterile soils, significantly lower amounts of bound residues and $^{14}CO_2$ in the sterile soils, indicate a biological component to the degradation / mineralization and formation of non-extractable residues from BYI 02960.
Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

Table 7.1.1- 22: Synopsis of overall results

Soil	[redacted] Viable	[redacted] Sterile	[redacted] Viable	[redacted] Sterile
Total Recovery (%)	98.9-100.4	98.1-101.1	99.6-100.8	95.2-99.6
Extracted RA (%)	70.9-99.2	90.7-100.8	33.7-99.8	82.4-99.4
Max. CO2 (%)	12.3	2.9	36.1	4.1
Bound Residues (%)	0.8-16.4	0.3-6.2	0.2-30.6	0.2-8.7
Major metabolites	-	Not determined	-	Not determined

B. Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that a viable aerobic soil environment will contribute significantly to the degradation of BYI 02960. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant, however no metabolites are expected to accumulate in soil (see Table 7.1.1- 23).

Table 7.1.1- 23: Synopsis of results of biotransformation of [FUR-^{14}C]BYI 02960 in two soils incubated at 20 °C and pF 2.0 - 2.5 % under aerobic conditions

Soil	[redacted] (NE)		[redacted] (CA)	
Soil type	Silt loam		Sandy loam	
Soil status	Viable	Sterile	Viable	Sterile
DT_{50} of BYI 02960 [days]	228	Not calculated	66	Not calculated
Major transformation products *	CO_2 NER	(NER)	CO_2 (max. 36.1%) NER (max. 30.6%)	(NER)
Minor transformation products	-	CO_2	-	CO_2

*): 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.
(NER): Major acc. to *, but did not exceed 10% of AR during the entire study period.

Report:	**KIIA 7.1.1/04, [redacted], [redacted], 2011**
Title:	[Ethyl-1-^{14}C]BYI 02960: Aerobic Soil Metabolism
Report No & Document No	MEF-10/858 M-414981-01-1
Guidelines:	OECD TG 307, Aerobic and Anaerobic Transformation in Soil US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.
GLP:	Yes (fully GLP compliant and certified laboratory)

EXECUTIVE SUMMARY

The biotransformation of [ethyl-1-^{14}C]BYI 02960 was studied in three European soils: [redacted] (AX), [redacted] am [redacted] (HF), and [redacted] II (DD) for a maximum period of approx. 120 days under aerobic conditions in the dark at approx. 20 °C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 1.07 mg/kg dry weight of soil, which is equivalent to 400 g/ha field application rate.
At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave. The BYI 02960 residues and transformation products were analyzed and quantified by LCS and radio-HPLC. Radio-TLC was used as confirmation method.
Material balances were complete throughout the study, and the test substance declined from 96.0, 96.6 and 97.1% of AR at DAT-0 to 17.7, 39.6 and 23.8% of AR at the end of the study for soils DD, AX and HF, respectively. Applying double first-order kinetics a half-life (geometric mean) of 41.6 days was calculated for BYI 02960 in the tested soils under aerobic conditions.
The mineralization of [ETH-^{14}C]BYI 02960 was high. At the end of the study (DAT-120), up to 42.3 (DD), 25.9 (AX) and 33.9% AR (HF) of $^{14}CO_2$ were generated. Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR).
In addition to carbon dioxide, one major transformation product was detected in the extracts of all three soils. It was identified as difluoroacetic acid (DFA) via HPLC-MS and accurate mass determination. DFA reached maximum values of 30.2, 22.0 and 33.8% of AR on DAT-45 or DAT-48 in soils DD, AX and HF, respectively. Towards the end of the study, the levels of DFA declined to 17.0, 16.3 and 23.8% of AR in soils DD, AX and HF, respectively.

Non-extractable ^{14}C-residues increased from 2.7, 2.8 and 3.2% of AR at DAT-0 to 17.9, 14.3 and 15.4% AR at the end of the study period for soils DD, AX and HF, respectively.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: Flupyradifurone: Code = BYI 02960;
Label ETH = [Ethyl-1-^{14}C]BYI 02960
Specific activity 3.93 MBq/mg
Batch ID KATH 6350 used for soil [redacted] II on 2009-08-25
Batch KATH 6431 used soils [redacted] and [redacted] am [redacted] 4a
Radiochemical purity: >98% (for both batches)
Chemical purity: >98% (for both batches)
Identity and purity of test items in the application solution were confirmed.

2. Soil: The biotransformation of BYI 2960 was studied in three different soils.. Soil [redacted] II was taken on 2009-08-18, soils [redacted] [redacted] and [redacted] am [redacted] 4a were taken on 2010-03-08 fresh from the field, and shipped subsequently. Few days later, i.e. five (DD) and four days before starting the test the gently air dried soils were sieved through a 2 mm sieve. One (soil DD) or three days (soils AX and HF) before application, aliquots equivalent to 100 g dry matter were weighed into the individual test flasks and fitted with trap attachments. Water was added in order to reach 55% of the maximum water holding capacity by the addition of application solution. The soils were pre-equilibrated at study conditions.

Table 7.1.1- 24: Soil physicochemical properties

Parameter	Results/Units		
Soil Batch ID	[redacted] II, 20090818	[redacted] [redacted], 20100308	[redacted] am [redacted] 4a, 20100308
Location	[redacted], Germany	[redacted] Germany	[redacted] Germany
Soil Taxonomic Classification (USDA)	N/A	Sandy floodplain deposits of the lower terrace of the Rhine river, material from the Pleistocene Ice Age	Loess or loess colluvium (Pleistocene, Holocene)
Soil Series	N/A	Sandy, mixed, mesic Typic Cambudolls	Loamy, mixed, mesic Typic Argudalfs
Texture Class (USDA)	Clay loam	Loamy sand	Silt Loam
Sand Silt Clay	43% 26% 31%	81% 10% 9%	23% 60% 17%
pH in Water pH in $CaCl_2$ pH in KCl pH in Saturated Paste	7.3 7.1 6.9 7.3	6.5 6.2 6.1 6.6	6.8 6.5 6.3 6.8
Organic Matter	8.8%	3.3%	4.1%
Organic Carbon	5.1%	1.9%	2.4%
Soil Microbial Biomass mg microbial carbon per kg of soil			
-1/0 days	2831	627	979
69/61 days	2208	398	731
114/117 days	1753	224	455
Cation Exchange Capacity (CEC)	25.6 meq/100 g	9.3 meq/100 g	13.4 meq/100 g
WHCmax	83.9 g H_2O /100 g DM	50.7 g H_2O /100 g DM	64.7 g H_2O /100 g DM

B. Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 19.2 ± 0.2 °C (soil DD) or 20.2 ± 0.3 C (soils AX and HF) for a maximum period of 118 days. 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 (each 23 flasks/soil). For all soils replicates were set up for each sampling (10 sampling dates including time 0, and each three flasks were used for determination of the microbial biomass. In addition four (soil DD) and 16 (soils AX and HF) spare flasks were set up. The final soil moisture was adjusted to 55% of WHC_{max} by adding pure water.

2. Test Item Stock Solution: The entire delivered amount of [ETH-^{14}C]BYI 02960 was dissolved in 4 mL methanol. Identity and purity of test items were confirmed by HPLC-MS, HPLC-MS/MS and NMR.

3. Test Item Application Solution: The application solution for soil DD was made by diluting 1806 µL of respective stock solution with 10.2 mL of purified water, resulting in a mean concentration of 21076 Bq/20 µL. The application solution used for soils AX and HF was made by diluting 3080 µL of respective stock solution with 28.92 mL of purified water, resulting in a mean concentration of 526.4 Bq/500 µL.

4. Mode of Application: On 2009-08-25, aliquots of 398 µL of resp. application solution were applied in droplets onto the 100 g subsamples of soil [redacted] II. On 2010-03-18, in the same way aliquots of 398 µL of resp. application solution were applied onto the 100-g subsamples of soils [redacted] and [redacted] am [redacted] 4a. By addition of the application solution the water content was finally adjusted to 55% of WHCmax. The test vessels for DAT-0 were immediately worked up. All other test vessels, including four reserve vessels and the biomass flasks which were not spiked with application solution, were fitted with trap attachments and incubated in the dark at nominal 20 ± 1°C. BYI 02960 was applied at a rate of 436,341.7 Bq per vessel containing soil DD and at a rate of 412,663.0 Bq per vessel containing soils AX and HF. This corresponds to 111.03 µg per DD and to 105.0 µg per AX or HF soil containing vessel, which is equivalent to 104.1% and 98.4% of the intended application rate of 106.7 µg per vessel (calculated for a single application rate of 400 g BYI 02960 per hectare).

5. Sampling: Microbial biomass was determined prior to commencement of the test (soils sampled one day before or at the day of application), after 69 or 61 days, and at the end of the study (114 or 117 days after treatment (DAT). Entire DD test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-3, DAT-7, DAT-14, DAT-30, DAT-45, DAT-62, DAT-90, and DAT-118, entire AX and HF test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-4, DAT-7, DAT-14, DAT-29, DAT-48, DAT-61, DAT-90, and DAT-117.
Prior to opening the incubation flasks (for moistening or sampling of soil), volatile (radioactive) compounds, possibly still present in the flasks, were transferred into the trap attachment by subjecting the flasks to vacuum in an exicator. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

6. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by 30-min shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave for 10 minutes. The supernatants were decanted and filtered using a folded filter. The folded filters were compressed to pills and combusted. The evolved $^{14}CO_2$ was trapped in a scintillation cocktail and analyzed for radioactivity by LSC.
The BYI 02960 residues and transformation products were analyzed and quantified by LSC and reversed phase radio-HPLC.
For HPLC analysis, 4 mL of the combined ambient organic extracts were concentrated to about 2 mL using a SpeedVac® concentrator. It was confirmed by a recovery test that that no radioactivity was lost during the concentration step. The microwave extracts ("aggressive" extracts") were analyzed without

any further processing. The limit of detection (LOD) in HPLC was equal to or better than 0.49% AR. Normal-phase Si-60 radio-TLC was used as confirmation method. For TLC analysis, the extracts were not processed further.
The identity of the test item in stock solution and in extracts was confirmed by spectroscopic methods. In addition, spectroscopic methods were used to identify one of the minor metabolites.

II. RESULTS AND DISCUSSION

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study.

A. Data

The respective data for the three soils are shown in **Error! Reference source not found.** to **Error! Reference source not found.**. The DAT-0 extraction efficiency was in the range 96.5 to 97.5% of AR (mean 97.0% AR; sum of extracts only). The stability of the test item was verified by DAT-0 values of 96.0 to 97.1% AR for the soil extracts (mean 96.6% AR). These results indicate that the extraction method was well suited to extract the applied [^{14}C]-labeled test item from the soil matrix. TLC confirmed the results of the HPLC measurements for the test item and DFA.

Table 7.1.1- 25: Biotransformation of [ETH-^{14}C]BYI 02960 in clay loam soil DD under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	3	7	14	30	45	62	90	118
BYI 02960	96.0 ±0.1	92.8 ±0.4	89.3 ±0.8	82.1 ±0.1	73.2 ±0.7	52.2 ±0.0	38.6 ±0.8	31.3 ±0.6	21.4 ±1.5	17.7 ±0.4
DFA	n.d.	0.8 ±0.1	3.2 ±0.2	7.7 ±0.4	15.5 ±0.0	26.4 ±0.5	30.2 ±0.2	28.0 ±0.7	23.4 ±0.9	17.0 ±0.2
Reg 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ±0.0
Non-charact. radioactivity	0.5 ±0.1	0.3 ±0.1	0.5 ±0.0	0.4 ±0.0	0.5 ±0.1	0.4 ±0.0	0.3 ±0.0	0.4 ±0.1	0.2 ±0.0	0.2 ±0.0
Total extract. residues	96.5 ±0.0	93.9 ±0.4	93.0 ±0.5	90.2 ±0.3	89.2 ±0.7	79.0 ±0.5	69.1 ±0.6	59.7 ±0.1	45.0 ±0.6	35.3 ±0.1
$^{14}CO_2$	n.a.	<0.1	0.1 ±0.0	0.7 ±0.0	2.2 ±0.0	7.8 ±0.3	14.3 ±0.1	22.3 ±0.0	33.4 ±0.0	42.3 ±0.2
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extract. residues (NER)	2.7 ±0.0	3.8 ±0.3	4.6 ±0.1	6.9 ±0.0	8.6 ±0.2	11.1 ±0.2	13.9 ±0.1	15.7 ±0.0	17.5 ±0.0	17.9 ±0.0
Total recovery	99.2 ±0.0	97.7 ±0.2	97.7 ±0.6	97.8 ±0.3	100.0 ±0.9	97.8 ±0.0	97.4 ±0.6	97.7 ±0.0	96.0 ±0.6	95.5 ±0.3

n.d. = not detected; n.a. = not analyzed

Table 7.1.1- 26: Biotransformation of [ETH-^{14}C]BYI 02960 in loamy sand soil AX under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	4	7	14	29	48	61	90	117
BYI 02960	96.6 ±1.0	94.3 ±1.0	90.5 ±1.3	83.7 ±0.3	76.7 ±1.4	59.6 ±2.7	53.8 ±1.6	49.4 ±0.6	44.5 ±0.1	39.6 ±0.3
DFA	n.d.	0.7 ±0.1	3.0 ±0.1	6.5 ±0.2	14.1 ±1.5	17.3 ±0.0	22.0 ±1.1	19.6 ±0.5	16.2 ±0.0	16.3 ±0.5
Reg 2	n.d.	n.d.	n.d.	n.d.	n.d.	1.2 ±0.1	1.5 ±0.1	1.6 ±0.1	1.8 ±0.1	1.3 ±0.4
Non-charact. radioactivity	0.4 ±0.1	0.4 ±0.0	0.5 ±0.1	0.4 ±0.1	0.3 ±0.0	0.4 ±0.2	0.3 ±0.1	0.2 ±0.1	0.3 ±0.1	0.4 ±0.0
Total extract. residues	97.0 ±1.1	95.4 ±0.9	93.9 ±1.2	90.6 ±0.3	91.1 ±0.0	78.6 ±5.0	77.5 ±0.3	70.8 ±0.1	62.8 ±0.0	57.6 ±0.1
$^{14}CO_2$	n.a.	<0.1	0.2 ±0.0	0.4 ±0.0	1.6 ±0.0	5.4 ±0.1	10.8 ±0.0	14.4 ±0.2	21.2 ±0.2	25.9 ±0.2
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	2.8 ±0.1	3.1 ±0.1	4.6 ±0.2	5.3 ±0.1	7.7 ±0.3	15.0 ±4.3	11.9 ±0.1	13.2 ±0.0	14.8 ±0.4	14.3 ±0.1
Total recovery	99.8 ±1.2	98.4 ±0.8	98.6 ±1.0	96.3 ±0.5	100.3 ±0.3	98.9 ±1.3	100.2 ±0.2	98.4 ±0.4	98.8 ±0.5	97.8 ±0.4

n.d. = not detected; n.a. = not analyzed

Table 7.1.1- 27: Biotransformation of [ETH-^{14}C] BYI 02960 in silt loam soil HE under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	4	7	14	29	48	61	90	117
BYI 02960	97.1 ±0.5	94.5 ±1.5	88.5 ±0.1	80.1 ±1.9	71.9 ±0.9	55.6 ±0.2	39.8 ±1.4	32.7 ±0.1	27.0 ±1.2	23.0 ±0.2
DFA	n.d.	0.9 ±0.1	5.0 ±0.2	8.7 ±0.3	17.1 ±0.1	25.8 ±0.0	33.9 ±1.2	33.1 ±1.0	28.0 ±1.4	23.8 ±0.1
Reg 2	n.d.	n.d.	n.d.	n.d.	n.d.	1.3 ±0.1	1.4 ±0.0	1.1 ±0.1	1.6 ±0.1	1.5 ±0.1
Non-characterized radioactivity	0.4 ±0.0	0.4 ±0.1	0.5 ±0.1	0.4 ±0.0	0.4 ±0.1	0.5 ±0.0	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0
Total extractable residues	97.5 ±0.5	95.8 ±1.2	93.9 ±0.0	89.1 ±1.5	89.4 ±1.1	83.3 ±0.1	75.4 ±0.2	67.1 ±0.7	57.0 ±0.3	48.6 ±0.0
$^{14}CO_2$	n.a.	<0.1	0.2 ±0.0	0.5 ±0.0	1.9 ±0.0	6.3 ±0.0	13.0 ±0.1	17.5 ±0.1	27.1 ±0.2	33.9 ±0.1
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	3.2 ±0.1	4.2 ±0.4	5.2 ±0.2	7.3 ±0.4	8.2 ±0.0	11.0 ±0.1	12.6 ±0.1	14.5 ±0.4	14.4 ±0.4	15.4 ±0.2
Total recovery	100.7 ±0.6	100.0 ±0.9	99.3 ±0.2	97.0 ±1.9	99.4 ±1.1	100.6 ±0.0	101.0 ±0.1	99.1 ±0.3	98.5 ±0.5	97.9 ±0.1

n.d. = not detected; n.a. = not analyzed

B. Mass Balance

Material balances ranged from 95.5 – 100.0% (soil DD), 96.3 – 100.3% (soil AX) and 96.3 – 100.3% (soil HE) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

C. Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 96.5, 97.0 and 97.5% AR at DAT-0 to 35.3, 57.6 and 48.6% of AR at study end in soils DD, AX and HF, respectively.
Non-extractable ^{14}C-residues increased from 2.7, 2.8 and 3.2% of AR at DAT-0 to 17.9, 15.0 and 15.4% AR at the end of the study period for soils DD, AX and HF, respectively. The major portions of radioactivity were found in the insoluble humin fraction.

D. Volatilization

The mineralization of [ETH-^{14}C]BYI 02960 was high. At the end of the study, $^{14}CO_2$ accounted for up to 42.3 (soil DD), 25.9 (soil AX) and 33.9% (soil HF) of AR. Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR).

E. Transformation of Test Item

The test item declined from 96.0, 96.6 and 97.1% of AR at DAT-0 to 17.7, 39.6 and 23.0% at the end of the study for soils DD, AX and HF, respectively.
One major transformation product was detected in all three soils. It was identified as difluoroacetic acid (DFA) via HPLC-MS and accurate mass determination in ambient organic extracts originating from soil [redacted] II. The amount of DFA reached maximum values of 30.2, 22.0 and 33.9% of AR on DAT-45 or DAT-48 in soils DD, AX and HF, respectively. At the end of the study, the amounts of DFA declined to 17.0, 16.3 and 23.8% of AR in soils DD, AX and HF.
In addition, one minor transformation product (Reg 2) was detected in all three soils with maximum amounts of 0.4 (DAT-118), 1.8 (DAT-90) and 1.6% (DAT-90) of applied radioactivity for soils DD, AX and HF, respectively. The total of unknown extracted radioactivity did not exceed 0.5% AR
The results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure 7.1.2- 1.

F. Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculations for the test item is given in under point 7.2.1.

III CONCLUSIONS

A. Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that BYI 02960 is well degraded in the four soils to form one major and one very minor metabolite. The major metabolite was identified as difluoroacetic acid (max. 33.9% of AR). The amounts of the very minor metabolite did not exceed 1.8% of AR; no identification or characterization was made. Significant amounts of $^{14}CO_2$ (up to 42.3% AR) were measured at the end of the study indicating that mineralization of the test item and/or degradates occurred. The maximum amount of non-extractable radioactivity was 17.9% of AR.

Table 7.1.1- 28: Synopsis of results of biotransformation of [ETH-^{14}C]BYI 02960 in soils incubated at 20 °C and 55 % of WHCmax under aerobic conditions)

Soil	DD	AX	HF
Total Recovery (%)	95.5-100.0	97.8-100.3	97.9-100.7
Extracted RA (%)	35.3-95.5	57.6-97.0	48.6-97.5
Max. CO2 (%)	42.3	25.9	33.9
Bound Residues (%)	2.7-17.9	2.8-15.0	3.2-15.4
Major metabolites	DFA (30.2%)	DFA (22.0%)	DFA (33.9%)

B. Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions. With respect to the radiolabel used mineralization to $^{14}CO_2$ is highly significant. Difluoroacetic acid was detected as a further major transformation product. Other metabolites are transient and would not be expected to accumulate in soil see Table 7.1.1- 29).

Table 7.1.1- 29: Synopsis of results of biotransformation of [ETH-^{14}C]BYI 02960 in soils incubated at 20 °C and 55 % of WHCmax under aerobic conditions

Soil	[redacted] **II**	[redacted]	[redacted] **am** [redacted]
Soil type	Clay loam	Loamy sand	Silt loam
Major transformation products *	CO_2 (max. 42.3% AR); NER (max 17.9%) DFA (max. 33.9% AR)		
Minor transformation products			

*): 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.

Report:	**KIIA 7.1.1/05, [redacted], [redacted], 2011**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Aerobic Soil Metabolism
Report No & Document No	MEF-10/880 M-411693-01-2
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The biotransformation of [pyridine-2,6-^{14}C]BYI 02960 was studied in one soil, [redacted] am [redacted] 4a (HF, [redacted] Germany), a silt loam soil of pH 6.5 ($CaCl_2$) and 2.4% organic carbon content. The incubation was conducted for max. 117 days under aerobic conditions in the dark at ca. 20°C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 106.7 µg/ 100 g soil, which is equivalent to a field application rate of 400 g/ha.

At each sampling date, i.e. 0, 1, 4, 7, 14, 29, 48, 61, 90 and 117 days after treatment (DAT), the duplicate soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave. The BYI 02960 residues and transformation products were analyzed and quantified by LCS and radio-HPLC. Radio-TLC was used as a confirmatory method.

Material balances were complete throughout the study (ranged on mean from 94.1 to 100.3% of AR), and the test substance declined from 96.7% at DAT-0 to 24.6% of AR at the end of the study.

The mineralization of [PYR-^{14}C]BYI 02960 was high. At the end of study (DAT-117), $^{14}CO_2$ accounted for 57.4% of AR. Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR).

Only minor transformation products (all mean values were ≤ 2.5% of AR) were detected in the extracts and were characterized by their retention time. The transformation product assigned to Reg 2 reached a mean maximum level of 2.0% of AR at DAT-48. The maximum amount of the second transformation product (Reg 3) was detected on DAT-7 (2.5% of AR). Towards the end of the study,

the amounts decreased below the detection limit. Reg 3 was identified as 6-chloronicotinic acid (6-CNA) by co-chromatography. The third minor transformation product was detected only once (DAT-48, 0.3% of AR). Non-extractable ^{14}C-residues increased from 3.3% of AR at DAT-0 to 16.7% of AR at the end of the study period.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: Flupyradifurone: Code = BYI 02960;
Label PYR = [Pyridine-2,6-^{14}C]BYI 02960 (sample ID: KATH 6432)
Specific activity: 4.49 MBq/mg
Radiochemical purity: >98% (acc. radio-HPLC and radio-TLC)
Chemical purity: >98% (HPLC, UV detection at 210 nm)
Identity and purity of test item in the application solution were confirmed.

2. Soil: The biotransformation of BYI 02960 was studied in one soil. The soil was taken on 2010-03-08 fresh from the field. On the day of sampling, the soil was broken up stepwise and gently air dried. Then, the soil was successively sieved to < 2 cm, filled into plastic bags and stored in a climatic cabinet at 20 ± 2°C.
On 2010-03-15, 100 g aliquots (dry matter) of the sieved soil were weighed into individual 300 mL Erlenmeyer flasks. Water was added in order to finally reach 55% of the maximum water holding capacity by the addition of application solution (953 µL) on the day of application.
Three additional test systems were prepared which were used for microbial biomass determinations. Since no application solution was added to these vessels, they were directly adjusted to 55% of maximum water holding capacity. All vessels were fitted with trap attachments and pre-equilibrated at 20 ± 2°C in the dark for three days.

Table 7.1.1- 30: Soil physicochemical properties

Parameter	Results/Units
Batch ID	[redacted] am [redacted] 4a, 20100308
Location	[redacted] Germany
Soil Taxonomic Classification (USDA)	Loess or loess colluvium (Pleistocene, Holocene)
Soil Series	Loamy, mixed, mesic Typic Argudalfs
Texture Class (USDA)	Silt Loam
Sand Silt Clay	23% 60% 17%
pH in Water pH in CaCl2 pH in KCl	6.8 6.5 6.3
Organic Matter	4.1%
Organic Carbon	2.4%
Initial & Final Soil Biomass or Microbial Activity	mg microbial C/g soil
0 days	970
61 days	756
117 days	554
Cation Exchange Capacity (CEC)	13.4 meq/100 g
WHCmax	64.7 g H2O/ 100 g DM

B. Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic conditions in the dark at 20.2 ± 0.3 °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. Replicates were set up and processed for each sampling (10 sampling dates including time 0, and 16 spare flasks were set up but not further processed in this study). Three flasks were used for determination of the microbial biomass. The final soil moisture was adjusted to 55% of WHC_{max} by adding pure water.

2. Test Item Stock Solution: The entire delivered amount of [pyridine-2,6-^{14}C]BYI 02960 was dissolved in 4 mL methanol.

3. Test Item Application Solution: An application solution was made by diluting 2048 µL of before-mentioned stock solution with 9.952 mL of purified water.

4. Mode of Application: On 2008-03-18, aliquots of 353 µL of the application solution were applied in droplets onto the 100 g pre-incubated subsamples of each soil. By the addition of the application solution, the water content was finally adjusted to 55% of WHC_{max}. The test vessels for DAT-0 were immediately processed for analysis. All other test vessels, including the biomass flasks which were not spiked with application solution, were fitted with trap attachments and incubated in the dark at nominal 20 ± 2°C.

[PYR-^{14}C]BYI 02960 was applied at a rate of 415954 Bq per vessel. This corresponds to 92.64 µg per vessel which is equivalent to 86.8% of the intended application rate of 106.7 µg per vessel (calculated for a single application rate of 400 g BYI 02960 per hectare).

5. Sampling: Microbial biomass was determined prior to commencement of the test (soils sampled at the day of application), after 61 days, and at the end of the study 117 days after treatment (DAT-117). Entire test flasks were taken for processing and analysis at DAT-0, DAT-1, DAT-4, DAT-7, DAT-14, DAT-29, DAT-48, DAT-61, DAT-90, and DAT-117.
Prior to opening the incubation flasks (for moistening or sampling of soil), volatile (radioactive) compounds, possibly still present in the flasks, were transferred into the trap attachment by subjecting the flasks to vacuum in a desiccator. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

6. 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. At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave.
The BYI 02960 residues and transformation products were analyzed and quantified by LSC and reversed phase radio-HPLC. Losses of radioactivity after concentration of extracts were minimal. Normal-phase Si-60 radio-TLC was used as confirmation method. The identity of the test item in stock solution and in extracts was confirmed by spectroscopic methods. In addition, spectroscopic methods were used to identify one of the minor metabolites.

II. RESULTS AND DISCUSSION

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study.

A. Data

The data for the soil are shown in Table 7.1.1- 30.. The DAT-0 extraction efficiency was 96.7% of AR (ambient extracts 95.0% of AR). The stability of the test item was verified by a DAT-0 recovery of 96.3 ±0.7% of AR. These results indicate that the extraction method was well suited to extract the applied [^{14}C]-labeled test item from the soil matrix.

Table 7.1.1- 31: Biotransformation of [PYR-[14]C]BYI 02960 in sandy loam soil HF under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)									
	0	1	4	7	14	29	48	61	90	117
BYI 02960	96.3 ±0.7	95.3 ±0.1	88.5 ±0.8	78.4 ±0.8	70.9 ±0.2	53.5 ±0.1	40.9 ±1.5	33.9 ±0.1	27.3 ±0.3	22.7 ±0.5
Reg 2	n.d.	n.d.	n.d.	n.d.	0.7 ±0.2	1.6 ±0.2	2.0 ±0.2	1.9 ±0.3	1.1 ±0.3	1.9 ±0.1
6-CNA	n.d.	n.d.	1.7 ±0.0	2.5 ±0.1	2.0 ±0.2	0.9 ±0.0	0.5 ±0.1	0.2 ±0.2	n.d.	n.d.
Reg 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ±0.3	n.d.	n.d.	n.d.
Non-characterized radioactivity	0.4 ±0.0	0.5 ±0.0	0.4 ±0.1	0.2 ±0.0	0.3 ±0.0	0.2 ±0.1	0.2 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
Total extractable residues	96.7 ±0.8	95.8 ±0.2	90.6 ±0.9	81.2 ±0.7	73.9 ±0.2	56.4 ±0.0	43.9 ±1.1	36.1 ±0.2	28.6 ±0.0	24.6 ±0.4
$^{14}CO_2$	n.a.	0.1 ±0.0	1.9 ±0.1	5.1 ±0.0	13.4 ±0.1	26.8 ±0.4	40.6 ±0.3	46.0 ±0.1	52.5 ±0.6	57.4 ±0.5
Volatile organics	n.a.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	3.3 ±0.0	4.2 ±0.0	6.1 ±0.0	7.8 ±0.3	10.4 ±0.0	14.1 ±0.2	15.7 ±0.0	16.3 ±0.1	16.7 ±0.1	16.2 ±0.1
Total recovery	100.0 ±0.7	100.2 ±0.2	98.6 ±0.9	94.1 ±0.3	97.7 ±0.3	97.3 ±0.2	100.3 ±0.8	98.3 ±0.1	97.8 ±0.5	98.3 ±0.0

n.d. = not detected; n.a. = not analyzed; Reg 3 was identified as compound 6-CNA

B. Mass Balance

Material balance ranged from 94.1 ±0.3 to 100.3 ±0.8% of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

C. Extractable and Bound Residues (NER)

Extractable [14]C-residues decreased from 96.7 ±0.8% at DAT-0 to 24.6 ±0.4% of AR at study end (DAT-117). Non-extractable [14]C-residues increased from 3.3 ±0.0% at DAT-0 to 16.2 ±0.1% of AR at the end of the study period.

D. Volatilization

The mineralization of BYI 02960 was high. At the end of the study (DAT-117), $^{14}CO_2$ accounted for 57.4 ±0.5% of AR. Volatile organic compounds were not detected in significant amounts (< 0.1% AR).

E. Transformation of Test Item

The test item declined from 96.3 ±0.7% of AR at DAT-0 to 22.7 ±0.5% of AR at the end of the study. Three minor transformation products were detected in the extracts. The transformation product assigned to Reg 2 reached a maximum level of 2.0% of AR at DAT-48. The amount of the second transformation product (Reg 3) increased to a maximum amount of 2.5% of AR on DAT-7 and decreased below the detection limit towards the end of the study. This transformation product was identified as 6-chloronicotinic acid (6-CNA) by co-chromatography. The third minor transformation product was detected only once (DAT-48), accounting for 0.3% of AR.

The total of unknown extracted radioactivity did not exceed 0.5% of AR and was assigned to the background signal in the HPLC-analysis. The mentioned results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure 7.1.2- 1.

F. Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculation for the test item is given in Point 7.2.1.

III CONCLUSIONS

A. Major Outcomes of Study

The data gathered in the current laboratory investigation demonstrate that BYI 02960 is degraded in HF soil. A synopsis of results is shown by Table 7.1.1- 32.
A few very minor metabolites were detected and quantified together with the test item. All further formed metabolites are regarded as transient, which is confirmed by the high mineralization rate of [PYR-^{14}C] BYI 02960 to $^{14}CO_2$ observed in this study, i.e. 57.4% of AR until the end of the study. Volatile organic compounds were very low (≤ 0.1% AR) at all sampling dates.

B. Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that BYI 02960 is degradable in soil under aerobic conditions. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant, however, metabolites are not expected to accumulate in soil, since – after their formation – they are well mineralized (see Table 7.1.1- 32).

Table 7.1.1- 32: Synopsis of results of biotransformation of [PYR-^{14}C]BYI 02960 in soil HF incubated at 20 °C and 55 % of WHCmax under aerobic conditions

Soil	[redacted] **am** [redacted] **4a**
Soil type	Silt loam
Major transformation products *	CO_2 (max. 57.4%) NER (max. 16.7%)
Minor transformation products	6-CNA (max. 2.5%)

*): 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.

Report:	**KIIA 7.1.1/06, [redacted], 2011**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Aerobic Soil Metabolism in Two US Soils
Report No & Document No	MERVP038 M-413425-02-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002** **US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The biotransformation of [pyridine-2,6-^{14}C]BYI 02960 was studied in two US soils, silt loam [redacted], NE, and sandy loam [redacted], CA, for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and soil moistures maintained between pF 2.0 to 2.5. Gamma

irradiated samples were investigated along with microbial active test systems. BYI 02960 was applied at the rate of 1.1 µg a.i./g soil, equivalent to 410 g a.i./ha assuming a 2.5 cm soil depth.

Duplicate test systems were analyzed at 0, 3, 7, 14, 28, 60, 92, and 120 days of incubation. The 50-g soil samples were extracted by shaking with acetonitrile:water (70:30) and acetonitrile (100%), followed by microwave extraction at 70 °C(aggressive extract) using acetonitrile:water (70:30). Extract aliquots were concentrated and analyzed by HPLC. Identification of the transformation products was performed by LC/MS, co-chromatography, and/or retention time comparison with reference standards.

Material balances were complete throughout the study, and the test item declined from 97.4 and 98.6% and of the applied amount at day 0 to 65.5 and 30.0% of the applied at the end of the study..

In the silt loam, extractable 14C-residues decreased from 99.3% of the applied amount at day 0 to 67.0% of the applied at the end of the study. Non-extractable 14C-residues increased from 0.7% of the applied amount at day 0 to a maximum of 11.3% at day 120. At study termination, evolved 14CO2 reached 20.2%, and radioactive volatile organics were not detected. Total unidentified radioactivity ranged from 0.0% to 2.4% of the applied amount.

In the sandy loam, extractable ^{14}C-residues decreased from 99.9% of the applied amount at day 0 to 40.0% of the applied at the end of the study. One major degradate was identified as 6-chloronicotinic acid (6-CNA), which formed 6.9% on day 14, reached a maximum of 17.1% on day 60, and declined to 8.2% by the end of the study.

In the sterile silt loam, extractable 14C-residues decreased from 99.1% of the applied amount at day 0 to 89.4% of the applied at the end of the study. Non-extractable 14C-residues increased from 0.9% of the applied amount at day 0 to a maximum of 6.2% at day 122. At study termination, evolved 14CO2 reached 0.4%, and radioactive volatile organics were not detected.

In the sterile sandy loam, extractable 14C-residues decreased from 99.8% of the applied amount at day 0 to 85.8% of the applied at the end of the study. Non-extractable 14C-residues increased from 0.2% of the applied amount at day 0 to a maximum of 9.3% at day 122. At study termination, evolved 14CO2 reached 0.9%, and radioactive volatile organics were not detected.

[PYR-^{14}C]BYI 02960 mineralizes relatively rapidly under aerobic condition to $^{14}CO_2$ (20.2% [redacted], 36.1% [redacted]) and becomes increasingly bound to soil (11.3% [redacted], 25.5% [redacted], CA) by study end. Total unidentified radioactivity ranged from 0.0% to 2.4% ([redacted]) and 0.0% to 3.0% ([redacted]) of the applied amount.

The amount of $^{14}CO_2$ and bound residue formed in gamma-irradiated soils was significantly less than in non-sterile soils indicating a biological component to degradation and the formation of non-extractable residues from BYI 02960. Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: Flupyradifurone: Code = BYI 02960
Label PYR = [pyridine-2,6-^{14}C]BYI 02960 (Vial C-1135)

Specific activity: 35.03 mCi/mMole
Radiochemical purity: >98%
Identity and purity of test item in the application solution were confirmed.

2. Soil: The biotransformation of [PYR-^{14}C]BYI 2960 was studied in two different soils
NE and CA soils were taken on 2009-11-30 and 2010-01-06, fresh from the fields. Each soil was collected from the top 0 to 8 inches of the field and shipped to the testing facility subsequently. No pesticides were applied in the past 5 years to these sites where soils were collected.
The NE soil was stored under refrigeration for 8 days, transferred to a greenhouse and stored under crop cover (alfalfa) until January 13, 2010. It was sieved with 2-mm sieve on January 15th, 2010.
The CA soil was maintained in a walk-in refrigerator prior to use at 5.9 ±0.8°C, sieved with 2-mm sieve on 2010-01-12.
Aliquots equivalent to 50 g dry matter were weighed into individual 250 mL glass flasks until starting the test. Test systems were pre-equilibrated at 20 ± 1°C and soil moisture between pF 2.0 to 2.5 for six days prior to the treatment performed on 2010-01-25.

Table 7.1.1- 33: Soil physicochemical properties

Parameter	Results/Units	
Batch ID	[redacted],NE	[redacted], CA
Location	[redacted]	[redacted]
Soil Taxonomic Classification (USDA)	Marshall fine-silty, mixed, superactive, mesic Typic Hapludolls	Hanford fine sandy loam, gravelly substrate
Soil Series		
Texture Class (USDA)	Silt Loam	Sandy Loam
Sand Silt Clay	13.4% 63.8% 22.8%	67.8% 25.0% 7.2%
Saturated paste pH in $CaCl_2$ pH in H_2O	6.7 6.5 6.7	7.4 7.0 7.3
Organic Matter	4.0%	0.97%
Organic Carbon	2.3%	0.57%
Microbial Biomass Initial (day 6) Middle (day 71) Final (day 120)	(mg Microbial C/kg soil) 833 686 660	(mg Microbial C/kg soil) 234 169 202
Cation Exchange Capacity (CEC)	17.8 meq/100 g	6.3 meq/100 g
Maximum Water Holding Capacity	49.6 g/100 g	27.7 g/100 g
Soil Moisture at 0.1 bar (pF 2.0)	37.9%	15.8%
Soil Moisture at 0.33 bar (pF 2.5)	26.4%	9.1%
Bulk Density (disturbed)	1.04 g/cc	1.29 g/cc

B. Methods

1. Experimental conditions: All test systems were incubated at 20 ± 1°C in the dark in a temperature-controlled environmental chamber. The test systems containing 50 g soil (dry-weight basis) consisted of a 250-mL glass flask (21 cm long and 7.2 cm internal diameter) connected to a flow-through system, containing an ethylene glycol trap for volatile organics followed by two 2 M potassium

hydroxide traps for collecting CO_2 and a 1 M sulfuric acid trap for volatile acids. The headspace above the soil was continuously purged with humidified air throughout the study.

For both soils twenty test systems were treated at 1X for the kinetic study rate with each soil (1.1 µg/g) Three test systems were prepared for metabolite identification (MID) purposes, and these systems were treated at 10x the kinetic rate. MID test systems were not used for kinetic evaluation. Four control test systems were prepared as biomass test systems to demonstrate biological activity of the soil.

For the sterile portion of this study, soil was sent to Food Technology Service, Inc. (FTSI; Mulberry, FL) for gamma irradiation (actual delivered absorbed dose of 24.67 kGy). Gamma-irradiated soils were transferred to sterilized 250-mL glass flasks within a BioGuard Laminar Flow Hood using aseptic techniques. Soils were treated at the kinetic rate in the laminar flow hood using aseptic techniques, and then connected to the flow-through system. Sterility was tested at each sampling interval on each test system by plating soil dilutions on 3M Petrifilm Agar plates which were incubated at ~35°C for a minimum of 48 hours.

In total, 64 test systems were prepared. All the soils were adjusted to the appropriate moisture level for each soil, and the moisture was maintained throughout the course of the study.

2. Test Item Application Solution: The application (treatment) solution was prepared by diluting 975 µL of [PYR-^{14}C]BYI 02960 (C-1135) with 37.5 mL of H_2O:MeOH (4:1) for a theoretical concentration of 0.11 µg/µL. The extensive radio-assays to determine the concentration of kinetic treatment solution resulted in 31,227, 300 dpm/mL or 116 µg/mL; the radiochemical purity and identity were confirmed by HPLC and mass spectral analysis.

3. Mode and Rate of Application: On 2010-01-25, aliquots of 480-µL of the kinetic treatment solution were applied in droplets onto the 50 g pre-incubated subsamples of soils, using a 500-µL gas-tight syringe. This rate is equal to the field-use rate of 410 g a.i./ha at 2.5 cm depth and 1.5 g/cm^3 soil density. The final concentration of a.i. in each test system was 1.1 µg BYI 02960 per g soil (dry weight). By the addition the water contained the soil moisture was finally adjusted to approx. pF 2.0. Material balance for the study was based on the day 0 recovery of radioactivity (14,495,777 dpm for [redacted], NE and 14,438,374 dpm for [redacted], CA).

4. Test System Maintenance and Sampling: Test systems were checked for air flow through the traps and water levels in the moisture flasks. The test systems were incubated at 20 ± 1°C in the dark in an environmental chamber. The moisture loss in each system was monitored periodically by comparing the weight of the test system with the weight on Day 0. The moisture of the test systems was measured at each interval. The moisture was maintained at pF 2.5 to pF 2.0 for each soil by the addition of HPLC grade water, as needed, at periodic intervals.

Duplicate test systems were extracted and analyzed on days 0, 3, 7, 14, 28, 60, 92 and 120 days after treatment. Gamma-irradiated test systems were processed at 0, 60 and 122 days after treatment. For the sterile portion of this study, the goal was only to compare the formation of bound residues with non-sterile test systems and therefore, soil extracts were radio-assayed but not analyzed by HPLC. Extracted soils were analyzed by combustion.

On the day of sampling, the test system and associated volatile traps were removed from the incubator for analysis. Prior to removing samples, the air flow was increased for approx. 15 minutes to ensure all headspace volatiles had been purged and trapped. The two KOH trap solutions were combined, volumes of the traps were recorded and triplicate aliquots were radio-assayed.

The soil was extracted and analyzed by LSC on the day of sampling. The soil extracts were analyzed by HPLC within 27 days after sample extraction. Concentrated extracts were stored in a laboratory freezer.

5. 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. At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge bottle and extracted for 30 minutes with approximately 40 mL of acetonitrile:water (70:30, v/v) using a Eberbach horizontal table top shaker, and centrifuged using an Eppendorf table-top centrifuge (5 minutes at 1850 g).

The supernatant was decanted, and the remaining soil was extracted two additional times with acetonitrile:water (70:30,v/v) followed by an extraction with approximately 40 mL of acetonitrile. The supernatants were pooled, and the volume was recorded. Extracts were radioassayed by LSC in triplicate (0.25 to 1.0 mL). An aliquot (approximately 4 mL) of the combined extracts was concentrated by a stream of nitrogen and analyzed by HPLC.

Soil remaining after the ambient acetonitrile/water and acetonitrile extractions was extracted by microwave extraction using 50 mL of acetonitrile:water (70:30, v/v) at 70°C for 10 minutes. The volume was recorded, and the extract was radioassayed by LSC in triplicate (0.25 to1.0 mL). An aliquot (approximately 4 mL) was concentrated by a stream of nitrogen and then analyzed by HPLC.

Extracted soils were air-dried under a fume hood and thoroughly homogenized using a Sunbeam Kitchen Assistant coffee grinder. Subsamples (0.3 to 1.0 g) of the non-extracted residue (NER) were quantified by combustion.

To characterize the NER, the extracted soils from the day 92 sandy loam samples were fractionated to quantify the amounts of radioactivity associated with humic acid, fulvic acid, and humin.

The BYI 02960 residues and transformation products were analyzed and quantified by LSC and radio-HPLC. Recovery of radioactivity from the HPLC column was determined by comparing radioactivity of the collected HPLC effluent (triplicate aliquots, radio-assayed) to the calculated radioactivity of the injected representative samples.

The LOD for HPLC was determined empirically by a series of injections of increasing radioactivity. The lowest amount of radioactivity detected resulted in minimum peak height of 3 times the background level in the chromatogram (520 dpm) and was considered to be the instrument LOD. The minimum amount of radioactivity injected for a sample was 6,034 dpm from the sandy loam day 120, replicate 1 microwave extract. This extract contained 2.5% of the applied activity which results in a LOQ of 0.2% of the applied radioactivity.

Liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) and LC/ESI-MS/MS analyses of standards and isolated compounds was performed using a Finnigan-MAT Ultra-AM (Thermo Electron, San Jose, CA) triple quadrupole mass spectrometer interfaced to a Surveyer autosampler and quaternary HPLC system (Thermo Electron).

II. RESULTS AND DISCUSSION

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study. For the gamma irradiated soils, sterility was lost during the study indicated by plate counts. However, results indicate the biological activity was compromised, as evidenced by the significant reduction in $^{14}CO_2$ formation in the sterile soils at the end of the study.

A. Data

The respective data for the four soils are shown in Table 7.1.1- 34 and Table 7.1.1- 35.
The ambient and aggressive extracts were effective in extracting essentially all of the ^{14}C-residues in both soils at DAT-0, the beginning of the study (silt loam = 99.3%, sandy loam = 99.9%).
The stability of the test item was verified by DAT 0 values of 97.4 and 98.6% AR (mean of duplicates) for the soil extracts These results indicate that the extraction method was very well suited to extract the applied [^{14}C]-labeled test item from the soil matrix.

Table 7.1.1- 34: Biotransformation of [PYR-^{14}C]BYI 02960 in silt loam [redacted] (NE) under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)							
	0 b)	3	7	14	28	60	92	120
BYI 02960	97.4 ±1.2	94.1 ±1.3	93.5 ±0.4	89.3 ±1.6	83.2 ±0.2	76.8 ±0.7	70.1 ±0.4	65.5 ±0.8
Non-characterized radioactivity a)	0.8 ±0.1	2.4 ±2.3	0.6 ±0.1	0.6 ±0.8	0.7 ±0.1	0.0 ±0.0	1.0 ±0.1	1.4 ±0.3
Total extractable residues	99.3 ±1.1	96.5 ±1.0	94.1 ±0.3	89.8 ±0.8	84.0 ±0.2	76.8 ±0.7	71.2 ±0.3	67.0 ±0.5
$^{14}CO_2$	0.0 ±0.0	0.7 ±0.0	1.8 ±0.0	4.5 ±0.0	7.8 ±0.3	13.8 ±0.2	17.2 ±0.2	20.2 ±1.9
Volatile organics	0.0 ±0.0	0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± n.a.	± 0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ± 0.0
Non-extractable residues (NER)	0.7 ±0.0	2.6 ±0.1	3.0 ±0.0	5.0 ±0.2	7.0 ±0.7	9.4 ±0.1	10.4 ±0.0	11.3 ±0.1
Total recovery	100.0 ±1.1	99.8 ±1.2	98.9 ±0.3	99.3 ±0.6	98.8 ±1.1	99.9 ±0.8	98.8 ±0.2	98.4 ±1.3

a) No individual peak accounted to more than 1.6%.
b) Aggressive extracts were not analyzed since the RA content was less than 3% of total applied.

Table 7.1.1- 35: Biotransformation of [PYR-^{14}C]BYI 02960 in sandy loam [redacted] (CA) under aerobic conditions; mean values and SD expressed as % of AR

Compound	Days After Treatment (DAT)							
	0 b)	3	7	14	28	60	92	120
BYI 02960	98.6 ±0.2	95.2 ±1.4	87.4 ±0.0	83.8 ±1.1	67.3 ±0.2	46.8 ±1.1	34.8 ±0.0	30.0 ±0.7
6-chloronicotinic acid	0.0 ±0.0	0.6 ±0.9	3.8 ±0.7	6.9 ±0.2	12.2 ±1.2	17.1 ±1.3	12.3 ±0.8	8.2 ±1.5
Non-characterized radioactivity a)	1.0 ±0.6	0.0 ±0.0	1.8 ±0.8	0.8 ±0.0	2.7 ±0.3	2.0 ±0.3	3.0 ±0.3	1.9 ±0.3
Total extractable residues	99.9 ±0.8	96.9 ±0.7	95.6 ±1.2	91.5 ±0.9	82.1 ±1.1	66.0 ±0.1	50.1 ±1.1	40.0 ±1.8
$^{14}CO_2$	0.0 ±0.0	0.2 ±0.0	0.5 ±0.1	1.8 ±0.0	5.2 ±0.3	15.6 ±0.1	26.1 ±1.3	36.1 ±6.3
Volatile organics	0.0 ±0.0	0.0 ± 0.0	± 0.0 ± 0.0	± 0.0 ±0.0	± 0.0 ± n.a.	± 0.0 ±0.0	± 0.0 ± 0.0	± 0.0 ± 0.0
Non-extractable residues (NER)	0.1 ±0.0	1.8 ±0.1	4.2 ±0.1	7.5 ±0.4	12.4 ±0.8	18.1 ±1.0	23.4 ±0.8	25.5 ±1.3
Total recovery	100.0 ±0.8	98.9 ±0.7	100.2 ±1.0	100.9 ±0.6	99.7 ±2.1	99.7 ±1.1	99.6 ±1.0	101.6 ±5.8

a) No individual peak accounted to more than 2.4%
b) Aggressive extracts were not analyzed since the RA content was less than 3% of total applied.

B. Mass Balance

Material balances ranged from 98.4 to 100% (NE soil) and from 98.9 to 101.6% (CA soil) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

In gamma-irradiated silt loam and sandy loam test systems, the material balances were complete as well.

C. Extractable and Bound Residues (NER)

Extractable ^{14}C-residues decreased from 99.3 and 99.9% of AR at DAT-0 to 67.0 and 40.0% of AR at study end (DAT-120) in NE and CA soil, respectively.

In gamma-irradiated test systems extractable ^{14}C-residues only decreased from 99.1 and 99.8% of AR at DAT-0 to 89.4 and 85.8 % of AR at study end (DAT-120) in NE and CA soil, respectively.

Non-extractable ^{14}C-residues increased from 0.7 and 0.1% of AR at DAT-0 to 11.3 and 25.5% of AR at study end (DAT-120) in NE and CA soil, respectively. The majority of the NER was associated with the humin. In the sandy loam after extraction with the strong acid and base, an average of 60% still remained with the solid fraction (humin). Thus, even with exposure to strong base a significant portion of non-extractable BYI 02960 residues still remained bound to the solid phase of the soil, supporting the observations of biologically-mediated bound residues.

In gamma-irradiated test systems NER only increased from 0.2 and 0.0% of AR at DAT-0 to 6.2 and 9.3% of AR at study end (DAT-120) in NE and CA soil, respectively. Thus, NER were significantly lower in gamma-irradiated compared to non-sterile soils, indicating a biological component to the formation of bound residue.

Table 7.1.1- 36: Summary of extractable and non-extractable residues

Soil		[redacted]	[redacted] gamma-irradiated	[redacted]	[redacted] gamma-irradiated
Extracted RA (%)	Day 0	99.3	99.1	99.9	99.8
89.4	Day 120 (122#)	67.0	89.4	40.0	85.8
Non-Extracted RA (%)	Day 0	0.7	0.9	0.1	0.2
	Day 120	11.3	6.2	25.5	9.3

gamma-irradiated soils

D. Volatilization

The mineralization of [PYR-^{14}C]BYI 02960 was high. At the end of the study (DAT-120), $^{14}CO_2$ accounted for 20.2% (NE soil) and 36.1% (CA soil) of AR.
In gamma-irradiated test systems the mineralization of [FUR-^{14}C]BYI 02960 was low. At the end of the study (DAT-120), $^{14}CO_2$ only accounted for 0.4% (NE soil) and 0.9% (CA soil) of AR,. This indicates a biological component to the formation of $^{14}CO_2$.
Volatile organic compounds were not detected in significant amounts (≤ 0.1% AR) throughout the study.

E. Transformation of Test Item

The test item declined from 97.4 and 98.6% of AR at DAT-0 to 65.5 and 30.0% at the end of the study for soils NE and CA, respectively.
In silt loam, other than parent, unidentified metabolites were measured and accounted for a maximum of 2.4%. In sandy loam, other than parent, one major degradate was identified as 6-chloronicotinic acid, which was formed 6.9% on day 14, reached a maximum of 17.1% on day 60, and declined to 8.2% by the end of the study. There, total unidentified radioactivity accounted for a maximum of 3.0% of the applied amount.
The mentioned results were included in the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure 7.1.2- 1.

F. Kinetics of Test Item Degradation

The degradation of the parent compound during the study is given under point 7.2.1

III CONCLUSIONS

A. Major Outcomes of Study

A synopsis of results is shown in Table 7.1.1- 38.
The data gathered in the current laboratory investigation demonstrate that [PYR-^{14}C]BYI 02960 is degraded moderately rapid in the two soils. One part of the residues becomes increasingly bound to soil (11.3% [redacted], NE, 25.5% [redacted], CA) by study end. The other part is well mineralized to $^{14}CO_2$ (20.2% [redacted], NE, 36.1% [redacted], CA). In the [redacted] soil one interim metabolite, which was identified as 6-chloronicotinic acid, was to be observed, reaching its maximum of 17.1% on day 60 and declining to 8.2% of AR by the end of the study. All further formed metabolites are regarded as transient, which is confirmed by the high mineralization rate of [PYR-^{14}C]BYI 02960 to $^{14}CO_2$ observed in this study. Volatile organic compounds were very low (≤ 0.1% of AR) at all sampling dates.

Degradation, amount of bound residues as well as of $^{14}CO_2$ formed in gamma-irradiated soils was significantly less than in non-sterile soils, indicating a biological component to the degradation/mineralization and formation of non-extractable residues from BYI 02960.
Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

Table 7.1.1- 37: Synopsis of overall results

Soil	[redacted] Viable	[redacted] Sterile	[redacted] Viable	[redacted] Sterile
Total Recovery (%)	100-99.6	96-100.0	100-100.4	96.0-100.0
Extracted RA (%)	70.9-99.2	89.4-99.1	33.7-99.8	85.8-99.8
Max. CO2 (%)	12.3	0.4	36.1	0.9
Bound Residues (%)	0.8-16.4	0.9-6.2	0.2-30.6	0.2-9.3
Major metabolites	-	Not determined	6-CNA (17.1%)	Not determined

B. Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that a viable aerobic soil environment will contribute significantly to the degradation of BYI 02960. With respect to the radiolabel used mineralization to $^{14}CO_2$ is significant. One metabolite, which was identified as 6-chloronicotinic acid, was observed at a proportion of greater 10% of applied, and declined to the end of study. However, other metabolites are not to be expected accumulate in soil, since – after their formation – they are well mineralized Table 7.1.1- 38).

Table 7.1.1- 38: Synopsis of results of biotransformation of [PYR-^{14}C]BYI 02960 in two soils incubated at 20 °C and pF 2.0 – 2.5 % under aerobic conditions

Soil	[redacted] (NE)		[redacted] (CA)	
Soil type	**Silt loam**		**Sandy loam**	
Soil status	**Viable**	**Sterile**	**Viable**	**Sterile**
Major transformation products *	CO_2 (max. 20.2%) NER (max. 11.3%)	(NER)	CO_2 (max. 36.1%) NER (max. 25.5%) 6-CNA (max. 17.1%	(NER)
Minor transformation products	-	CO_2	-	CO_2

*): 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.
(NER): Major acc. to *, but did not exceed 10% of AR during the entire study period.

IIA 7.1.2 Anaerobic Degradation

Report:	**KIIA 7.1.2/01, [redacted], [redacted], 2012**
Title:	[Furanone-4-^{14}C] and [Ethyl-1-^{14}C] and [Pyridine-2,6-^{14}C]BYI 02960: Anaerobic Soil Metabolism
Report No & Document No	MEF-11/514 M-421504-01-2
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002** **US EPA, OPPTS, 835.4200, 2008**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The present laboratory study investigated the degradation of BYI 02960 in one soil (silt loam) under flooded anaerobic conditions. [Pyridine-2,6-^{14}C], [furanone-4-^{14}C] and [ethyl-1-^{14}C]BYI 02960 (equivalent to label PYR, FUR, and ETH, respectively) were applied at of 110.1, 104.6 and 105.9 µg/100 g soil (dry matter), i.e. equivalent to 103.2, 98.0 and 99.2% of the nominal application rate of 400 g/ha.

The soil in duplicate test flasks/interval was maintained under aerobic conditions for 30 days in the dark at 20 ± 2°C and approx. 55% of the maximum water holding capacity. Following the aerobic phase, the samples were flooded with oxygen-depleted de-ionized water (approx. 3 cm layer above soil level), set under nitrogen atmosphere, and maintained in the dark at 20 ± 2°C under anaerobic conditions for max. 123 days. During the aerobic study phase, air-permeable traps were attached for the collection of CO_2 and volatile organics (static test system design). At start of the anaerobic study phase, the trap systems were replaced by sealable two-valve glass stoppers connected with plastic gas sampling bags.

Soil samples and water layers were separated by decanting to allow for individual analysis. The soil was extracted four times with at ambient temperature (combined as "ambient organic extract"). Subsequently, the soil was extracted once at an elevated temperature ("aggressive extract"). BYI 02960 residues in water layers were directly analyzed by reversed phase HPLC; the soil extracts were subjected to solvent exchange prior to analysis (all labels). TLC was employed as second contrasting separation method for the confirmation of the results. A limit of quantification (LOQ) of equal or better than 0.9% of the applied radioactivity (% AR) was calculated for HPLC radioactivity detection within the sample matrices.

For all three radiolabels complete material balances found at all sampling intervals demonstrated that no significant portion of radioactivity dissipated from the flasks or was lost during processing. During the 30 days of aerobic incubation $^{14}CO_2$ accounted for up to 26.7 (PYR), 15.9 (FUR) and 6.9 % of AR (ETH). During the anaerobic incubation phase, mineralization to $^{14}CO_2$ was negligible (< 0.1 % of AR). Organic volatiles were not observed in the aerobic or in the anaerobic study phase (< 0.1 % AR at all sampling intervals).

The radioactivity extractable from soil decreased to 55.9, 55.2 and 82.0% of AR towards the end of the aerobic incubation phase (DAT-30), and further to 41.5, 40.2 and 53.4% of AR until the end of the anaerobic incubation period for labels PYR, FUR and ETH, respectively.

Within the aerobic phase of the study (30 days) the percentages of BYI 02960 in the entire systems decreased to 53.7, 52.6 and 54.7% of AR for labels PYR, FUR and ETH, respectively. During the

anaerobic incubation period (i.e. flooded state) the portions of BYI 02960 slightly decreased further to 47.8, 47.2 and 47.7% of AR for labels PYR, FUR and ETH, respectively.
Only one transformation product exceeded 5% of AR over the entire study period. It was detected in the test systems of label ETH and it was identified as difluoroacetic acid (DFA). DFA levels increased to a level of 25.1% of AR during the initial 30 days of aerobic conditions. During the anaerobic phase, the amounts of DFA remained more or less stable (24.2 - 26.2% of AR).

In the aerobic incubation phase, non-extractable residues (NER) in soil increased from 2.7 to 12.9% (label PYR), 3.1 to 25.6% (Label FUR) and 2.8 to 10.8% of AR (Label ETH). During the anaerobic incubation phase the NER slightly increased further.

Applying single first order kinetics (SFO) to the BYI 02960 residues in the entire systems during the anaerobic phase of the study, the estimated DT_{50} values range from 581.8 to 693.2 days (geometric mean: 633.7 days).

Based on the results obtained within this study it can be expected that the amounts of BYI 02960 and its only significant metabolite DFA remain stable under flooded field conditions. Degradation would be expected to continue whenever the conditions become aerobic.

I. MATERIALS AND METHODS

A. Materials

1. Test Items: Flupyradifurone: Code = BYI 02960;
Label PYR = [Pyridine-2,6-^{14}C]BYI 02960 (sample ID: KATH 6403)
Specific activity: 4.49 MBq/mg
Radiochemical purity: >98% (acc. radio-HPLC)
Label FUR = [Furanone-4-^{14}C]BYI 02960 (sample ID: KATH 6405)
Specific activity: 3.94 MBq/mg
Radiochemical purity: >98% (acc. radio-HPLC)
Label ETH = [Ethyl -1-^{14}C]BYI 02960 (sample ID: KATH 6404)
Specific activity: 3.93 MBq/mg
Radiochemical purity: >99% (acc. radio-HPLC)
Identity and purity of test items in the application solution were confirmed.

2. Soil: The biotransformation of BYI 02960, under anaerobic conditions, was studied in one soil.. The soil was taken on 2009-12-07 fresh from the field. On the day of sampling, the soil was broken up stepwise and gently air dried. Then, the soil was successively sieved to ≤ 2 cm. Soil moisture after sieving was equivalent to 40.8% WHCmax. One day after preparation, soil portions were weighed into the incubation flasks, adjusted to 55% WHCmax and pre-incubated at about 20 ± 2°C until application, i.e. for approx. 7 days.

Table 7.1.2- 1: Soil physicochemical properties

Parameter	Results/Units
Batch ID	[redacted] am [redacted] 4a, 20091207
Location	[redacted] Germany
Soil Taxonomic Classification (USDA)	N/A
Soil Series	N/A
Texture Class (USDA)	Silt Loam
Sand Silt Clay	25% 61% 14%
pH in $CaCl_2$ pH in KCl pH in Water	6.4 6.2 6.5
Organic Matter	4.7%
Organic Carbon	2.7%
Soil Microbial biomass mg microbial C/kg	
0 days	1263
30 days	1097
Anaerobic bacteria plate count	67000 CFU/g soil (non-treated) 33000 CFU/g soil (solvent control)
Cation Exchange Capacity (CEC)	14.7 meq/100 g
Water Holding Capacity	
at 0.33 bar [1/3 bar WHC]	22.7 g/100 g
Maximum Water Holding Capacity [WHCmax]	67.0 g/100 g
Bulk Density (disturbed)	1.07 g/cm3

B. Methods

1. Experimental conditions: The study was performed in static incubation test systems under aerobic followed by anaerobic conditions in the dark at 19.9 ± 0.1 °C. The test system consisted of Erlenmeyer flasks (300 mL) attached, first 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, second with a gastight gas sampling bag. Aliquots of 100 g of dry soil were weighed into the test flasks. Replicates were set up and processed for each sampling (3 sampling dates including time 0 for the aerobic phase, 9 sampling dates including time 0 for the anaerobic phase). Additional flasks were used for determination of the microbial biomass. The final soil moisture, for the aerobic phase was adjusted to 55% of WHC_{max} by adding pure water.
The switch to anaerobic (flooded) conditions was made 30 days after test item application: The trap system of all remaining test flasks was removed and stored for later analysis. The soil of each flask was flooded with 150 mL of oxygen-depleted de-ionized water leading to a water layer of approx. 3 cm above soil level. The flasks were then equipped with sealable double-valve glass stoppers. The flasks were connected to plastic gas sampling bags, which had been flushed with nitrogen gas. The valves were set to connect flask headspace and gas sampling bag, but closing the system from the outer atmosphere. Such setup allowed for pressure-less closed-flask incubation. To ensure maintenance of fully oxygen-free conditions, the test systems furthermore were placed in an argon, then nitrogen flooded box within the incubation chamber.

2. Test Item Stock Solution: The entire delivered amount of [^{14}C]BYI 02960 was dissolved in 4 mL methanol.

3. Test Item Application Solution: An application solution was made by diluting 2-3-mL aliquots of stock solutions in 20 mL of purified water.

4. Mode of Application: On 2009-12-15, the test systems were removed from the incubation chamber and dosed with 447 µL, 549 µL or 464 µL of respective application solutions per flask. Treatment was made as small droplets applied directly onto the soil surface using a micropipette. Each flask was then gently shaken to incorporate the radiochemical into the test soil. Care was taken not to form clumps during the mixing process. Finally, the test systems were fitted with the volatiles traps and placed back into the incubation chamber. Biomass and anaerobic bacteria determination test systems were either left untreated (native soil sample DAT-0), or dosed with 30 µL of methanol.
The test vessels for DAT-0 were immediately processed for analysis. All other test vessels, including the biomass flasks which were not spiked with application solution, were fitted with trap attachments and incubated in the dark at nominal 20 ± 2°C.
[PYR-^{14}C]BYI 02960 was applied at a rate of 494,261.3 Bq per vessel. This corresponds to 110.1 µg per vessel which is equivalent to 102.3 % of the intended field application rate of 106.7 µg per vessel (calculated an application rate of 400 g BYI 02960 per hectare). [FUR-^{14}C]BYI 02960 was applied at a rate of 412,122.5 Bq per vessel, this corresponds to 104.6 µg per vessel which is equivalent to 98.0 % of the intended application rate. [ETH-^{14}C]BYI 02960 was applied at a rate of 416,163.9 Bq per vessel. This corresponds to 105.9 µg per vessel which is equivalent to 99.2 % of the intended application rate.

5. Sampling: Characterization of the soil microbial viability was achieved by (a) determinations of soil microbial biomass during the aerobic incubation phase, and (b) by determinations of anaerobic bacteria during the anaerobic incubation phase. Biomass measurements were conducted at the beginning of the incubation period (DAT-0) for a pre-equilibrated but untreated test system, and at the end of aerobic incubation period (DAT-30) for a test system treated with a test item and a test system treated with the application solvent (30 µL of methanol). Determinations of anaerobic bacteria were performed at the end of the study (DASF-123) for an untreated test system and a test system treated with the application solvent, each. For procedure descriptions see Sections 3.6.2.7 and 3.6.2.8 of report.
Entire test flasks were taken for processing and analysis at 0, 23 and 30 days after treatment (DAT, (i.e. the aerobic phase) and 0, 1, 4, 7, 14, 29, 60, 90 and 123 days after soil flooding (DASF).

Aerobic systems: After collection of the respective test systems from the incubation chamber, samples taken on days 23 and 30 were exposed to vacuum for about 10 min to purge the volatiles possibly still present in the headspace into the traps. Then, the flask and volatile traps were separated. For the samples which were directly processed after application (DAT-0), no volatiles were collected.
At each sampling date the entire amount of soil in each test vessel was transferred into a centrifuge beaker and extracted using a mechanical shaker.

Anaerobic systems: After collection of the respective test systems from the incubation box, they were connected to a volatiles combustion oven unit. Using nitrogen, volatiles present in the headspace and gas sampling bag were slowly purged over a soda lime trap for absorption of $^{14}CO_2$, through the catalytic oven for oxidative combustion of organic volatiles (e.g. methane), and finally through three liquid scintillation flasks filled with LSC cocktail, in order to absorb $^{14}CO_2$ contained in the combustion exhaust. LSC cocktail traps were directly analyzed.
Next, the test flasks were opened, and the oxygen content of the water layers as well as the redox potential and the pH value of the water phases and soils layer were immediately determined by electrode measurements.

The water layers were separated from the soil layers by decanting. For removal of suspended particles, the decanted water layers were centrifuged for about 20 min at ca. 10000xg and filtered through a paper filter. The supernatants were analyzed without further extraction. The centrifugation pellets were added to the soil phases, by re-use of the centrifuge flasks for the extraction of the respective soil layers.
Soil extracts and decanted water layers were subjected to chromatography profiling within a maximum of 2 days after sampling. Samples were stored in a refrigerator prior to analysis. After analysis, they were stored deep frozen at about -20°C. Extracted soil was air-dried at room temperature. Combustion analysis was conducted within a maximum of 127 days. Absence of losses upon storage can be concluded from the complete radioactive material balances. After analysis, the soil samples were stored deep frozen at about -20°C. Volatiles traps were processed and analyzed within a maximum of 35 days. Sample stability can be concluded from the complete radioactive material balances.

6. Description of analytical procedures: The soil processing procedure was optimized to obtain >90% extraction efficiency and >90% recovery of the test item at time zero. Extraction was by cycles of heavily shaking for about 30 minutes on a mechanical shaker at room temperature followed by centrifugation (ca. 10 min, 10000xg) and decanting through a paper filter. At each sampling date, the soil samples were extracted with 3 x 80 mL acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. A further extraction step followed with acetonitrile/water (80/20, v/v) at 70°C using a microwave for 10 minutes. 'Ambient' and 'aggressive' extracts were kept separate for individual chromatography profiling.
The BYI 02960 residues and transformation products were analyzed and quantified by LSC and reversed phase radio-HPLC. Losses of radioactivity after concentration of extracts were minimal. Normal-phase Si-60 radio-TLC was used as a confirmatory method. The identity of the test item in stock solution and in extracts was confirmed by spectroscopic methods. In addition, spectroscopic methods were used to identify one of the minor metabolites.
The disappearance kinetics of BYI 02960 in the entire test systems during the anaerobic phase was individually calculated for each radiolabel. The calculation was done according to FOCUS kinetics guidelines.

II. RESULTS AND DISCUSSION

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active throughout the entire laboratory study, i.e. in the aerobic as well as the anaerobic phase where anaerobic bacteria plate count assays showed the presence of at least 33000 colony forming units per gram of soil dry weight for untreated soil or soil treated with application solvent. This confirmed the establishment of an anaerobic micro flora in the test systems.
Oxygen content in the water layer was below 1 mg/L 4 days after soil flooding (DASF) for all three labels. Throughout the rest of the anaerobic incubation phase oxygen concentration stayed below this value. Redox potential measurements indicated transition of the system to reducing conditions, in both soil and water layer from DASF-29 or DASF-60 onwards. The pH values scatter until DASF-29. Afterwards, they increased slightly (from about pH 6.8 to pH 7.3/7.4) and approached a plateau at around DASF-60. The pH values in the sediment were slightly lower than the pH-values in the water layers.

Table 7.1.2- 2: Redox Potential, Oxygen Content and pH of Test Systems (PYR label)

DASF	Replicate	Water Phase				Soil Layer		
		pH	Oxygen content	Redox potential		pH	Redox potential	
				SenTix ORP electrode[1]	E_h[1)]		SenTix ORP electrode[1]	E_h[1)]
			[mg/L]	[mV]	[mV]		[mV]	[mV]
0	P1	6.44	3.62	205	415	6.62	200	410
	P2	6.91	3.38	200	410	6.66	205	415
	Mean	6.68	3.50	203	413	6.64	203	413
1	P1	6.78	2.07	130	340	6.75	120	330
	P2	6.67	2.37	140	350	6.62	126	336
	Mean	6.73	2.22	135	345	6.69	123	333
4	P1	6.60	0.75	135	345	6.76	124	334
	P2	6.71	0.68	195	405	6.64	186	396
	Mean	6.66	0.72	165	375	6.70	155	365
7	P1	6.91	0.44	195	405	6.82	191	401
	P2	6.62	0.97	197	407	6.69	186	396
	Mean	6.77	0.71	196	406	6.76	189	399
14	P1	6.82	0.33	112	322	6.61	107	317
	P2	6.81	0.42	116	326	6.60	105	315
	Mean	6.82	0.38	114	324	6.61	106	316
29	P1	6.85	0.40	-25	185	6.64	-39	171
	P2	6.75	0.23	-41	169	6.59	-55	155
	Mean	6.80	0.32	-33	177	6.62	-47	163
60	P1	7.45	0.36	-147	63	7.31	-151	59
	P2	7.39	0.40	-165	45	7.16	-164	46
	Mean	7.42	0.38	-156	54	7.24	-158	53
90	P1	7.45	0.32	-145	65	7.25	-159	51
	P2	7.51	0.40	-146	64	7.28	-162	48
	Mean	7.48	0.36	-146	65	7.27	-161	50
123	P1	7.67	0.19	-254	-44	7.38	-373	-163
	P2	7.57	0.25	-253	-43	7.40	-278	-68
	Mean	7.62	0.22	-254	-44	7.39	-326	-116

DASF: Days after soil flooding
1): Eh = Emeasured + 210mV (reference potential of the SenTix ORP electrode vs. the standard electrode at 20 °C according to information of manufacturer WTW)

Table 7.1.2- 3: Redox Potential, Oxygen Content and pH of Test Systems (FUR label)

DASF	Repli-cate	Water Phase pH	Water Phase Oxygen content [mg/L]	Water Phase Redox potential SenTix ORP electrode[1] [mV]	Water Phase Redox potential E_h 1) [mV]	Soil Layer pH	Soil Layer Redox potential SenTix ORP electrode[1] [mV]	Soil Layer Redox potential E_h 1) [mV]
0	F1	6.87	3.94	199	409	6.75	200	410
	F2	6.87	3.82	195	405	6.75	206	416
	Mean	**6.87**	**3.88**	**197**	**407**	**6.75**	**203**	**413**
1	F1	6.76	1.66	140	350	6.63	127	337
	F2	6.72	1.26	165	375	6.57	151	361
	Mean	**6.74**	**1.46**	**153**	**363**	**6.60**	**139**	**349**
4	F1	6.53	0.56	197	407	6.48	187	397
	F2	6.58	0.72	199	409	6.54	190	400
	Mean	**6.56**	**0.64**	**198**	**408**	**6.51**	**189**	**399**
7	F1	6.63	0.64	211	421	6.57	216	426
	F2	6.60	0.68	230	440	6.57	215	425
	Mean	**6.62**	**0.66**	**221**	**431**	**6.57**	**216**	**426**
14	F1	6.71	0.33	111	321	6.69	104	314
	F2	6.82	0.54	107	317	6.65	96	306
	Mean	**6.77**	**0.44**	**109**	**319**	**6.67**	**100**	**310**
29	F1	6.84	0.70	-61	149	6.63	-82	128
	F2	6.79	0.31	-44	166	6.62	-62	148
	Mean	**6.82**	**0.51**	**-53**	**158**	**6.63**	**-72**	**138**
60	F1	7.35	0.30	-151	59	7.13	-153	57
	F2	7.32	0.35	-155	55	7.14	-162	48
	Mean	**7.34**	**0.33**	**-153**	**57**	**7.14**	**-158**	**53**
90	F1	7.35	0.28	-159	51	7.19	-162	48
	F2	7.40	0.27	-163	47	7.21	-167	43
	Mean	**7.38**	**0.28**	**-161**	**49**	**7.20**	**-165**	**46**
123	F1	7.47	0.18	-287	-77	7.23	-283	-73
	F2	7.44	0.27	-252	-42	7.24	-282	-72
	Mean	**7.46**	**0.23**	**-270**	**-60**	**7.24**	**-283**	**-73**

DASF: Days after soil flooding

1): $E_h = E_{measured} + 210mV$ (reference potential of the SenTix ORP electrode vs. the standard electrode at 20 °C according to information of manufacturer WTW)

Table 7.1.2- 4: Redox Potential, Oxygen Content and pH of Test Systems (ETH label)

DASF	Repli-cate	Water Phase pH	Water Phase Oxygen content [mg/L]	Water Phase Redox potential SenTix ORP electrode[1] [mV]	Water Phase Redox potential E_h[1)] [mV]	Soil Layer pH	Soil Layer Redox potential SenTix ORP electrode[1] [mV]	Soil Layer Redox potential E_h[1)] [mV]
0	E1	6.79	4.18	205	415	6.80	207	417
	E2	6.88	4.13	212	422	6.76	212	422
	Mean	**6.84**	**4.16**	**209**	**419**	**6.78**	**210**	**420**
1	E1	6.74	1.52	192	402	6.55	185	395
	E2	6.65	1.68	175	385	6.53	161	371
	Mean	**6.70**	**1.60**	**184**	**394**	**6.54**	**173**	**383**
4	E1	6.47	0.61	199	409	6.46	192	402
	E2	6.48	0.68	199	409	6.51	195	405
	Mean	**6.48**	**0.65**	**199**	**409**	**6.49**	**194**	**404**
7	E1	6.82	0.49	219	429	6.60	207	417
	E2	6.66	0.51	272	482	6.61	236	446
	Mean	**6.74**	**0.50**	**246**	**456**	**6.61**	**222**	**432**
14	E1	6.89	0.31	108	318	6.65	98	308
	E2	6.85	0.43	100	310	6.59	92	302
	Mean	**6.87**	**0.37**	**104**	**314**	**6.62**	**95**	**305**
29	E1	6.77	0.25	-46	164	6.59	-61	149
	E2	6.80	0.25	-67	143	6.59	-76	134
	Mean	**6.79**	**0.25**	**-57**	**154**	**6.59**	**-69**	**142**
60	E1	7.27	0.24	-153	57	7.06	-162	48
	E2	7.26	0.32	-162	48	7.00	-164	46
	Mean	**7.27**	**0.28**	**-158**	**53**	**7.03**	**-163**	**47**
90	E1	7.45	0.47	-167	43	7.13	-171	39
	E2	7.40	0.13	-168	42	7.16	-169	41
	Mean	**7.43**	**0.30**	**-168**	**43**	**7.15**	**-170**	**40**
123	E1	7.39	0.23	-291	-81	7.18	-306	-96
	E2	7.38	0.28	-284	-74	7.18	-295	-85
	Mean	**7.39**	**0.26**	**-288**	**-78**	**7.18**	**-301**	**-91**

DASF: Days after soil flooding
1): $E_h = E_{measured} + 210mV$ (reference potential of the SenTix ORP electrode vs. the standard electrode at 20°C according to information of manufacturer WTW)

A. Data

The respective data for the three different radiolabels are shown in Table 7.1.2- 5 to Table 7.1.2- 7.

The DAT-0 extraction efficiency was 95.4, 96.7 and 95.2% of AR (sum of ambient and aggressive extract), and for labels PYR, FUR and ETH, respectively. The stability of BYI 02960 during processing was verified by mean purities >98.1, >98.2 and >98.2% in the ambient extracts and 100% in the aggressive extracts of labels PYR, FUR and ETH, respectively. These results indicate that the extraction method was well suited to extract the applied [^{14}C]-labeled test item from the soil matrix.

Table 7.1.2- 5: Biotransformation of [PYR-^{14}C]BYI 02960 in silt loam HF under aerobic then anaerobic conditions; mean values and SD expressed as % of AR

Com-pound	Compart-ment	Days After Treatment (DAT)			Days After Soil Flooding (DASF)								
		0	23	30	0	1	4	7	14	29	60	90	123
BYI 02960	Water layer	N/A	N/A	N/A	4.0	8.1	11.7	10.5	10.3	8.9	7.8	7.1	7.8
	Soil extracts	94.5	58.6	53.7	49.7	47.3	43.1	43.9	42.4	42.0	41.8	41.3	40.0
	Entire system	94.5 ±1.9	58.6 ±1.4	53.7 ±1.1	53.7 ±1.0	55.3 ±0.2	54.7 ±0.2	54.4 ±0.3	52.7 ±0.7	50.9 ±0.7	49.6 ±0.2	48.5 ±1.1	47.8 ±0.2
ROI 1	Water layer	N/A	N/A	N/A						0.9	0.9	0.9	0.9
	Soil extracts		1.0	0.9	0.5			0.7		0.8	0.5		
	Entire system		1.0 ±0.1	0.9 ±0.4	0.5			0.7		1.7 ±0.2	1.2 ±0.5	0.9	0.9 ±0.1
ROI 2	Water layer	N/A	N/A	N/A									
	Soil extracts		0.7	0.6	0.9	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.8
	Entire system		0.7 ±0.1	0.6 ±0.3	0.9 ±0.1	0.8 ±0.1	0.8 ±0.2	0.8 ±0.3	0.7	0.7 ±0.1	0.7 ±0.2	0.7 ±0.2	0.8 ±0.2
ROI 3	Water layer	N/A	N/A	N/A									
	Soil extracts						0.5	0.5	0.4				
	Entire system						0.5	0.5 ±0.2	0.4				
ROI 4	Water layer	N/A	N/A	N/A									
	Soil extracts							0.3					
	Entire system							0.3					
ROI 5	Water layer	N/A	N/A	N/A									
	Soil extracts									0.4	0.4	0.3	0.3
	Entire system									0.4	0.4	0.3	0.3
Diffuse RA	Water layer	N/A	N/A	N/A	0.1	0.2	0.5	0.3	0.3	<0.1	0.1	0.1	0.1
	Soil extracts	0.9	0.4	0.7	0.7	0.4	0.6	0.6	0.8	0.7	1.3	0.5	0.7
	Entire system	0.9 ±1.2	0.4 ±0.3	0.7 ±0.9	0.8 ±0.2	0.6 ±0.1	1.1 ±0.5	0.9 ±0.1	1.1 ±0.9	0.7 ±0.8	1.4 ±0.3	0.6 ±0.4	0.8 ±0.5
Total extractable residues	Water layer	N/A	N/A	N/A	4.0	8.3	12.5	11.4	10.8	10.1	9.1	8.4	9.0
	Soil extracts	95.4	60.3	55.9	51.6	48.4	44.4	45.7	43.9	44.1	44.0	42.5	41.5
	Entire system	95.4 ±0.7	60.3 ±1.2	55.9 ±0.5	55.6 ±0.8	56.7 ±0.3	56.9 ±0.4	57.1 ±0.1	54.7 ±0.2	54.2 ±0.2	53.1 ±0.5	50.9 ±0.5	50.5 ±0.4
$^{14}CO_2$	Sum entire period	N/A	20.7	26.2 ±0.3	26.3 ±0.2	25.8 ±0.6	26.1 ±1.1	25.2	26.3 ±0.7	25.6 ±1.3	26.7 ±0.2	24.9 ±0.1	26.6 ±0.4
Volatile organics	Sum entire period	N/A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NER	Soil extracts	12.7 ±0.1	12.4	12.9 ±0.1	13.2 ±0.4	13.2 ±0.2	12.2	12.9	13.7 ±0.4	14.6 ±0.2	16.4 ±0.1	17.8	17.2 ±0.3
Total % recovery	Entire system	98.1 ±0.6	93.4 ±1.2	95.0 ±0.1	95.1 ±0.2	95.7 ±0.6	95.3 ±0.6	95.2 ±0.1	94.7 ±1.0	94.4 ±1.4	96.3 ±0.4	93.6 ±0.5	94.2 ±1.1

N/A = not applicable; blank cell = not detected, no SD could be calculated;

Table 7.1.2- 6: Biotransformation of [FUR-14C]BYI 02960 in silt loam HF under aerobic then anaerobic conditions; mean values and SD expressed as % of AR

Compound	Compartment	Days After Treatment (DAT) 0	23	30	Days After Soil Flooding (DASF) 0	1	4	7	14	29	60	90	123
BYI 02960	Water layer	N/A	N/A	N/A	3.6	9.1	11.0	9.8	10.8	8.9	7.3	6.8	7.9
	Soil extracts	95.1	58.2	52.6	48.3	43.0	41.7	43.2	40.2	41.0	40.5	40.5	39.3
	Entire system	95.1 ±0.6	58.2 ±0.4	52.6 ±0.6	51.9 ±0.3	52.1 ±1.5	52.6 ±1.8	53.0 ±1.0	51.0 ±0.5	49.9 ±0.7	47.7	47.3 ±1.1	47.2 ±0.7
ROI A	Water layer	N/A	N/A	N/A						0.4	0.7	0.8	0.7
	Soil extracts									0.6			0.1
	Entire system									1.1 ±0.4	0.7 ±0.2	0.8 ±0.2	0.8
ROI B	Water layer	N/A	N/A	N/A									
	Soil extracts		0.7	1.0	1.2	0.7	0.6	0.9	0.5	0.7	1.0	0.7	0.6
	Entire system		0.7 ±0.2	1.0 ±0.3	1.2	0.7 ±0.1	0.6 ±0.1	0.9 ±0.1	0.5 ±0.1	0.7 ±0.3	1.0	0.7 ±0.2	0.6
ROI C	Water layer	N/A	N/A	N/A									
	Soil extracts		0.7	0.5	1.1	1.3	0.7						
	Entire system		0.7 ±<0.1	0.5 ±0.1	1.1	1.3	0.7						
ROI D	Water layer	N/A	N/A	N/A									
	Soil extracts											0.6	
	Entire system											0.6	
Diffuse RA	Water layer	N/A	N/A	N/A	<0.1	0.2	0.3	0.1	0.2	0.2	0.2	0.1	0.1
	Soil extracts	1.5	0.6	1.1	0.9	0.6	0.4	0.5	1.0	0.3	0.9	0.9	0.5
	Entire system	1.5 ±0.3	0.6 ±0.4	1.1 ±1.3	1.0 ±0.3	0.7 ±0.4	0.7 ±0.2	0.6 ±0.3	1.3 ±0.2	0.5 ±0.1	1.2 ±0.4	1.0 ±0.3	0.6 ±0.2
Total extract. residues	Water layer	N/A	N/A	N/A	3.6	9.2	11.3	9.9	11.0	9.5	8.2	7.7	8.7
	Soil extracts	96.7	60.3	55.2	50.4	44.9	43.1	44.6	41.8	42.6	42.4	42.3	40.2
	Entire system	96.7 ±0.3	60.3 ±1.0	55.2 ±0.4	54.0 ±0.6	54.1 ±1.1	54.3 ±1.0	54.5 ±0.6	52.8 ±0.4	52.2 ±0.1	50.6 ±0.2	50.0 ±1.1	48.9
$^{14}CO_2$	Sum entire period	N/A	12.3 ±0.3	15.1 ±0.2	15.0 ±0.1	15.6 ±0.1	15.5 ±0.1	15.8 ±0.1	15.9 ±0.3	15.7 ±0.1	15.5 ±0.1	15.5 ±0.3	15.9 ±0.2
Volatile organics	Sum entire period	N/A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NER	Soil extracts	3.1 ±0.1	23.9 ±0.6	25.6 ±0.3	25.2 ±0.4	26.7 ±0.3	25.1 ±0.1	25.5 ±0.2	26.7 ±0.3	27.4 ±0.9	29.0 ±0.1	30.1 ±1.0	29.3 ±0.9
Total % recovery	Entire system	99.7 ±0.4	96.5 ±0.7	95.9 ±0.9	94.2 ±0.2	96.4 ±0.9	94.9 ±0.8	95.8 ±0.6	95.5 ±0.3	95.3 ±0.8	95.1 ±0.3	95.6 ±1.8	94.1 ±0.7

N/A = not applicable; blank cell = not detected, no SD could be calculated;

Table 7.1.2- 7: Biotransformation of [ETH-^{14}C]BYI 02960 in silt loam HF under aerobic then anaerobic conditions; mean values and SD expressed as % of AR

Compound	Compartment	Days After Treatment (DAT)			Days After Soil Flooding (DASF)								
		0	23	30	0	1	4	7	14	29	60	90	123
BYI 02960	Water layer	N/A	N/A	N/A	5.5	9.2	11.0	8.9	10.7	9.2	7.7	7.0	7.0
	Soil extracts	93.5	60.9	54.7	48.7	44.6	41.7	44.8	42.1	42.1	42.2	40.7	40.7
	Entire system	93.5 ±0.5	60.9 ±0.5	54.7 ±0.2	54.1 ±0.3	53.8 ±0.6	52.7 ±1.5	53.7 ±<0.1	52.8 ±0.5	51.4 ±0.7	50.0 ±1.2	47.6 ±0.2	47.7 ±0.9
DFA	Water layer	N/A	N/A	N/A	4.8	9.1	12.5	14.2	15.7	16.6	15.7	14.6	15.1
	Soil extracts		21.8	25.1	20.4	16.0	13.5	11.3	10.5	9.0	8.5	10.2	11.1
	Entire system		21.8 ±0.5	25.1 ±0.2	25.2 ±0.4	25.1 ±0.6	26.0 ±0.6	25.5 ±1.6	26.2 ±0.8	25.6 ±0.5	24.2 ±0.6	24.7 ±0.4	26.2 ±0.6
ROI Z	Water layer	N/A	N/A	N/A									
	Soil extracts			1.3	1.1	0.7	0.9	0.9	0.9	1.0	0.7	1.0	1.0
	Entire system			1.3 ±0.2	1.1 ±0.2	0.7	0.9 ±0.3	0.9 ±0.5	0.9 ±0.1	1.0	0.7 ±0.3	1.0 ±0.4	1.0 ±0.3
Diffuse RA	Water layer	N/A	N/A	N/A	0.2	0.8	0.6	1.0	0.2	0.1	1.4	0.3	0.6
	Soil extracts	1.7	0.5	0.9	0.7	1.0	1.1	0.8	0.9	0.8	0.6	0.4	0.6
	Entire system	1.7 ±<0.1	0.5 ±0.3	0.9 ±0.3	0.9 ±0.3	1.8 ±<0.1	1.7 ±0.3	1.8 ±0.5	1.0 ±0.6	0.9 ±1.0	2.0 ±0.3	0.7 ±0.4	1.2 ±0.1
Total extract. residues	Water layer	N/A	N/A	N/A	10.5	19.2	24.1	24.1	26.6	26.0	24.9	21.9	22.7
	Soil extracts	95.2	83.3	82.0	70.9	61.9	57.1	57.8	54.3	52.9	52.0	52.2	53.4
	Entire system	95.2 ±0.4	83.3 ±0.3	82.0 ±0.1	81.3 ±0.8	81.1 ±0.6	81.2 ±0.9	81.9 ±1.6	80.9 ±0.5	78.8 ±0.8	76.9 ±1.2	74.1 ±0.2	76.1 ±0.1
$^{14}CO_2$	Sum entire period	N/A	4.3 ±0.1	6.5 ±0.2	6.1	6.4 ±0.2	6.8 ±0.1	5.9 ±0.7	6.9	6.4 ±0.2	6.5 ±0.4	6.4 ±0.3	6.6 ±0.1
Volatile organics	Sum entire period	N/A	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
NER	Soil extracts	2.8 ±0.1	11.0 ±0.1	10.8	10.7	10.6	9.7 ±0.1	9.8 ±0.4	10.6 ±0.5	12.4 ±0.2	13.8 ±0.1	15.2 ±0.3	14.7 ±0.4
Total % recovery	Entire system	98.0 ±0.5	98.6 ±0.3	99.4 ±0.1	98.2 ±0.8	98.1 ±0.3	97.7 ±1.0	97.5 ±0.5	98.4 ±1.0	97.7 ±0.4	97.2 ±1.7	95.7 ±0.2	97.5 ±0.2

N/A = not applicable; blank cell = not detected; no SD could be calculated;

B. Mass Balance

Material balance ranged from 93.4 to 98.1% (PYR), 94.1 – 99.7% (FUR) and 95.7 – 99.4% (ETH) of the applied radioactivity [AR]. The high material balances shown for all sampling intervals demonstrate that no significant RA dissipated from the flasks or was lost during processing.

Table 7.1.2- 8: Synopsis mass balance and recovery of radioactivity

Label	PYR	FUR	ETH
Total Recovery (%)	93.4 – 98.1	94.1 – 99.7	95.7 – 99.4
Extracted RA (%)	41.5 – 95.4	40.2 – 96.7	52.0 - 95.2
Max. Volatile RA (CO_2) (%)	26.7	15.9	6.9
Bound Residues (%)	2.7 – 17.8	3.1 - 30.1	2.8 - 15.2
Extraction Efficiency on DAT-0 (%)	95.4	96.7	95.2

C. Extractable and Bound Residues (NER)

Extractable ^{14}C-residues (plus the portions in the water layer after flooding) decreased from 95.4, 96.7 and 95.2% of AR at DAT-0 to 50.5, 48.9 and 76.1% of AR at study end (DASF-123) for labels PYR, FUR and ETH, respectively.

Non-extractable ^{14}C-residues increased from 2.7, 3.1 and 2.8% of AR at DAT-0 to max. 17.8% (PYR), 30.1% (FUR) and 15.2% (ETH) of AR at DASF-90, respectively, a slight decrease was observed at the final sampling point.

D. Volatilization

^{14}C-carbon dioxide accounted for up to 26.7, 15.9 and 6.9% of AR at maximum for labels PYR, FUR and ETH, respectively. Mineralization occurred during the 30 days of aerobic incubation, during the anaerobic phase after flooding the mineralization of BYI 02960 was very low. Organic volatiles were not observed in the aerobic or in the anaerobic study phase (< 0.1% AR at all sampling intervals).

E. Transformation of Test Item

Within the aerobic phase of the study (30 days) the percentages of BYI 02960 in the entire systems decreased from 94.5, 95.1 and 93.5% of AR to 53.7, 52.6 and 54.7% of AR for labels PYR, FUR and ETH, respectively. During the anaerobic incubation period (i.e. flooded state) the portions of BYI 02960 decreased further to 47.8, 47.2 and 47.7% of AR for labels PYR, FUR and ETH, respectively.

In the course of the study several transformation products were detected and quantified (see Table 7.1.2- 5 to Table 7.1.2- 7). Up to five minor transformation products were detected in the test systems of label PYR, and up to four minor transformation products were detected in the test systems of label FUR. In the test systems of label ETH, two transformation products were detected. One of these was identified as difluoroacetic acid (DFA) by HPLC-MS with accurate mass detection. During the aerobic incubation phase, the amounts of DFA increased up to 25.1% of AR at DAT-30. During the anaerobic phase, the amounts of DFA remained more or less constant (24.2 - 26.2% of AR). The second, minor, transformation product (ROI Z) appeared from DAT-30 onwards in amounts ranging from 0.7 - 1.3% of AR.

The results did not change the proposed overall pathway of degradation of BYI 02960 in soil shown in Figure 7.1.2- 1.

F. Kinetics of Test Item Degradation

A summary of the DT_{50} and DT_{90} calculation for the test item is given in Table 7.1.2- 9 (including the data for alternate kinetic model evaluations). The single first order model (SFO) was chosen as the best fit kinetic model for all labels (indicated bold typed in Table 7.1.2- 9).

Overall, the amount of BYI 02960 was declined very slowly during the test period of 123 days.

The estimated DT_{50} values range from 581.8 to 693.2 days (geometric mean: 633.7 days).

Table 7.1.2- 9: Summary of the kinetic evaluation (according to FOCUS) of the degradation of [14C]BYI 02960 in Anaerobic Soil at 20 °C

Soil	Kinetic model	Parent BYI 02960 DT50	DT90	Chi² value
Label PYR	**SFO**	**581.8**	**> 1000**	1.4
	FOMC	> 1000	> 1000	0.9
	DFOP	> 1000	> 1000	0.9
Label FUR	**SFO**	**693.2**	**> 1000**	1.3
	FOMC	> 1000	> 1000	1.0
	DFOP	> 1000	> 1000	1.0
Label ETH	**SFO**	**631.0**	**> 1000**	0.9
	FOMC	> 1000	> 1000	0.7
	DFOP	653.2	> 1000	0.9

III CONCLUSIONS

A. Major Outcomes of Study

The data gathered in the laboratory investigation demonstrate that residues of BYI 02960 are degraded very slowly in silt loam HF under anaerobic conditions at 20°C without the formation of further metabolites. For parent compound a SFO geometric mean DT_{50} value of 633.7 days was calculated. Observed portions of $^{14}CO_2$, NER and one major metabolite (DFA) were formed during the 30 days of aerobic incubation phase. A synopsis of results is shown in Table 7.1.2- 10.

B. Significance of Results to Environmental Behavior of BYI 02960

Based on the results obtained within this study it can be expected that the amounts of BYI 02960 and its only major metabolite DFA remain stable under flooded field conditions.
Degradation would be expected to continue according to the proposed overall pathway of degradation of BYI 02960 (see Figure 7.1.2- 1) whenever the conditions in soil turn aerobic again.

Table 7.1.2- 10: Synopsis of results of biotransformation of [14C]BYI 02960 in 30 days aerobic, then 123 days anaerobic silt loam HF at 20°C

	Radio-label position PYR	FUR	ETH
Total ^{14}C-Recovery (%) *	93.4 – 98.1	94.1 – 99.7	95.7 – 99.4
Extracted RA (%) *	41.5 – 95.4	40.2 – 96.7	52.0 - 95.2
Max. Volatile RA (%)	26.7	15.9	6.9
Bound Residues (%) *	2.7 - 17.8	3.1 - 30.1	2.8 - 15.2
Extraction Efficiency DAT-0 (%)	95.4	96.7	95.2
Anaerobic SFO DT_{50} of BYI 02960 [days]	582	693	631
Major transformation products ** predominantly formed during aerobic phase	CO_2 (max. 26.7%) NER (max. 17.8%)	CO_2 (max. 15.9%) NER (max. 30.1%)	CO_2 (max. 6.9%) NER (max. 15.2%) DFA (max. 26.2%)
Minor transformation predominantly formed during aerobic phase	Up to 5 individual (each ≤ 1.7% of AR)	Up to 4 individual (each ≤ 1.3% of AR)	1 (≤ 1.3% of AR)

*: Minimum and maximum values (as % of AR, mean values)
**: 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.

Report:	**KIIA 7.1.2/02, [redacted], [redacted]; 2012**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Anaerobic Soil Metabolism
Report No & Document No	MERVP094 M-421993-01-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002** **US EPA, OPPTS, 835.4200, 2008**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The anaerobic biotransformation of [pyridine-2,6-^{14}C]BYI 02960 was studied in a loamy sand (pH 6.7 in 0.01M $CaCl_2$, organic carbon 0.45%) from [redacted], California, USA. During the first phase of the study, the soil was maintained under aerobic conditions for 30 days in the dark at 20 ± 1 °C and at soil moisture of 55% maximum water holding capacity. Following the aerobic phase, the samples were flooded with water (water:soil ratio 3:1, v/w) and maintained in the dark under anaerobic conditions for 121 days at 20 ± 1 °C. [PYR-^{14}C]BYI 02960 was applied at a rate of 1.17 µg a.i./g, dry soil, equivalent to an application rate of 410 g a.i./ha.

Samples were analyzed at 0, 14 and 32 days of aerobic incubation, and at 0, 14, 30, 45, 59, 91 and 121 days of incubation following flooding (post treatment) of the samples (anaerobic phase). The water was decanted from each test system and the soil was extracted by a shaking method. In addition, aggressive extraction was conducted. The water layer, ambient extract and the microwave extracts were analyzed by HPLC. Identification of the parent compound and major metabolite was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

The average total material balance in the soil/water system for BYI 02960 was 96.5% ± 1.8% of AR. Non-extractable (bound) residues in soil increased from 0.6% at day 0 to 15.7% at day 32. At the end of the aerobic phase, 6.1% of the applied radioactivity was present as CO_2. No volatile organic compounds were present. The concentration of BYI 02960 in the aerobic phase decreased to 61.4% of AR at day 32.

In the anaerobic phase, radioactivity in the combined water and ambient extract decreased from 60.2% at day 0 to 35.8% by the end of the study. Aggressive extractions with both acetonitrile:water and methanol:water ranged from 3.1% to 2.9% of the applied radioactivity in the study, indicating that residue left after ambient and aggressive extraction was not easily extractable. Since non-extractable residues did not change during aerobic (15.7%) and anaerobic phases (16.1%), no further characterization was conducted. No CO_2 or volatile organic compounds were produced during the anaerobic phase of the study.

During the anaerobic phase, the concentration of BYI 02960 in soil decreased from 51.1% at day 0 to 26.2% of the applied amount at study termination. One major metabolite, 6-chloronicotinic acid (6-CNA), was detected during the aerobic phase of the study and it reached maximum of 12% (water/sediment) at day 0 of anaerobic phase and remained steady (12 to 14 %) throughout anaerobic phase. One minor metabolite, BYI 02960-chloro was detected during the aerobic phase and accounted for 2.8% at day 32. During the anaerobic phase, it remained steady (2%).

The observed DT_{50} values for BYI 02960 in the aerobic, then anaerobic soil/water system were determined using single first-order kinetics (SFO), first-order multi compartmental (DFOP) and double first-order in parallel (FOMC) and half-lives were 152 days, 164 days and 584 days,

respectively. BYI 02960 degrades moderately under aerobic conditions and remains more or less stable during anaerobic phase in soil.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: [Pyridine-2,6-^{14}C]BYI 02960 (Flupyradifurone) (sample IDC-1135A)
Specific activity: 35.03 mCi/mMole
Radiochemical purity: 99
Identity and purity of test item in the application solution was confirmed.

2. Soil: The biotransformation of BYI 2960 was studied in one soil. The soil was taken from [redacted], California, USA and transported by air to [redacted], KS at ambient temperature. It was stored for 13 days at 4 °C prior to use. The soil was sieved through a 2 mm sieve. Soil moisture was adjusted to 50% WHCmax and pre-incubated at about 20 ± 2 °C until application, i.e. for approx. 20 days.

Table 7.1.2- 11: Soil physicochemical properties

Parameter	Results/Units
Geographic Location (City / State / Country)	[redacted]/California/USA
GPS coordinates of sampling site	[redacted]”
Taxonomic Name	Hanford fine sandy Loam, gravely substrate
USDA Texture Class	Loamy Sand
Sand / Silt / Clay (%)	80 / 15 / 5
pH (soil/water, 1/1) pH (Saturated Paste) pH (soil/0.01 M $CaCl_2$ 1:2)	7.3 7.2 6.7
Organic Matter	0.77%
Organic Carbon	0.45%
Initial soil biomass (Day 0 aerobic)	128 mg microbial C/kg soil
Soil biomass on flooding day (Day 0 anaerobic)	128 mg microbial C/kg soil (untreated control soil)
Biomass at the end of the study (anaerobic)	3.83×10^6 cells/mL (untreated control water)
	6.83×10^7 cells/mL (untreated control soil)
	4.00×10^6 cells/mL (untreated control water)
	7.51×10^7 cells/mL (untreated control water)
Cation Exchange Capacity (CEC)	5.7 meq/100 g
Maximum Water Holding Capacity	30.5
Bulk Density (disturbed)	1.27 g/cm^3

B. Methods

1. Experimental conditions: The study was performed in flow-through incubation test systems under aerobic, and later under static anaerobic conditions in the dark at 20 ± 0.1 °C. During the aerobic phase, the test system consisted of Erlenmeyer flasks (250 mL) with a trap attached for absorption of ^{14}C-volatiles and $^{14}CO_2$, consisting of ethylene-glycol, potassium hydroxide and sulfuric acid, respectively. During the anaerobic phase, test systems were flooded with nitrogen to purge oxygen

from the systems. Aliquots of 50 g of dry soil were weighed into the test flasks. Replicates were set up and processed for each sampling. The final soil moisture was adjusted to 55% of WHC_{max} by adding pure water. The switch to anaerobic (flooded) conditions was made 30 days after test item application: The trap system of all remaining test flasks was removed and stored for later analysis.

2. Test Item Application Solution: [Pyridine 2,6-^{14}C]BYI 02960 was mixed into an aliquot of 1.3 mL acetonitrile dissolved 8.5 mL methanol:water (1:1).

3. Mode of Application: The application solution was applied at 200 µL evenly across the soil surface with a 250 µL syringe. The flasks were gently rotated to mix the treated soil, connected to volatile traps and kept at 20 C in the environmental chamber. [PYR-^{14}C]BYI 02960 was applied at a rate of 15,922,150 dpm per vessel. This corresponds to 58.4 µg per vessel.

4. Sampling: Duplicate test systems were sampled at day 0, 14 and 32 under aerobic conditions and after the 30 day aerobic incubation period, duplicate anaerobic test systems were analyzed at 0, 14, 45, 59, 91 and 121 days. During the anaerobic phase, pH, redox potential and dissolved oxygen were measured.
During the aerobic phase on the day of sampling, test systems were removed from the incubator along with the attached traps for the volatile and CO_2 analysis. During the anaerobic phase, volatile traps were attached and nitrogen was purged through the head space to trap any volatiles.
Samples were processed and soil was extracted on the day of sampling. The water was decanted from each test system and the soil was extracted by a shaking method. Water and extracts were analyzed with 10 days after sampling.

5. Description of analytical procedures: Extraction was done by cycles of heavily shaking for about 30 minutes on a mechanical shaker at room temperature followed by centrifugation (ca. 5 min, 2100g) and filtering through a paper filter. The soil samples were extracted with 3 x 150 mL acetonitrile/water (70/30, v/v) and 1 x 150 mL acetonitrile by shaking at ambient temperature. A further extraction step followed with acetonitrile/water (70/30, v/v) at 70°C using a microwave for 10 minutes. An aliquot of the combined 'ambient' and 'aggressive' extracts was transferred to a flask and rotovapped at 30°C.
The extracted soil was air dried and subsamples were combusted to quantify the non-extractable residues (NER). The water layer, ambient extract and the microwave extracts were analyzed by LSC and HPLC using a flow-through ^{14}C detector. Identification of the parent compound and major metabolite was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

II. RESULTS AND DISCUSSION

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study.

Table 7.1.2- 12: Dissolved oxygen, pH and redox potential of the test systems of the [PYR 2,6-^{14}C]BYI 02960 anaerobic soil metabolism study (mean of duplicates).

Measurement Interval [Days of post flooding]	Dissolved Oxygen (mg/L)	pH	Redox (E_o) in Water (mV)	Redox (E_h) in Water (mV)	Redox (E_o) in Soil (mV)	Redox (E_h) in Soil (mV)
0	3.2	7.9	NA	NA	155.6	362.6
14	0.5	7.8	81.6	278.6	87.0	284.0
30	0.4	6.5	-19.0	178.1	-28.9	168.1
45	0.9	7.4	-28.8	168.2	-31.6	165.4
59	0.4	7.1	-30.2	166.8	-60.3	136.8
91	0.1	7.1	-54.2	142.9	-118.8	78.3
121	0.4	7.1	-70.8	126.2	-128.1	69.0

NA = not analyzed, $E_h = E_{Obs} + E_{Ref}$
Where: E_h = Redox potential referred to the hydrogen scale
E_{Obs} = Observed redox potential of electrode (Ag/AgCl)
E_{Ref} = Redox potential of the electrode as related to the hydrogen electrode (Ag/AgCl = +197 mV)

A. Data

Data on biotransformation is shown in **Error! Reference source not found.**. The DAT 0 extraction efficiency was 99.4% of AR. The aggressive extraction removed approximately 2% to 3% radioactive residues during the study. These results indicate that the extraction procedure was efficient in extracting the majority of radioactive residues from the soil. At the end, bound residue was 16.1% of applied.

Table 7.1.2- 13: Biotransformation of [PYR-^{14}C]BYI 02960 in silt loam under aerobic, then anaerobic conditions; mean values and SD expressed as % of AR)

Compound	Matrix	Aerobic Phase Interval (Days Post Treatment)			Anaerobic Phase Interval (Days Post-Flooding)						
		0	14	32	0	14	30	45	59	91	121
BYI 02960	Water	-	-	-	11.7 ±0.4	26.9 ±1.8	27.0 ±3.2	25.0 ±0.1	22.5 ±1.0	23.1 ±2.9	22.7 ±4.2
	Soil	98.5 ±1.5	75.3 ±0.5	61.4 ±0.4	51.1 ±0.8	32.5 ±3.0	30.9 ±1.1	32.0 ±0.7	32.0 ±0.4	29.6 ±1.3	26.2 ±5.3
	Subtotal	98.5 ±1.5	75.3 ±0.5	61.4 ±0.4	62.7 ±1.2	59.4 ±4.8	57.9 ±4.2	57.0 ±0.5	54.5 ±1.4	52.8 ±1.6	48.9 ±1.2
6-CNA	Water	-	-	-	4.8 ±0.1	9.8 ±1.8	8.7 ±3.9	10.5 ±0.0	9.6 ±1.0	9.5 ±1.1	9.4 ±0.7
	Soil	0.0 ±0.0	7.4 ±0.0	11.1 ±0.4	7.2 ±0.3	3.4 ±0.4	2.9 ±0.1	3.8 ±0.1	3.4 ±0.1	3.2 ±0.5	3.0 ±0.6
	Subtotal	0.0 ±0.0	7.4 ±0.0	11.1 ±0.4	12.0 ±0.4	13.3 ±2.2	11.6 ±3.9	14.2 ±0.1	13.0 ±1.1	12.7 ±1.5	12.4 ±0.1
BYI 02960-chloro	Water	-	-	-	0.2 ±0.0	0.8 ±0.2	0.7 ±0.3	0.9 ±0.1	0.9 ±0.0	0.7 ±0.1	0.7 ±0.3
	Soil	0.0 ±0.0	1.3 ±0.2	2.8 ±0.3	2.1 ±0.5	1.7 ±0.1	1.4 ±0.2	1.7 ±0.1	1.8 ±0.2	1.4 ±0.1	1.5 ±0.2
	Subtotal	0.0 ±0.0	1.3 ±0.2	2.8 ±0.3	2.4 ±0.5	2.5 ±0.3	2.1 ±0.5	2.6 ±0.0	2.7 ±0.1	2.1 ±0.2	2.2 ±0.1
A (Diffuse radioactivity)	Subtotal	0.0 ±0.0	0.5 ±0.7	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.9 ±1.3	2.5 ±0.7
B (Diffuse radioactivity)	Subtotal	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	0.0 ±0.0	1.7 ±0.1	3.1 ±0.1
C (Diffuse radioactivity)	Subtotal	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	0.0 ±0.0	0.7 ±0.9	3.1 ±0.4
Unknown radioactivity	Subtotal	0.5 ±0.7	0.6 ±0.9	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	0.0 ±0.0
Unidentified radioactivity	Subtotal	0.5 ±0.7	2.4 ±1.3	2.8 ±0.3	2.4 ±0.5	2.5 ±0.3	2.1 ±0.5	2.6 ±0.0	2.7 ±0.1	5.4 ±0.7	8.7 ±0.2
Total extractable Radioactivity	Water	-	-		16.7 ±0.4	37.5 ±0.3	39.1 ±2.9	36.4 ±0.0	33.0 ±0.0	35.2 ±0.9	38.0 ±6.2
	Soil	99.4 ±1.5	84.6 ±0.1	75.3 ±0.3	60.2 ±0.6	37.6 ±2.6	35.2 ±0.7	37.5 ±0.7	37.2 ±0.1	38.1 ±0.2	35.8 ±5.1
	Subtotal	99.4 ±1.5	84.6 ±0.1	75.3 ±0.3	76.9 ±1.0	75.2 ±2.3	74.3 ±3.6	73.9 ±0.7	70.2 ±0.1	73.3 ±1.0	73.8 ±1.1
CO_2		-	1.7 ±0.0	6.1 ±0.0	5.7 ±0.4	5.7 ±0.7	4.3 ±0.4	5.2 ±0.3	4.8 ±0.0	6.0 ±1.8	6.0 ±0.6
Volatile organics		-	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	0.0 ±0.0	0.0 ±0.0
Total volatile		-	1.7 ±0.0	6.1 ±0.0	5.7 ±0.4	5.7 ±0.7	4.3 ±0.4	5.2 ±0.3	4.9 ±0.0	6.0 ±1.7	6.0 ±0.6
Bound Residues		0.6 ±0.0	9.3 ±0.3	15.7 ±0.9	15.9 ±0.5	15.1 ±2.3	16.5 ±0.5	18.1 ±3.1	18.7 ±0.1	16.9 ±3.7	16.1 ±0.0
Total % Recovery		100.0 ±1.5	95.5 ±0.2	97.1 ±0.5	98.5 ±1.1	95.9 ±0.7	95.2 ±3.5	97.2 ±3.6	93.8 ±0.0	96.3 ±3.0	95.9 ±0.5

B. Mass Balance

The average material balance was 96.5% ± 1.8% (mean range = 93.8% to 100%)

C. Extractable and Bound Residues (NER)

In the aerobic phase, extractable ^{14}C-residues in soil decreased from 99.4% at day 0 to 75.3% at day 32. Bound residues increased from 0.6% at day 0 to 15.7% at day 32.
In the anaerobic phase extractable ^{14}C-residues in soil decreased from 60.2% at day 0 to 35.8% at day 121. In the water layer, ^{14}C-residues increased from 16.7% at day 0 to 38% at the end of the study. Bound residues reached a maximum of 18.7% at day 59 and declined slightly to 16.1% at day 121 post-flooding.

D. Volatilization

At the end of the aerobic and anaerobic phase 6.1 and 6.0% of AR were present as CO_2. No organic volatiles were detected.

E. Transformation of Test Item

Within the aerobic phase of the study (30 days) the percentages of BYI 02960 in soil decreased from 98.5% (day 0) to 61.4% of AR (day 32). The major metabolite 6-chloronicotinic acid (6-CNA) increased from 0.9 to 11.1% of AR. During the anaerobic phase, the concentration of BYI 02960 in water and soil decreased from 62.7% (day 0) to 48.9% of AR at the end of the study. The proportion of metabolite 6-CNA was more or less constant during the anaerobic phase (12.4% at day 121).
The minor metabolite BYI 02960-chloro was observed at day 14 of the aerobic phase, reaching a maximum of 2.8% on day 32. During the anaerobic phase, it remained between 2% and 3%.
Further, there was a diffuse area of radioactivity, consisting of multiple peaks, which reached a maximum of 8.7%. None of the peaks accounted for more than 3.1% of applied.
The proposed degradation pathway is depicted in Figure 7.1.2- 1.

F. Kinetics of Test Item Degradation

A summary of the SFO DT_{50} and DT_{90} calculation for the test item is given in Table 7.1.2- 14.
The degradation may be due to residual aerobic conditions following flooding and should not be considered anaerobic soil degradation, anaerobic conditions were achieved after 14 days post flooding. The calculated half-life values should be attributed to the initial aerobic conditions with the test systems and not anaerobic soil half-life.

III CONCLUSIONS

A. Major Outcomes of Study

BYI 02960 degraded under aerobic/anaerobic conditions). Most of the degradation occurred during the aerobic phase and once anaerobic conditions were established, very little degradation occurred. During the aerobic phase, 6-CNA was the major metabolite formed and it remained more or less constant during the anaerobic phase (between 11 and 14% of AR), assuming that it needed further days post flooding until strictly anaerobic conditions were achieved. A minor degradate, BYI 02960-chloro, accounted for 2.8% at day 32 of the aerobic phase and remained more or less constant during the anaerobic phase, too.
A synopsis of results is shown in Table 7.1.2- 15.

B. Significance of Results to Environmental Behavior of BYI 02960

BYI 02960 degrades under aerobic condition; however, once anaerobic conditions are reached its residues remain more or less stable in soil.

Table 7.1.2- 14: Summary of the kinetic evaluation (according to FOCUS) of the degradation of [^{14}C]BYI 02960 in aerobic, then anaerobic soil at 20 °C

	Parent BYI 02960 (total system)					
Kinetic model (entire test system)	**Estimated initial % applied radioact. M(0)**	**Rate constant (d^{-1})**	**DT_{50} [d]**	**DT_{90} [d]**	**Chi^2 value [%]**	**r^2**
SFO	41.6	0.0045	152	506	11.9	0.52

Table 7.1.2- 15: Synopsis of results of biotransformation of [^{14}C]BYI 02960 in 30 days aerobic, then 121 days anaerobic loamy sand [redacted] at 20°C

Material Balance	**(% of AR)**
Mean ^{14}C-recovery during entire study Total extractable radioactivity during aerobic phase Total extractable radioactivity during anaerobic phase Max. volatile RA Range of bound residues (NER) Extraction Efficiency DAT-0	96.5 ± 1.8 75.3 – 99.4 35.8 – 60.2 6.1 15.7 – 18.7 99.4
Aerobic/anaerobic DT_{50} of BYI 02960 [days]	152 (SFO)
Transformation products *)	
Major transformation products observed in entire study but predominantly formed during aerobic phase	CO_2 (max. 6.1%) NER (max. 18.7%) 6-CNA (max. 14.2%)
Minor transformation products observed in entire study but predominantly formed during aerobic phase	BYI 02960-chloro (2.8% of AR)

*) 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

Report:	**KIIA 7.1.2/03, [redacted]; 2012**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Anaerobic Soil Metabolism in [redacted], Nebraska (USA) Soil
Report No & Document No	MERVL006 M-424987-01-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002 US EPA, OPPTS, 835.4200, 2008**
Deviation	**The study was terminated after the 60 day sampling due to a failure of: the temperature control which resulted in a temperature of 50ºC and compromised the remaining samples, this does not affect the interpretation of results of the stud**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The anaerobic biotransformation of [pyridine-2,6-^{14}C]BYI 02960 was studied in sandy clay loam (pH 6.5 in 0.01 M $CaCl_2$, organic carbon 1.9%) from [redacted], NE, USA. During the first phase of the study, the soil was maintained under aerobic conditions for 29 days in the dark at 20 ± 2 °C and at soil moisture of 55% maximum water holding capacity. Following the aerobic phase, the samples were flooded with water (water:soil ratio 3:1, w/w) and maintained in the dark under anaerobic conditions for 60 days at 20 ± 2 °C. The study was terminated after the 60 day sampling due to a failure of: the temperature control which resulted in a temperature of 50ºC and compromised the remaining samples. [PYR-^{14}C]BYI 02960 was applied at a rate of 1.1 µg a.i./g, dry soil, equivalent to an application rate of 410 g a.i./ha.

Samples were analyzed at 0, 14 and 29 days of aerobic incubation, and at 0, 19, 31, 45 and 60 days of incubation following flooding (post treatment) of the samples (anaerobic phase). The water was decanted from each test system and the soil was extracted using a shaking method. In addition, an aggressive extraction was conducted. The water layer, ambient extract and the microwave extracts were analyzed by HPLC using a flow-through 14C detector. Identification of the parent compound was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

The material balance for the study was complete (on average 105.8% ± 3.2%, mean range = 100.0 to 109.4%). Extractable [^{14}C] residues in soil decreased from 99.7% at day 0 to 79.0% by day 29. Non-extractable (bound) residues in soil increased from 0.3% at day 0 to 9.8% at day 29. At the end of the aerobic phase, 16.2% of the applied radioactivity was present as CO_2. No volatile organic compounds were present. In the aerobic phase the concentration of BYI 02960 decreased from 99.7% of the applied amount at day 0 to 79.0% at day 29. In the anaerobic phase, radioactivity in the combined ambient and aggressive extracts remained steady from 69.5% at day 0 to 71.7% by the end of the study. Aggressive extractions ranged from 6.7% to 11.7% of the applied radioactivity in the study, indicating that residue left after ambient and aggressive extraction was not easily extractable. NER and CO_2 in soil remained more or less constant during the anaerobic phase of the study, and no volatile organic compounds were produced.

BYI 02960 degrades moderately under aerobic conditions and its residues remain more or less stable during anaerobic phase in soil.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: [Pyridine-2,6-^{14}C]BYI 02960 (Flupyradifurone) (sample ID C-1135A)
Specific activity: 35.03 mCi/mMole
Radiochemical purity: 100%
Identity and purity of test item in the application solution was confirmed.

2. Soil: The test matrix used in this study was sandy clay loam from [redacted], Nebraska, USA. This site had no prior history of pesticide application for 5 years. The soil was transported from Nebraska to the testing facility, KS, by air cargo at ambient temperature, and upon arrival at Bayer CropScience, [redacted], [redacted], was stored in a refrigerator at 4 °C. Prior to the use, the soil was maintained at an average temperature of 4°C at the testing facility for 6 days. Soil was sieved through a 2-mm sieve. Soil was acclimated (8/12/11 to 8/18/11) for a period of 6 days before treatment. Moisture was not adjusted due to no significant moisture loss from the time the test systems were set up until the time of flooding. The test systems were flooded with Fisher optima® HPLC grade water.

Table 7.1.2- 16: Soil physicochemical properties

Parameter	Results/Units
Geographic Location (City / State / Country)	[redacted] / Nebraska, USA
GPS coordinates of sampling site	[redacted]
Taxonomic Name	Fine, kaolinitic, thermic Typic Kanhapludults
USDA Texture Class	Sandy clay loam
Sand / Silt / Clay (%)	53.1 / 23.2 / 23.7
pH (soil/water, 1/1) pH (Saturated Paste) pH (soil/0.01 M $CaCl_2$ 1:2)	6.7 6.6 6.5
Organic Matter	3.3%
Organic Carbon	1.9%
Initial soil biomass (Day 0 aerobic)	263 mg microbial C/kg soil
Soil biomass on flooding day (Day 0 anaerobic)	432 mg microbial C/kg soil (untreated control soil)
Biomass at the end of the study (anaerobic)	Since the study was terminated due to the increased temperature from a malfunctioning incubator (>50 °C), biomass determination was not assessed as the biological component may have been compromised from the higher temperature
Cation Exchange Capacity (CEC)	17.6 meq/100 g
Maximum Water Holding Capacity	61.4%
Bulk Density (disturbed)	1.02 g/cm³

B. Methods

1. Experimental Conditions: The study was performed under static aerobic incubation conditions, and later under flow-through anaerobic incubation conditions in the dark at 20 ± 0.1 °C. The test system consisted of 250-mL Pyrex® Erlenmeyer flasks (containing 50 g soil (dry weight)) with side arms for attachment to traps for the collection of CO_2 and volatile organic compounds. During the aerobic phase, test systems were kept in an environmental chamber at 20 ± 2 °C. The final soil moisture was adjusted to 55% of WHC_{max}. During the anaerobic (flooded) phase, they were kept in a temperature-controlled incubator with a nitrogen-filled atmosphere at 20 ± 2 °C. During incubation, aluminum foil was wrapped around flasks to prevent exposure to light.
The switch to anaerobic (flooded) conditions was made 29 days after test item application. The trap system of all remaining test flasks was removed and stored for later analysis.

2. Test Item Application Solution: [Pyridine 2,6-^{14}C]BYI 02960 (vial no. C-1135A) was mixed into an aliquot of 1.3 mL acetonitrile dissolved 8.5 mL methanol:water (1:1). The solution was mixed using a vortex and sonicator to get a homogeneous solution.

3. Mode and Rate of Application: The application solution (200 µL) was applied evenly across the surface of the soil using a 250-µL Hamilton Gastight® syringe. After treatment, 9 mL of water was added to the flasks to bring soil moisture to ~ 55% of max water holding capacity (MWHC). The flasks were gently rotated to mix the treated soil. The flasks were labeled, wrapped in aluminum foil

and weighed. Flasks were connected to volatile traps and kept at 20 ± 2 °C in the environmental chamber.
[PYR-^{14}C]BYI 02960 was applied at a rate of 15,122,786 dpm per vessel. This corresponds to 56.1 µg per vessel. Material balances for the kinetic treatment test systems of the study were based on the theoretical dpm applied to the soil.

4. Sampling: Duplicate test systems at day 0, 14 and day 29 were analyzed under aerobic conditions. After the 29-day aerobic incubation, duplicate anaerobic test systems were analyzed at 0, 19, 31, 45 and 60-days post-flooding intervals. During the anaerobic phase of the study, test systems were measured for pH, redox potential, and dissolved oxygen. Radioactive CO_2 and volatile organics were measured at each interval. The water was separated from the soil by decanting.

Samples were processed and soil was extracted on the day of sampling. Water and extracts were analyzed within 7 days of sampling. The extracts and water were stored in a laboratory refrigerator. The concentrated extracts were stored in the laboratory freezer until analysis, and were moved to a central freezer for long-term storage.

During the aerobic phase on the day of sampling, test systems were removed from the incubator along with the attached traps for the volatile and CO_2 analysis. During the anaerobic phase test systems were removed from the incubator, volatile traps were attached and nitrogen was purged through the head space to trap any volatiles from the head space into the bubblers.

5. Description of Analytical Procedures: The volatile organics were collected from the headspace of the treated test systems using 2 M KOH, ethylene glycol, and 1 M H_2SO_4. The trapping solutions were radioassayed in triplicate 1-mL aliquots for KOH, ethylene glycol and H_2SO_4. The aqueous portion of the sample was filtered through a Whatman GF/F glass filter into a 250-mL graduated cylinder. The water was radioassayed in triplicate 1-mL aliquots by LSC.
The soil was transferred to a 250-mL Teflon® bottle, and 40 mL of acetonitrile/water (70/30) was added to the bottle which was then extracted on a bench-top shaker for 30 minutes. The samples were centrifuged for 5 minutes at 1850 g. The extracts were filtered through a Whatman GF/F filter into a 250-mL graduated cylinder. The extraction procedure was repeated two additional times with acetonitrile/water (70/30) and another time with 100% acetonitrile. The combined aqueous portion (ambient extract) was radioassayed in triplicate 1-mL aliquots. A further extraction step followed once with 50 mL of acetonitrile/water (70/30) and once with 50 mL of methanol/water (50/50) at 70°C using a microwave for 10 minutes (650 Watts). After the acetonitrile/water (70/30) microwave extraction the sample was centrifuged for 5 minutes at 1850 g and decanted through a Whatman GF/F filter into a 250-mL graduated cylinder. Following the methanol/water (50/50) microwave extraction the entire sample were filtered through a Whatman GF/F filter into the same 250-mL graduated cylinder and aliquots (3 x 1-mL) were radioassayed. The remaining soil was allowed to air dry on the filter.
The extracted soil samples were air dried, homogenized thoroughly and weighed. Subsamples (approximately 0.5 g) of the soil were combusted to quantify the non-extractable residue (NER).
The water layer, ambient extract and the microwave extracts were analyzed by LSC and HPLC using a flow-through ^{14}C detector. Identification of the parent compound and major metabolite was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

II. RESULTS AND DISCUSSION

Results indicated that anticipated standardized conditions were maintained, and the soils were microbial active over the duration of the laboratory study.

Table 7.1.2- 17: Dissolved oxygen, pH and redox potential of the test systems of the [PYR 2,6-^{14}C]BYI 02960 anaerobic soil metabolism study (mean of duplicates).

Measurement Interval [Days of post flooding]	Dissolved Oxygen (mg/L)	pH	Redox (E_o) in Water (mV)	Redox (E_o) in Soil (mV)
0	4.52	6.13	250.7	264.8
19	0.45	6.64	150.2	182.4
33	0.30	6.69	-10.6	20.9
45	0.26	7.04	-64.6	-46.1
60	0.03	6.98	-152.1	-128.9

NA = not analyzed, $E_h = E_{Obs} + E_{Ref}$
Where: E_h = Redox potential referred to the hydrogen scale
E_{Obs} = Observed redox potential of electrode (Ag/AgCl)
E_{Ref} = Redox potential of the electrode as related to the hydrogen electrode (Ag/AgCl = +197 mV)

A. Data

Data on biotransformation is shown in **Error! Reference source not found.**.
The DAT 0 extraction efficiency was 99.7% of applied radioactivity. The shake procedure extracted an average of 99.7% of the applied radioactivity at aerobic day 0. This indicated that the extraction procedure was efficient in extracting the majority of the radioactive residues from the soil, and did not degrade the parent. The bound residue at the end of the study was 12.9% of the applied radioactivity

Table 7.1.2- 18: Biotransformation of [pyridine 2,6--[14]C]BYI 02960 in sandy clay loam under aerobic, then anaerobic conditions; means values and SD expressed as % of AR

Compound	Matrix	Aerobic Phase Interval (Days Post Treatment)			Anaerobic Phase Interval (Days Post-Flooding)				
		0	14	29	0	19	31	45	60
BYI 02960	Water	-	-	-	7.3 ±2.1	10.7 ±0.1	9.5 ±0.3	7.2 ±0.4	5.7 ±0.2
	Soil	99.7 ±2.5	83.7 ±2.9	74.4 ±0.9	64.5 ±3.7	58.4 ±3.6	66.5 ±2.2	67.1 ±0.4	66.7 ±1.4
	Subtotal	99.7 ±2.5	83.7 ±2.9	74.4 ±0.9	71.9 ±1.6	69.1 ±3.7	76.0 ±2.5	74.3 ±0.1	72.5 ±1.6
Unidentified radioactivity	Water	-	-	-	0.4 ±0.5	0.6 ±0.1	0.4 ±0.0	0.9 ±0.4	0.6 ±0.1
	Soil	0.0 ±0.0	5.4 ±1.0	4.6 ±0.6	5.0 ±0.1	5.1 ±1.3	5.0 ±0.1	5.6 ±0.2	5.0 ±0.6
	Subtotal	0.0 ±0.0	5.4 ±1.0	4.6 ±0.6	5.3 ±0.4	5.7 ±1.4	5.4 ±0.1	6.4 ±0.5	5.6 ±0.6
Total extractable Radioactivity	Water	-	-	-	7.7 ±2.6	11.3 ±0.2	9.9 ±0.3	8.1 ±0.1	6.3 ±0.3
	Soil	99.7 ±2.5	89.1 ±1.9	79.0 ±0.2	69.5 ±3.8	63.5 ±4.9	71.6 ±2.2	72.7 ±0.5	71.7 ±2.0
	Subtotal	99.7 ±2.5	89.1 ±1.9	79.0 ±0.2	77.2 ±1.2	74.8 ±5.1	81.5 ±2.5	80.8 ±0.5	78.1 ±2.2
CO_2		-	7.0 ±1.0	16.2 ±0.4	16.0 ±1.3	18.4 ±0.2	15.2 ±3.9	16.8 ±0.8	17.9 ±0.0
Bound Residues		0.3 ±0.0	7.7 ±0.1	9.8 ±0.9	10.6 ±1.5	14.8 ±1.8	11.1 ±0.4	11.9 ±0.2	12.9 ±0.0
Total % Recovery		100.0 ±2.6	103.8 ±3.0	105.0 ±0.2	103.8 ±1.7	108.0 ±3.1	107.8 ±1.8	109.5 ±0.5	108.9 ±2.2

B. Mass Balance

The average material balance for the study was 105.8 ± 3.2% (mean range = 100.0 to 109.5%).

C. Extractable and Bound Residues (NER)

In the aerobic phase, extractable [[14]C]residues in soil decreased from 99.7% at day 0 to 79.0% by day 29. Non-extractable (bound) residues in the soil increased from 0.3% at day 0 to 9.8% at day 29.
In the anaerobic phase, extractable [[14]C]residues in soil remained constant with residues from 69.5% at day 0 to 71.7% at day 60. In the water layer [[14]C]residues also remained constant from 7.7% at day 0 to 6.3% at the end of the study. Non-extractable residues in soil reached a maximum of 14.8% at day 19 and declined to 12.9% at day 60, post flooding.

D. Volatilization

At the end of the aerobic and anaerobic phase, 16.2 and 17.9% of the applied radioactivity was present as CO_2, and no organic volatile compounds were detected.

E. Transformation of Test Item

During the aerobic phase, the concentration of BYI 02960 in the soil decreased from 99.7% at day 0 to 74.4% of the applied amount at day 29. Other unidentified radioactivity remained constant from aerobic day 14 until the end of the study, ranging from 4.6 to 6.4% of the applied amount of radioactivity. During the anaerobic phase, the concentration of BYI 02960 remained constant, starting at 71.9% on day 0 and ending at 72.5% at the termination of the study. Non-extractable residues were less than 20% of AR, therefore, no further fractionation was conducted.

F. Kinetics of Test Item Degradation

Following the flooding of the [redacted], NE, sandy clay loam test system, BYI 02960 levels remained stable through the end of the study. Since degradation of BYI 02960 did not occur during the anaerobic phase of the study, kinetic endpoints could not be derived.

III CONCLUSIONS

A. Major Outcomes of Study

BYI 02960 steadily degraded during the aerobic phase, but once the test systems were flooded with water, no further degradation of BYI 02960 occurred during the anaerobic phase Although the study was terminated after the 60 day sampling the results are consistent with those obtained with the other soils and labels.

B. Significance of Results to Environmental Behavior of BYI 02960

BYI 02960 degrades under aerobic conditions. Once anaerobic conditions are reached, BYI 02960 is stable. Thus, will not be a major route of dissipation in a flooded anaerobic soil environment.

IIA 7.1.3 Soil Photolysis

Report:	**KIIA 7.1.3/01, [redacted], [redacted], 2011**
Title:	[Pyridinylmethyl-^{14}C]BYI 02960 and [furanone-4-^{14}C]BYI 02960: Phototransformation on Soil
Report No & Document No	MEF-10/351 M-405776-01-2
Guidelines:	**OECD TG: Phototransformation of Chemicals on Soil Surfaces, Draft of 2002** **US EPA, 161-3: Photodegradation Studies on Soil, 1982**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

Phototransformation of [pyridinylmethyl-^{14}C]- and [furanone-4-^{14}C]BYI 02960 was studied on a loam soil from [redacted], California, USA at an application rate of about 400 g/ha. The study was conducted for a period of eight days at 20°C ± 1°C and at soil moisture of about 75% of 1/3-bar water holding capacity (humid test samples). In addition, photolysis was studied on air dried samples.

[PYM-^{14}C] and [FUR-^{14}C]BYI 02960 were directly applied to the surface of the soil aliquots at an initial concentration of about 40.8 µg/ 3 g soil. The treated samples were continuously exposed to artificial irradiation (xenon lamp with <290 nm cut-off filter, 1082 W m^{-2} for label PYM and 1116 W m^{-2} for label FUR). In addition, dark controls were set up. Test vessels were connected to traps for the collection of CO_2 and organic volatiles. Samples from humid test systems were taken in duplicate 0, 0.2, 1, 4, 5, 7, and 8 days after application for the determination of the parent compound and the transformation product residues. Samples from additional test systems containing air dried soil were only taken at the end of the study period. The soil was extracted at ambient temperature with 2 x acetonitrile/water (1/1, v/v), 1 x acetonitrile/water (8/2, v/v) and pure acetonitrile at ambient temperature (ambient extraction). Afterwards, the soil was extracted once with acetonitrile/water (8/2, v/v) at an elevated temperature of 70°C (aggressive extraction). The BYI 02960 residues were analyzed by reversed phase HPLC with radioactivity detection. Selected samples were additionally

analyzed by TLC as a confirmatory method. Identification and confirmation of the parent compound was by HPLC-MS, HPLC-MS/MS and NMR (stock solution) or co-chromatography (selected extracts).

The mass balances were complete indicating that no losses occurred during exposure or processing of samples. The mass balances were 99.3 ± 1.2% and 99.4 ± 0.9% of the AR in the irradiated and dark soil samples of label PYM, respectively. For label FUR, the corresponding mass balances were 99.5 ± 0.6% and 99.5 ± 0.8% of the AR.

Extractable residues for radiolabel PYM decreased from 99.7% of the AR at day 0 to 98.0% and 98.9% of the AR at the end of the study for irradiated and dark systems respectively. For both radiolabels tested the amounts of non-extractable residues (NER) in the irradiated and dark samples were low during the entire study.
In the irradiated test systems, BYI 02960 decreased to 93.8 and 93.5% of the AR by the end of the study for PYM and FUR radiolabel, respectively. No major transformation product was detected in the soil extracts, very minor transformation products were observed. The amounts of $^{14}CO_2$ at study termination amounted to 0.1% and 2.2% of the AR for PYM and FUR test systems, respectively. Organic volatile formation was negligible throughout the study (≤ 0.1% of the AR).

In the dark PYM and FUR test systems, BYI 02960 decreased just to 98.4 and to 97.3% of the AR by the end of the study. No transformation products were detected. At study termination, the amounts of $^{14}CO_2$ were 0.1% of the AR for both labels. Organic volatile formation was negligible throughout the study (≤ 0.1% of the AR).

In order to test the effects of the dryness of soil, a supplementary test was performed. As degradation was generally low, no significant differences between humid and dry soil samples were observed.

Overall the degradation of BYI 02960 was very slow. The degradation in irradiated test systems was slightly less slow compared to the degradation observed in dark test systems. The experimental DT50 values of BYI 02960 in the irradiated samples were 99.6 days (PYM) and 109.3 days (FUR), showing a good comparability for the results obtained with the two labels. The corresponding experimental DT_{50} values of BYI 02960 in the dark controls were > 1000 days (PYM) and 419.2 days (FUR).

The results of this study indicate that phototransformation on soil is of minor importance for the degradation of BYI 02960 under outdoor conditions and no major phototransformation products are expected.

I. MATERIALS AND METHODS

A. Materials

1. Test Items: Flupyradifurone: Code = BYI 02960;
Label PYM = [Pyridinylmethyl-^{14}C]BYI 02960 (sample ID: KATH 6703)
Specific activity: 4.37 MBq/mg
Radiochemical purity: >99% (acc. radio-HPLC)
Label FUR = [Furanone-4-^{14}C]BYI 02960 (sample ID: KATH 6702)
Specific activity: 3.94 MBq/mg
Radiochemical purity: >99% (acc. radio-HPLC)
Identity and purity of test items in the application solution were confirmed.

2. Soil: The biotransformation of BYI 2960 was studied in one soil. The soil was taken on 2008-11-20 fresh from the field. At the test facility, the soil was air dried broken up for sieving. Then, the soil was successively sieved to ≤ 10, 5, 3.35 and 2 mm. The sieved soil was filled in a plastic bag and stored at 4-8°C until further use.

Table 7.1.3- 1: Soil physicochemical properties

Parameter	Test soil [redacted] Results/Units
SCS soil series	Camarillo
Texture class	Loam
Sand Silt Clay	40% 45% 15%
pH (water), ($CaCl_2$)	6.4 (6.5
Organic matter	1.03%
Organic carbon	0.6%
Cation exchange capacity	20.7 meq/100 g soil dry wt
% Moisture at 0.33 bar	12.3%
Bulk density (disturbed)	1.23 g/cm^3
Microbial biomass Day 0	 92 mg microbial C/kg soil dry wt
Soil mapping unit	[redacted]"

B. Methods

1. Preparation and application of the test item: The test systems for the kinetic photolysis test with humid soil consisted of 32 quartz glass vessels for each label (36-mm inner diameter, 35-mm height, base area 10.2 cm^2) each containing 3 g of soil (dry weight), providing about 3-mm soil depth. In addition, 4 vessels containing air dried soil were prepared for each label. A glass neck with ground joint (NS 10) was attached to the side of the wall. There, except for day 0) the flask is closed with a solid trap attachment, a small glass tube of 90 mm length and 12 mm inner diameter, in which volatile compounds were bound to soda lime and polyurethane foam. The trap is packed in the following sequence: PU-foam plug → quartz wool → 2 g soda lime → quartz wool → 0.5 g soda lime → quartz wool. Soil moisture was about 75% of 1/3-bar water holding capacity at the time of application. Moisture adjustment was performed on day 4 by replenishing the lost water with 0 to 50 µL of Milli-Q-water using a Hamilton injection device.

Duplicate treated test systems were analyzed at each sampling interval for both irradiated and dark test systems.

In addition to the main test with soil [redacted] CA adjusted to a definitive moisture (75% of 1/3 bar moisture), a supplementary non kinetic experiment was conducted in order to assess the effect of dryness on phototransformation of BYI 02960. For this purpose, soil was air dried to a remaining soil moisture of 0.04 g / 3 g dry soil (about 11% of 1/3 bar moisture). Application of the test items, maintenance procedures and sampling details of this test were in line with the main test, but samples were taken on the last sampling intervals only.

The test items [PYM -^{14}C] BYI 02960 and [FUR-^{14}C] BYI 02960 were dissolved in 5.0 mL Milli-Q-water/methanol (1/1, v/v). The purity of the ^{14}C-labeled test items was determined by HPLC, resulting in mean purities of 99.8% and 99.6% for PYM and FUR, respectively.
According to an intended application rate of 400 g/ha, 39.2 µL (PYM) and 37.9 µL (FUR) of application solution was pipetted into each incubation vessel. The solutions were applied evenly as drops across the surface of the soil. The soil was not mixed or agitated after application.
The material balance of the study for both dark and irradiated test systems was based on the average amount of radioactivity (RA) recovered with these measurements: 186,094 Bq or 42.58 µg BYI 02960 for PYM and 156,914 Bq or 39.83 µg BYI 02969 for FUR (per 3 g soil (dry weight)).

2. <u>Irradiation and sampling:</u> The photolysis vessels were placed in a Suntest CPS+ unit (Xenotest GmbH, Hanau, Germany) containing a xenon lamp simulating natural sunlight. The light emission was filtered with a 290 nm cut-off UV-filter, which eliminates all wavelengths <290 nm. The exposure time and intensity under experimental conditions can be related to natural solar radiation, e.g. of Houston (Texas; USA), Los Angeles (California; USA) or Tampa (Florida; USA), representing areas of high intensity of sunlight. For example the total radiant exposure representative of a summer day (July) at a horizontal plane is 26 MJ m^{-2} (at Los Angeles), 22 MJ m^{-2} (at Houston) or 23 MJ m^{-2} (at Tampa). For humid samples, two test systems for each label were processed for analysis immediately after the application on day 0 (0 h). Subsequently, duplicates of both irradiated and dark soil samples for each label were processed at 0.2, 1, 4, 5, 7, and 8 days post-application. Air dried samples were only processed and analyzed at day 8 after application.
Soil samples were extracted immediately on the day of sampling. Extracts were analyzed within three days of sample extraction and then stored in a freezer (-18°C or below). Samples for CO_2 and volatile organics were stored at ambient temperature (≤ 12 days) until processing for analysis.
Prior to opening a test vessel, volatile compounds possibly still present in the headspace were transferred into the trap attachment. For this purpose, the test vessels were placed in a desiccator for 15 min and the headspace was carefully purged through the trap.

3. <u>Description of analytical procedures:</u> Volatile organic compounds possibly contained in the PU foam plug were extracted with 5 mL of ethyl acetate. 500 µL aliquots of the extracts were analyzed by LSC in duplicate. The radioactivity (i.e. $^{14}CO_2$) absorbed by the soda lime was liberated with 18% aqueous HCl and purged and absorbed in a series of three Zinsser vials each filled with 20-mL of ice-cooled scintillation cocktail intended for radio-assay by LSC.

The extraction of the soil samples was similar to the extraction procedure used in the aerobic soil degradation study. The total amount of soil of each vessel was transferred into a 40 mL Teflon® centrifuge beaker and extracted once with 8 mL acetonitrile/water (1/1, v/v) followed by 5 mL acetonitrile/water (1/1, v/v), 5 mL ACN/water (8/2, v/v) and 5 mL ACN. The extractions were performed at ambient temperature on a mechanical shaker for 30 min. Each extraction step was followed by centrifugation (15 min, 10 000 x g) and decanting of the supernatant. All ambient extracts were combined and the volume was determined. Aliquots thereof were analyzed by LSC and reversed phase HPLC with radioactivity detection. Extracts sampled on day 0, 4 and 8 were also analyzed by TLC. Afterwards, the soil was extracted once with acetonitrile/water (8/2, v/v) at an elevated temperature of 70°C (aggressive extraction). Identification and confirmation of the parent compound was done by HPLC-MS, HPLC-MS/MS and NMR (stock solution) or co-chromatography (selected extracts).

The extracted soil was air dried and the non-extractable Residues (NER) were quantified by combustion.

The limit of quantification (LOQ) was set to three times the maximum background radioactivity i.e. about 1.5 Bq (0.34 ng/500 µL for label PYM and 0.38°ng/500 µL for label FUR). The lowest amount measured in ambient and aggressive organic extract samples was about 25 Bq/500 µL, i.e. 16.7 times higher than the LOQ. For CO_2 liberated from soda lime and organic volatiles extracted with ethylacetate the lowest radioactivity measured (after background subtraction) were 1.2 and 0.01 Bq, respectively. Although these values were lower than the LOQ they were evaluated, resulting in amounts of radioactivity < 0.1% of AR.

II. RESULTS AND DISCUSSION

A. Data

Data on phototransformation and dark controls is shown in Table 7.1.3- 2 and Table 7.1.3- 3.

Table 7.1.3- 2: Transformation of [PYM-^{14}C]BYI 02960 in loam soil [redacted]; mean values and standard deviations expressed as % of AR

Compound	Test system	Sampling Time (Days Post Treatment)							
		Humid soil							Dry soil
		0	0.2	1	4	5	7	8	8
BYI 02960	Irradiated	99.3 ±1.1	98.2 ±0.3	97.1 ±1.0	94.4 ±05	95.4 ±0.8	93.3 ±0.6	93.8 ±0.2	92.1 ±0.1
	Dark	99.3 ±1.1	98.4 ±0.4	98.2 ±0.6	97.7 ±0.3	98.0 ±0.0	98.9 ±0.6	98.4 ±1.0	99.3 ±0.9
Reg a	Irradiated	n.d	n.d	n.d	1.6 ±0.1	1.3 ±0.2	1.4 ±0.1	1.3 ±0.2	1.3 ±0.3
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Reg b	Irradiated	n.d	n.d	n.d	0.5 ±0.1	0.5 ±0.1	0.9 ±0.1	0.6 ±0.1	1.6 ±0.2
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Reg c	Irradiated	n.d	n.d	n.d	0.9 ±0.1	0.8 ±0.0	1.2 ±0.0	0.8 ±0.2	0.7 ±0.2
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Reg d	Irradiated	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.0 ±0.4
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Reg e	Irradiated	n.d	n.d	n.d	1.0 ±0.2	1.0 ±0.1	1.8 ±0.1	1.0 ±0.1	0.8 ±0.0
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unidentified radioactivity	Irradiated	0.4 ±0.0	0.3 ±0.0	0.4 ±0.0	0.4 ±0.1	0.3 ±0.2	0.7 ±0.0	0.5 ±0.2	0.5 ±0.0
	Dark	0.4 ±0.0	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.4 ±0.0	0.4 ±0.1	0.5 ±0.0	0.5 ±0.0
Total extractable Radioactivity	Irradiated	99.7 ±1.1	98.5 ±0.3	97.5 ±1.0	98.7 ±0.9	99.3 ±0.7	99.2 ±0.4	98.0 ±0.7	98.0 ±0.6
	Dark	99.7 ±1.1	98.6 ±0.5	98.5 ±0.5	98.0 ±0.9	98.3 ±0.0	99.4 ±0.6	98.9 ±1.0	99.8 ±0.9
CO_2	Irradiated	n.a	<0.1	<0.1	0.0 ±0.0	0.1 ±0.0	0.2 ±0.0	0.1 ±0.0	0.1 ±0.0
	Dark	n.a	<0.1	<0.1	<0.1	0.0 ±0.0	0.1 ±0.0	0.1 ±0.0	<0.1
Volatile organics	Irrad.	n.a	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Dark	n.a	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	Irradiated	0.1 ±0.0	0.2 ±0.0	0.3 ±0.1	0.9 ±0.0	0.8 ±0.1	0.9 ±0.1	0.8 ±0.1	0.6 ±0.1
	Dark	0.1 ±0.0	0.2 ±0.0	0.4 ±0.0	0.6 ±0.0	0.9 ±0.3	0.8 ±0.1	1.0 ±0.1	0.1 ±0.0
Total % Recovery	Irradiated	99.7 ±1.1	98.7 ±0.2	97.9 ±0.9	99.7 ±0.9	100.1 ±0.7	100.3 ±0.6	98.9 ±0.8	98.7 ±0.7
	Dark	99.7 ±1.1	98.8 ±0.5	98.9 ±0.5	98.6 ±0.4	99.3 ±0.3	100.2 ±0.7	99.9 ±0.9	99.9 ±0.9

n.d. = not detected; n.a. = not analyzed; <0.1 = values below 0.05% of AR

Table 7.1.3- 3: Transformation of [FUR ^{14}C]BYI 02960 in loam soil [redacted]; mean values and standard deviations expressed as % of AR

Compound	Test system	Sampling Time (Days Post Treatment)							
		Humid soil							Dry soil
		0	0.2	1	4	5	7	8	8
BYI 02960	Irradiated	99.1 ±0.3	98.1 ±0.4	98.5 ±0.5	95.9 ±0.3	96.2 ±0.6	94.7 ±0.2	93.5 ±0.1	93.3 ±0.2
	Dark	99.1 ±0.3	99.0 ±0.5	99.0 ±0.5	98.0 ±0.3	98.8 ±0.4	98.3 ±0.3	97.3 ±1.4	99.1 ±1.0
Reg a	Irradiated	n.d	n.d	0.2 ±0.2	1.1 ±0.1	1.2 ±0.3	1.1 ±0.0	1.3 ±0.0	1.2 ±0.0
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Reg b	Irradiated	n.d	n.d	n.d	0.3 ±0.3	n.d	0.3 ±0.3	0.6 ±0.0	1.3 ±0.0
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Reg c	Irradiated	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.4 ±0.4
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Reg f	Irradiated	n.d	n.d	n.d	0.4 ±0.4	n.d	0.5 ±0.0	0.5 ±0.0	0.4 ±0.0
	Dark	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Unidentified radioactivity	Irradiated	0.3 ±0.0	0.3 ±0.1	0.3 ±0.0	0.5 ±0.1	0.4 ±0.0	0.4 ±0.0	0.5 ±0.0	0.4 ±0.0
	Dark	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0	0.3 ±0.1	0.3 ±0.0	0.1 ±0.0	0.3 ±0.2
Total extractable Radioactivity	Irradiated	99.3 ±0.2	98.4 ±0.3	99.0 ±0.3	98.3 ±0.3	97.8 ±0.3	96.9 ±0.4	96.4 ±0.2	96.9 ±0.3
	Dark	99.3 ±0.2	99.4 ±0.6	99.3 ±0.6	98.3 ±0.3	99.1 ±0.3	98.7 ±0.3	97.4 ±1.4	99.4 ±0.9
CO_2	Irradiated	n.a	<0.1	0.1 ±0.0	0.4 ±0.1	1.4 ±0.6	1.6 ±0.0	2.2 ±0.2	2.3 ±0.0
	Dark	n.a	<0.1	<0.1	<0.1	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	<0.1
Volatile organics	Irrad.	n.a	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Dark	n.a	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues (NER)	Irradiated	0.0 ±0.0	0.1 ±0.0	0.4 ±0.1	1.1 ±0.0	1.0 ±0.1	1.0 ±0.1	0.9 ±0.1	0.7 ±0.1
	Dark	0.0 ±0.0	0.2 ±0.0	0.4 ±0.1	1.1 ±0.0	1.0 ±0.1	1.0 ±0.1	0.9 ±0.1	0.7 ±0.0
Total % Recovery	Irradiated	99.4 ±0.2	98.5 ±0.3	99.4 ±0.5	99.8 ±0.4	100.1 ±0.3	99.6 ±0.3	99.5 ±0.1	99.9 ±0.3
	Dark	99.4 ±0.2	99.6 ±0.5	99.7 ±0.7	99.4 ±0.3	100.2 ±0.4	99.8 ±0.2	98.4 ±1.3	100.1 ±0.8

n.d. = not detected; n.a. = not analyzed; <0.1 = values below 0.05% of AR

B. Mass Balance

The material balances were shown to be complete. For irradiated test systems the overall mean (±RSD) was of 99.3 (±1.2) and 99.5 (±0.6) % for label PYM and FUR, respectively. For dark test systems the overall mean (±RSD) was of 99.4 (± 0.9) and 99.5 (± 0.8) % for label PYM and FUR, respectively.

C. Distribution and Composition of Residues

For irradiated test systems PYM and FUR, the extractable amounts of radioactivity were 99.7% and 99.3% of the AR at day 0 and 98.0% and 96.4% at day 8, respectively. The amounts of NER were 0.1% and 0.0% of the AR at day 0 and remained at very low levels of 0.8% and 0.9% of the AR at the end of the test. $^{14}CO_2$ formation increased up to 0.1% and 2.2% by day 8 for label PYM and FUR, respectively. Organic volatile formation was negligible throughout the study (<1%).
For dark test systems PYM and FUR, the extractable amounts of radioactivity were 99.7% and 99.3% of the AR at day 0 and 98.9% and 97.4% at day 8, respectively. NER were 0.1% and 0.0% of the AR at day 0 and remained at the very low levels of 1.0% and 0.9% of the AR at the end of the test, respectively. $^{14}CO_2$ formation increased up to 0.1% at day 8 for both labels. Organic volatile formation was negligible throughout the study (<1%).

In the irradiated PYM test systems, BYI 02960 decreased from an average of 99.3% of the AR at day 0 to 93.8% of the AR by the end of the study. No major transformation product was detected in the soil extracts. From day 4 onwards, minor transformation products were detected. The maximum amount of a single peak accounted for 1.8% of AR (day 7).

In the irradiated FUR test systems, BYI 02960 decreased from an average of 99.1% of the AR at day 0 to 93.5% of the AR by the end of the study. From day 1 onwards, minor transformation products were detected. The maximum amount of a single peak was 1.3% of AR (day 8)

The DT_{50} and DT_{90} values of BYI 02960 in dark and irradiated samples were calculated using a single first order model (see Table 7.1.3- 4).

Table 7.1.3- 4: Degradation of BYI 02960 on surface of loam soil [redacted]

Test System	Half-life		Experimental DT_{50} [days]	Experimental DT_{90} [days]
	Kinetic Model	Chi^2 Error		
Dark, Label PYM	SFO	0.41	> 1000	> 1000
Irradiated, Label PYM	SFO	0.61	99.6	331.0
Dark, Label FUR	SFO	0.29	419.2	> 1000
Irradiated, Label FUR	SFO	0.32	109.3	363.2

SFO = single first order

III. CONCLUSIONS

A. Major Outcomes of Study

Overall the degradation of BYI 02960 was very slow. The degradation in irradiated test systems was slightly less slow compared to the degradation observed in dark test systems. Based on the experimental DT_{50} values of 99.6 and 109.3 days for [pyridinymethyl-^{14}C] BYI 02960 and [furanone-4-^{14}C] BYI 02960, respectively, the DT_{50} values of BYI 02960 under environmental conditions were calculated to be 358 and 405 solar summer days at Los Angeles, California, USA, 466 and 527 days at Athens, Greece and 638 and 722 solar summer days at Tokyo, Japan.

B. Significance of Results to Environmental Behavior of BYI 02960

Direct phototransformation of BYI 02960 on soil surfaces is not regarded as a relevant degradation process in the environment. Based on the experimental DT50 values of 99.6 and 109.3 days for [PYM-^{14}C]BYI 02960 and [FUR-^{14}C]BYI 02960, respectively, the DT50 values of BYI 02960 under

environmental conditions were calculated to be 358 and 405 solar summer days at Los Angeles, California, USA, 466 and 527 days at Athens, Greece and 638 and 722 solar summer days at Tokyo, Japan.

Table 7.1.3- 5: Synopsis of degradation of [^{14}C]BYI 02960 on loam soil [redacted]

	(% of AR)			
	PYR		FUR	
Material Balance	**Irradiated**	**Dark**	**Irradiated**	**Dark**
Mean ^{14}C-recovery during study	99.3 (±1.2)	99.4 (±0.9)	99.5 (±0.6)	99.5 (± 0.8)
Total extractable 14C during study	99.7-98.0	99.7-98.0	99.3-96.4	99.4-97.4
Max of bound 14C residues (NER)	0.9	0.9	1.1	1.1
Max. 14CO2	0.2	0.1	2.2	0.1
Experimental SFO DT50 of BYI 02960 [days]	99.6	> 1000	109.3	419.2
Environmental DT50 of BYI 02960 [days]		-		-
Los Angeles, California, USA	358		405	
Athens, Greece	466		527	
Tokyo, Japan	638		722	
Major transformation *)	-			
Minor transformation products	NER, CO_2			

*) 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

Route of Degradation of BYI 02960 in Soil - Summary

The route of Flupyradifurone (BYI 02960) degradation in soil was studied using different radiolabel positions,. [pyrindinyl-methyl-^{14}C = PYM], [pyridine-2,6-^{14}C = PYR], [furanone-4-^{14}C = FUR] and [ethyl-1-^{14}C = ETH]BYI 02960.. The data gathered in the aerobic soil metabolism studies demonstrated that BYI 02960 is degraded in soil

When using the PYM label in four different European aerobic soils no major metabolites were detected. All formed metabolites were regarded as transient, which was confirmed by the high mineralization rate to $^{14}CO_2$, i.e. up to 58.6% of AR from [PYM-^{14}C]BYI 02960. The portions of not extractable residues (NER) were comparatively low throughout the study (max 16.8% of AR) in case of [PYM-^{14}C]BYI 02960). As minor transformation products BYI 02960-chloro (max. 1.8%) and BYI 02960-des-difluoroethyl (max. 0.4%) were identified (for structures see Figure 7.1.2- 1).

In studies with the FUR label (four European and two US soils) similar results were obtained with the formation of NER (maximum 34.1% of AR) and extensive mineralization to $^{14}CO_2$ (up to 38.9% of AR). With the exception of $^{14}CO_2$ no major metabolites were formed in any of the soils. Degradation, amount of NER as well as of $^{14}CO_2$ formed in sterilized was significantly less than in non-sterile soils, indicating a biological component to the degradation/mineralization and formation of non-extractable residues from BYI 02960. Additionally, soil fractionation showed that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

When using [ETH-^{14}C] BYI 02960 in three aerobic soils one major metabolite, identified as difluoroacetic acid (max. 33.9% of AR), and one very minor metabolite was detected and quantified. Again, significant amounts of 14CO2 (up to 42.3% AR) were measured, indicating that mineralization of the test item and/or its metabolites occurred. The maximum amount of NER was relatively low at 17.9% of AR.

The biotransformation of [PYR-^{14}C]BYI 02960 was studied in one aerobic EU and two US soils. The mineralization to 14CO2 was significant (max. 57.4, 20.2 and 36.1% of AR) with the formation of minor metabolites in two soils. However, in one US soil, one major metabolite, which was identified as 6-chloronicotinic acid, was formed at maximum of 17.1%. NER formation was in the range of max. 11.3 to 25.5% of AR in the three soils.

Three studies investigating the degradation of [PYR-^{14}C], [FUR-^{14}C] and [ETH-^{14}C]BYI02960 in soils under aerobic, then flooded anaerobic conditions showed that it can be expected that the amounts of BYI 02960 and its major aerobic soil metabolites DFA and 6-CNA remain stable under flooded field conditions. Degradation would be expected to continue according to the proposed overall pathway of degradation of BYI 02960 (see Figure 7.1.2- 1) whenever the conditions in soil turn aerobic again.

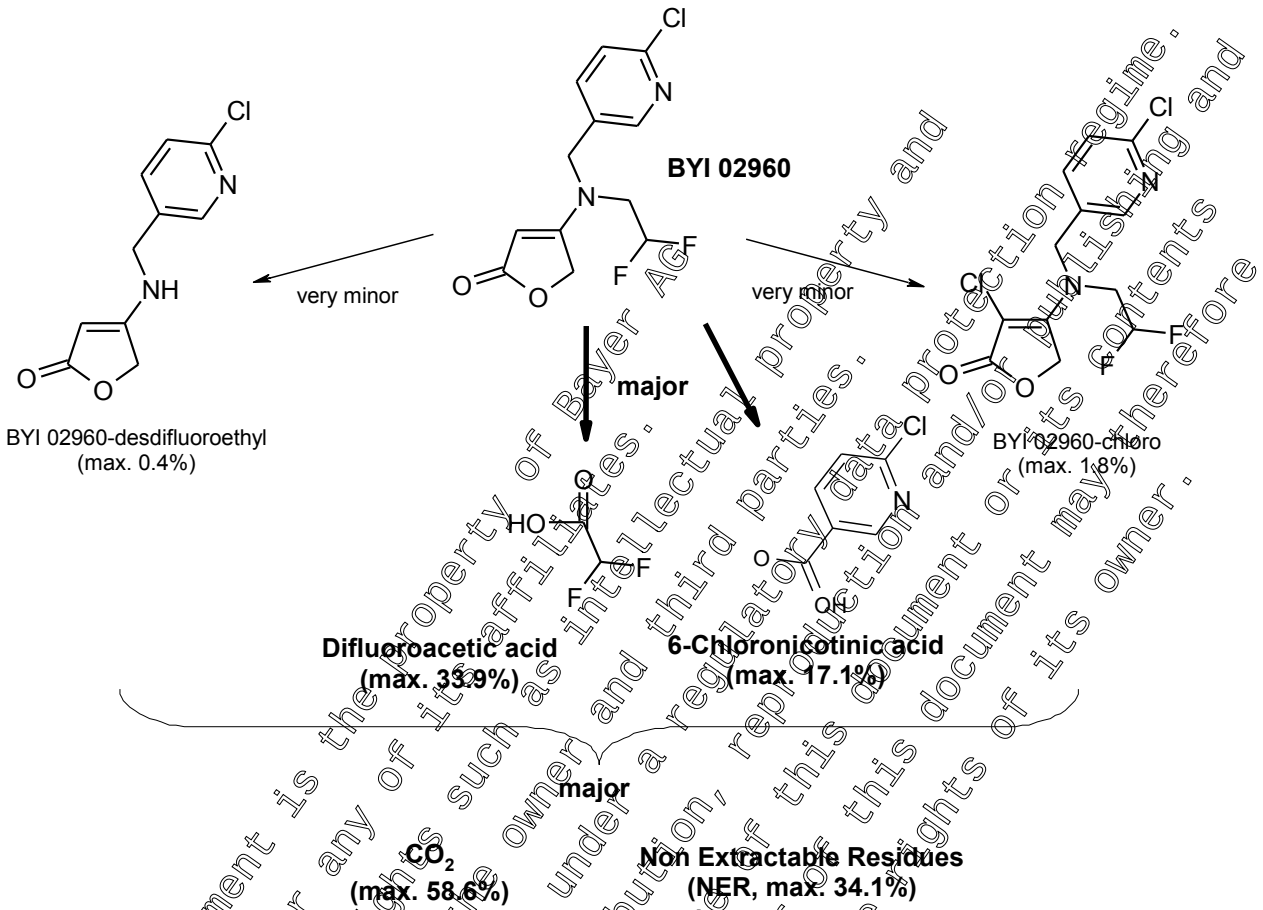
Phototransformation of [PYM-^{14}C]- and [FUR-^{14}C]BYI 02960 was studied on a loam soil. From the results it is concluded that direct phototransformation of BYI 02960 is not regarded as a relevant degradation process in the environment.

Considering the results from laboratory soil metabolism studies the major routes of degradation of BYI 02960, under aerobic conditions, are:

- cleavage of the difluoroethyl group producing difluoroacetic acid (DFA),
- cleavage of the molecule at the pyridinylmethylene bridge with subsequent oxidation to 6-CNA
- mineralization to CO_2 and formation of non-extractable residues.

Degradation under anaerobic and due to photolysis are not relevant for BYI 02960.

Figure 7.1.2- 1: Proposed degradation pathway of BYI02960 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 Flupyradifurone (BYI 02960) 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, a separate experimental degradation study was performed with 6-CNA, a major aerobic soil metabolite.

IIA 7.2.1 Aerobic Degradation of the Active Substance in Soils at 20 °C

Evaluation of the degradation kinetics of the aerobic soil degradation studies described under point IIA 7.2.1 has been performed to derived, to derive EU trigger endpoints and model input parameters.

Kinetic Evaluation of Laboratory Studies - Trigger Values

Report:	**KIIA 7.2.1/01, [redacted], 2011**
Title:	[Pyridinylmethyl-^{14}C]BYI 02960: Aerobic soil metabolism/degradation and time-dependent sorption in soils
Report No & Document No	MEF-07/334 M-414615-01-2
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil** **US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.** **OECD TG 106: Adsorption/Desorption, 2001 (only in parts)**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The route of degradation and experimental design has been described under KIIA 7.1.1/01.
The best fit kinetic for "trigger evaluation" was obtained by (geometric mean) of 68.8 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

Table 7.2.1- 1: Synopsis of "trigger" best-fit degradation kinetics calculated for BYI 02960

Soil (Soil type)	**Kinetic model**	**Parent BYI 02960** DT_{50} **[d]**	DT_{90} **[d]**	**Chi² error [%]**
AX (Sandy loam)	DFOP	63.4	443.3	1.1702
HF (Silt loam)	DFOP	52.4	209.3	0.5924
HN (Loam)	DFOP	120.0	489.7	1.2373
DD (Clay loam)	DFOP	56.4	265.1	1.6945
GEOmean	**DFOP**	**68.8**	**331.3**	

I. MATERIALS AND METHODS

A. Materials

Materials used in this study are comprehensively described within report KIIA 7.1.1/01.

B. Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time, in accordance with FOCUS kinetics, the following models were used, Simple first-order model (SFO),

First-order multi compartment model (FOMC, Gustafson-Holden) and Bi-exponential model (double first-order in parallel, DFOP):

The parameters of all three kinetic models were estimated by non-linear optimization to the measured data with the software KinGUI v.1.1. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

II. KINETICS OF TEST ITEM DEGRADATION

A summary of the DT_{50} and DT_{90} calculations for the test item considering the alternative kinetic models is given in Table 7.2.1- 2.

Table 7.2.1- 2: Summary of the Kinetic Evaluation for Trigger Values According to FOCUS

Soil	Kinetic model	Parent BYI 02960		
		DT_{50} (d)	DT_{90} (d)	Chi^2 value
[redacted]	SFO	70.3	233.6	5.0955
	FOMC	62.9	>1000.0	1.0484
	DFOP *	**63.4**	**443.3**	1.1702
HF	SFO	54.4	180.6	2.3139
	FOMC	50.8	270.2	0.8125
	DFOP *	**52.4**	**209.3**	0.5924
HN	SFO	112.6	373.9	3.7804
	FOMC	134.8	>1000.0	1.3412
	DFOP *	**120.0**	**489.7**	1.2373
DD	SFO	60.1	199.8	3.3408
	FOMC	55.2	398.9	1.5906
	DFOP *	**56.4**	**265.1**	1.6945
GEOmean	**DFOP ***	**68.8**	**331.3**	

*: best fit (values bold typed)
SFO: Single first-order
FOMC: First-order multi compartment
DFOP: Double first-order in parallel

The fit of the single first-order (SFO) model was less good than the fits of the biphasic models first-order multi compartment (FOMC) and double first-order in parallel (DFOP). The latter two models however were equivalent, concerning the visual assessment as well as the marginal difference in chi^2 error (< 3 %).
Where there is significant extrapolation the FOMC model is not suitable for prediction of DT_{90} values, since unrealistic DT_{90} values are estimated that are far outside the study duration. Therefore as the FMOC and DFOP models gave equivalent fits the DFOP model was selected for evaluation of the trigger end-points for all soils
Overall, the GEOmean of the DT_{50} and the DT_{90} values for degradation of BYI 02960 in the tested soils under aerobic conditions at 20 °C were 68.8 and 331.3 days, respectively.

III. CONCLUSIONS

The current laboratory study demonstrated that BYI 02960 is degraded in soil under aerobic conditions with GEOmean DT_{50} and the DT_{90} values in the tested soils under aerobic conditions at 20 °C of 68.8 and 331.3 days, respectively. An overall summary of the “best-fit” trigger values for all soils is given in Table 7.2.1- 13.

Report:	**KIIA 7.2.1/02, [redacted], [redacted], 2011**
Title:	[Furanone-4-^{14}C]BYI 02960: Aerobic Soil Metabolism/Degradation
Report No & Document No	MEF-10/804 M-411625-01-2
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil** **US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The route of degradation and experimental design has been described under point KIIA 7.1.1/02.
The best fit kinetic for "trigger evaluation" was obtained by double first-order kinetics a half-life (geometric mean) of 68.8 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

Table 7.2.1- 3: Synopsis of "trigger" half-lives calculated for BYI 02960

Soil (soil type)	Kinetic model	Parent BYI 02960 DT$_{50}$ [d]	DT$_{90}$ [d]	Chi^2 error [%]
AX (Sandy loam)	DFOP	62.2	390.6	1.55
HF (Silt loam)	DFOP	33.2	229.5	1.71
HN (Silt loam)	DFOP	98.3	462.5	2.03
DD (Silty clay)	DFOP	49.3	303.1	2.26
GEOmean		**56.2**	**334.8**	

I. MATERIALS AND METHODS

A. Materials

Materials used in this study are comprehensively described within report KIIA 7.1.1/02.

B. Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, DFOP and FMOC models were considered. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

II. KINETICS OF TEST ITEM DEGRADATION

The degradation of the parent compound during the study was evaluated by first-order non-linear regression analysis, a summary of the kinetic analyses for all models is shown in Table 7.2.1- 4. Where there is significant extrapolation the FOMC model is not suitable for prediction of DT$_{90}$ values, since unrealistic DT$_{90}$ values are estimated that are far outside the study duration. Therefore as the FMOC and DFOP models gave equivalent fits the DFOP model was selected for evaluation of the trigger end-points for all soils

Table 7.2.1- 4:: Summary of the Kinetic Evaluation (for Trigger Values According to FOCUS) of the Degradation of [FUR-^{14}C]BYI 02960 in Aerobic Soils at 20 °C and 55 % of WHCmax)

Soil	Kinetic model	Parent BYI 02960		
		DT50 (d)	DT90 (d)	Chi² value
[redacted]	SFO	70.6	234.5	6.06
	FOMC	59.5	> 1000	1.20
	DFOP *	**62.2**	**390.6**	1.55
HF	SFO	40.5	134.6	5.30
	FOMC	33.2	244.2	1.58
	DFOP *	**33.2**	**229.5**	1.71
HN	SFO	96.3	320.1	5.47
	FOMC	104.7	> 1000	1.97
	DFOP *	**98.3**	**462.5**	2.03
DD	SFO	55.1	183.0	3.87
	FOMC	49.3	341.0	2.11
	DFOP *	**49.3**	**303.1**	2.26
GEOmean	**DFOP ***	**56.2**	**334.8**	

*: best fit (values bold typed)
SFO: Single first-order
FOMC: First-order multi compartment
DFOP: Double first-order in parallel

III. CONCLUSIONS

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions with a geomean DT_{50} (trigger endpoint) of 56.2 days for the current study. . An overall summary of the "best-fit" trigger values for all soils is given Table 7.2.1- 13

Report:	**KIIA 7.2.1/03, [redacted], 2011**
Title:	[Furanone-4-^{14}C]BYI 02960: Aerobic Soil Metabolism in Two US Soils
Report No & Document No	MERVP037 M-405497-03-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002 US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
Devations	**None**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The route of degradation and experimental design has been described under point KIIA 7.1.1/03. The best fit kinetic for "trigger evaluation" was obtained by SFO in [redacted] and DFOP in [redacted] soil.

Table 7.2.1- 5: Synopsis of half-lives (trigger values) calculated for BYI 02960

Soil	Kinetic model	Parent BYI 02960		
		DT_{50} [d]	DT_{90} [d]	Chi² error [%]
[redacted]	SFO	228	757	1.3
[redacted]	DFOP	58.3	273	1.1

I. MATERIALS AND METHODS

A. Materials

Materials used in this study are comprehensively described within report KIIA 7.1.1/03.

B. Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, DFOP and FMOC models were considered. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

II. KINETICS OF TEST ITEM DEGRADATION

The degradation of the parent compound during the study was evaluated by first-order non-linear regression analysis, a summary of the kinetic analyses for all models is shown in Table 7.2.1- 6. In the [redacted] soil all models resulted in equivalent fits, the DFOP and FOMC were regarded as less reliable due to the extrapolation required, therefore the SFO was selected. In the [redacted] soil the DFOP and FOMC models resulted in better fits, therefore the DFOP was selected as FOMC is less reliable where extrapolation beyond the experimental period is required.

Table 7.2.1- 6: Summary of the Kinetic Evaluation (for Trigger Values According to FOCUS) of the Degradation of [FUR-^{14}C]BYI 02960 in Aerobic Soils at 20 °C and 55 % of WHCmax

Soil	Kinetic model	Parent BYI 02960		
		DT50 (d)	DT90 (d)	Chi^2 value
[redacted]	**SFO**	**228**	**757**	1.3
	DFOP	374	> 1000	1.2
	FOMC	> 1000	> 1000	1.1
[redacted]	SFO	65.7	218	3.4
	DFOP	**58.3**	**273**	1.1
	FOMC	57.2	455	4.4

*: best fit (values bold typed)
SFO: Single first-order
FOMC: First-order multi compartment
DFOP: Double first-order in parallel

III. CONCLUSIONS

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions with DT_{50} values of 58.3 and 228 days. An overall summary of the "best-fit" trigger values for all soils is given in Table 7.2.1- 13.

Report:	**KIIA 7.2.1/04, [redacted], [redacted], 2011**
Title:	[Ethyl-1-^{14}C]BYI 02960: Aerobic Soil Metabolism
Report No & Document No	MEF-10/858 M-414981-01-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The route of degradation and experimental design has been described under point KIIA 7.1.1/04.
The best fit kinetic for "trigger evaluation" was obtained by double first-order kinetics a half-life (geometric mean) of 41.6 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

Table 7.2.1- 7: Synopsis of half- lives calculated for BYI 02960

Soil (Soil type)	Kinetic model	Parent BYI 02960 DT_{50} [d]	Parent BYI 02960 DT_{90} [d]	Parent BYI 02960 Chi2 error [%]
DD (Clay loam)	DFOP	33.9	649.6	1.9
AX (Loamy sand)	DFOP	62.0	538.1	1.6
HF (Silt loam)	DFOP	34.1	329.8	2.3
GEOmean		**41.6**	**486.7**	

I. MATERIALS AND METHODS

A. Materials

Materials used in this study are comprehensively described within report KIIA 7.1.1/04.

B. Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, DFOP and FMOC models were considered. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

II. KINETICS OF TEST ITEM DEGRADATION

A summary of the DT_{50} and DT_{90} calculations for the test item is given in Table 7.2.1- 8.
In all cases the fit of the single first-order (SFO) model was less good than the fits obtained with the two biphasic models first order multi compartment (FOMC) and double first order in parallel (DFOP). Although the difference between the latter two models was quite low concerning the chi^2 errors, the visual assessments indicated a better fit of the last sampling points using the DFOP model. Therefore, the DFOP kinetic model was chosen as best fit for all soils

Table 7.2.1- 8 Summary of the kinetic evaluation (for trigger values according to FOCUS)

Soil	Kinetic model	Parent BYI 02960 DT50 (d)	Parent BYI 02960 DT90 (d)	Parent BYI 02960 Chi^2 value
DD	SFO	38.6	128.1	4.1
	FOMC	33.8	178.4	2.0
	DFOP	**33.9**	**649.6**	1.9
[redacted]	SFO	74.5	247.4	6.8
	FOMC	61.6	> 1000	1.7
	DFOP	**62.0**	**538.1**	1.6
HF	SFO	43.0	142.9	5.9
	FOMC	34.1	287.5	2.2
	DFOP	**34.1**	**329.8**	2.3
GEOmean	**Best fit**	**41.6**	**486.7**	

Bold: best fit according to chi2 error or visual assessment
SFO: Single first-order, FOMC: First-order multi compartment, DFOP: Double first-order in parallel

III. CONCLUSION

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions The GEOmean of the DT_{50} and the DT_{90} values for degradation of BYI 02960 was 41.6 and 486.7 days, respectively. . An overall summary of the "best-fit" trigger values for all soils is given in Table 7.2.1- 13

Report:	**KIIA 7.2.1/05, [redacted], [redacted], 2011**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Aerobic Soil Metabolism
Report No & Document No	MEF-10/880 M-411693-01-2
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008,**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The route of degradation and experimental design has been described under point KIIA 7.1.1/05.
The best fit kinetic for "trigger evaluation" was obtained by double first-order kinetics a half-life (geometric mean) of 33.0 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

Table 7.2.1- 9: Synopsis of half- lives calculated for BYI 02960

Soil	Kinetic model	Parent BYI 02960		
		DT_{50} (d)	**DT_{90} (d)**	**Chi^2 value**
HF	DFOP	33.0	221.3	2.0

I. MATERIALS AND METHODS

A. Materials

Materials used in this study are comprehensively described within report KIIA 7.1.1/05.

B. Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, DFOP and FMOC models were considered. The best fit model was chosen on the basis of the goodness of fit as judged by visual assessment and on the chi^2 scaled-error criterion.

II. KINETICS OF TEST ITEM DEGRADATION

A summary of the DT_{50} and DT_{90} calculation for the test item is given in Table 7.2.1- 10.
The fit of the single first-order (SFO) model was less good than the fits of the biphasic models first-order multi compartment (FOMC) and double first-order in parallel (DFOP). The letter two models however were equivalent, concerning the visual assessment as well as the marginal difference in chi^2 error. In all cases the chi^2 values of the fits of both, FOMC and DFOP model, were very low (< 3 %). Where there is significant extrapolation the FOMC model is not suitable for prediction of DT_{90} values, since unrealistic DT_{90} values are estimated that are far outside the study duration. Therefore as the FMOC and DFOP models gave equivalent fits the DFOP model was selected for evaluation of the trigger end-point.

Table 7.2.1- 10: Summary of the kinetic evaluation (for trigger values according to FOCUS) of the degradation of [PYR-14C]BYI 02960 in aerobic soil

Soil	Kinetic model	Parent BYI 02960		
		DT_{50} (d)	DT_{90} (d)	Chi^2 value
HF	SFO	43.4	144.2	6.2
	FOMC	33.1	327.0	1.7
	DFOP*	**33.0**	**221.3**	2.0

*: best fit (values bold typed)

III. CONCLUSIONS

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions with a DT_{50} of 33 days. . An overall summary of the "best-fit" trigger values for all soils is given in Table 7.2.1- 13.

Report:	**KIIA 7.2.1/06, [redacted], 2011**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Aerobic Soil Metabolism in Two US Soils
Report No & Document No	MERVP038 M-413425-02-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002** **US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The route of degradation and experimental design has been described under point KIIA 7.1.1/06.
The best fit kinetic for "trigger evaluation" was obtained by DFOP or FMOC

Table 7.2.1- 11 Summary of the kinetic evaluation (for trigger values according to FOCUS)

Soil	Kinetic model	Parent BYI 02960		
		DT50 (d)	DT90 (d)	Chi2 value
[redacted]	DFOP	242	898	0.7
[redacted]	FOMC	56.3	324	1.8

FOMC: First-order multi compartment

I. MATERIALS AND METHODS

A. Materials

Materials used in this study are comprehensively described within report KIIA 7.1.1/06.

B. Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of BYI 02960 in soil over time SFO, FOMC and DFOP models were considered. 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, the coefficient of determination (r^2) was calculated and reported by the kinetics modeling tool.

II. KINETICS OF TEST ITEM DEGRADATION

A summary of the DT_{50} and DT_{90} calculation for the test item is given in Table 7.2.1- 12.
The fit of the single first-order (SFO) model was less good than the fits of the biphasic models first-order multi compartment (FOMC) and double first-order in parallel (DFOP). The letter two models however were equivalent, concerning the visual assessment as well as the marginal difference in chi^2 error. In all cases the chi^2 values of the fits of SFO, FOMC and DFOP model were very low (< 3 %).
Due to the extent of extrapolation required and the lower reliability of the derived parameters the SFO fit was regarded as more robust.

Table 7.2.1- 12 Summary of the kinetic evaluation (for trigger values according to FOCUS)

Soil	Kinetic model	Parent BYI 02960		
		DT50 (d)	DT90 (d)	Chi^2 value
[redacted]	SFO	211	400	1.6
	FOMC	429	>1000	0.6
	DFOP	**242**	**898**	0.7
[redacted]	SFO	62.2	207	3.0
	DFOP	**56.3**	**> 1000**	1.7
	FOMC	56.3	324	1.8

Bold: best fit according to chi2 error or visual assessment
SFO: Single first-order, FOMC: First-order multi compartment, DFOP: Double first-order in parallel

III. CONCLUSIONS

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions with "best fit" DT_{50} values of 242 and 56.3

Summary of Trigger DT_{50} and DT_{90} values in aerobic soil studies

A summary of the DT_{50} and DT_{90} "best-fit" trigger values calculated as described above is given in Table 7.2.1- 13.

Table 7.2.1- 13: Overall summary of derived DT_{50} and DT_{90} values for degradation of BYI 02960 in aerobic soil (trigger evaluation according to FOCUS kinetics)

Soil	Study Reference	Kinetic model (best-fit)	Parent BYI 02960	
			DT50 (d)	DT90 (d)
AX (Sandy loam)	KIIA 7.2.1/01	DFOP	63.4	443.3
AX (Sandy loam)	KIIA 7.2.1/02	DFOP	62.2	390.6
AX (Loamy sand)	KIIA 7.2.1/04	DFOP	62.0	538.1
HF (Silt loam)	KIIA 7.2.1/01	DFOP	52.4	209.2
HF (Silt loam)	KIIA 7.2.1/02	DFOP	33.2	229.5
HF (Silt loam)	KIIA 7.2.1/04	DFOP	34.1	329.8
HF (Silt loam)	KIIA 7.2.1/05	DFOP	33.0	221.3
HN (Loam)	KIIA 7.2.1/01	DFOP	120.0	489.7
HN (Silt loam)	KIIA 7.2.1/02	DFOP	98.3	462.5
DD (Clay loam)	KIIA 7.2.1/01	DFOP	56.4	265.1
DD (Silty clay)	KIIA 7.2.1/02	DFOP	40.3	303.1
DD (Clay loam)	KIIA 7.2.1/04	DFOP	33.9	649.6
[redacted] (Silt loam)	KIIA 7.2.1/03	SFO	228	757
[redacted] (Silt loam)	KIIA 7.2.1/06	DFOP	242	898
[redacted] (Sandy loam)	KIIA 7.2.1/03	DFOP	58.3	273
[redacted] (Sandy loam)	KIIA 7.2.1/06	FOMC	553	> 1000
Overall Geomean			**73 days**	

Kinetic Evaluation of Laboratory Studies – Modelling Input

In addition to the evaluation of trigger values by considering "best-fit" as described above parameters suitable for use in environmental modeling have been separately evaluated in accordance with the procedures of FOCUS.

Report:	**KIIA 7.2.1/07, [redacted]; 2012**
Title:	Kinetic evaluation of the aerobic metabolism of BYI 02960 in four soils for the determination of modelling endpoints
Report No & Document No:	MEF-11/619 M-423020-01-1
Guidelines:	**US EPA OPPTS 835.SUPP** **FOCUS Kinetics 2006**
GLP:	**No**

SUMMARY

The present report is a supplement to [redacted] 2011, M-414615-01-1 summarized in KIIA 7.1.1/01. It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics. The trigger endpoints of BYI 02960 are summarized in Table 7.2.1- 13.

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Methods

The measured data were taken into account as reported (individual replicates). All experimental data sets and all data points were weighted equally (weighting factor 1), which corresponds to an absolute error model. The initial concentration was fitted.

The goodness of the fits was assessed visually and by the Chi^2 error as described in FOCUS (2006). A single-sided t-test was used to identify the probability that the degradation rate is not significant, that is equal or smaller than zero. A probability t-prob of 0.05 is considered as sufficiently small to obtain significant degradation parameters, which are suitable for modelling purposes (FOCUS 2006).
As outlined in the underlying main report, parameter optimization was carried out with the MATLAB evaluation tool 'KinGUI' (MATLAB 2005 and Bramley 2007) considering alternative kinetic models.

Conclusion
The degradation parameters for modelling input are summarized in Table 7.2.1- 14.

Table 7.2.1- 14: Modelling endpoints of BYI 02960

Soil	Kinetic Model	BYI 02960			
		DT_{50} (d)	χ^2 err (%)	Visual Acceptability	t-prob
[redacted]	DFOP	169.1*	1.1702	Yes	k_1: <0.001 k_2: <0.001
HF	SFO	54.4	2.3139	Yes	<0.001
HN	DFOP	157.5*	1.2373	Yes	k_1: <0.001 k_2: <0.001
DD	SFO	60.1	3.3408	Yes	<0.001

* slow compartment of DFOP model

Report:	**KIIA 7.2.1/08, [redacted], 2012**
Title:	Kinetic evaluation of the aerobic metabolism of BYI 02960 in four soils for the determination of modelling endpoints
Report No & Document No:	MEF-11/620 M-423347-01-1
Guidelines:	**US EPA OPPTS 835.SUPP**
GLP:	**No**

EXECTUIVE SUMMARY

The present report is a supplement to [redacted] 2011, M-411625-01-1 summarized in **KIIA 7.1.1/02**. It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006).. The trigger endpoints of BYI 02960 have been summarized in Table 7.2.1-13.

METHODS

The measured data were taken into account as reported (individual replicates). All experimental data sets and all data points were weighted equally (weighting factor 1), which corresponds to an absolute error model. The initial amount of the parent was fitted
The goodness of the fits was assessed visually and by the Chi^2 error as described in FOCUS (2006). A single-sided t-test was used to identify the probability that the degradation rate is not significant, that is equal or smaller than zero. A probability t-prob of 0.05 is considered as sufficiently small to obtain significant degradation parameters, which are suitable for modelling purposes (FOCUS 2006).

As outlined in the underlying main report, parameter optimization was carried out with the MATLAB evaluation tool 'KinGUI' (MATLAB 2005 and Bramley 2007) considering alternative kinetic models..

CONCLUSION

The degradation parameters for modelling input are summarized in Table 7.2.1- 15.

Table 7.2.1- 15: Modelling endpoints of BYI 02960

Soil	Kinetic Model	BYI 02960			
		DT50 (d)	χ^2 err (%)	Visual Acceptability	t-prob
[redacted]	DFOP	141.5*	1.5528	Yes	k_1: <0.001 k_2: <0.001
HF	SFO	40.5	5.2961	Yes	<0.001
HN	DFOP	157.5*	2.0266	Yes	k_1: <0.001 k_2: <0.001
DD	SFO	55.1	3.8698	Yes	<0.001

*slow compartment of DFOP model

Report:	**KIIA 7.2.1/09, [redacted]., [redacted]; 2012**
Title:	Kinetic evaluation of the degradation rate of [Ethyl-1-^{14}C]BYI 02960 and its metabolite difluoroacetic acid (BYI 02960-DFA) for the determination of trigger and modelling endpoints
Report No & Document No:	MEF-11/855 M-422874-01-1
Guidelines:	US EPA OPPTS 835.SUPP
GLP:	No

EXECUTIVE SUMMARY

The present report is a supplement to [redacted] 7.1.1/05 and [redacted] 2011, M-414981-01-1, summarized in KIIA 7.1.1/04. It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 and the major metabolite DFA in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006).. The trigger endpoints of BYI 02960 have been summarized Table 7.2.1- 13 , the degradation kinetic for the metabolite is summarized in point KIIA 7.2.2

The present kinetics evaluation followed the recommendations of the FOCUS working group on degradation kinetics for modelling endpoints.

METHODS

The measured data were taken into account as reported (individual replicates). All experimental data sets and all data points were weighted equally (weighting factor 1).

For all residues used in the kinetic evaluation, the following procedure was applied. Any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 0.5% of applied radioactivity (AR). The remaining values below LOD were excluded from the analysis. The values of the parent compound BYI 02960 at zero days after application (DAT 0) were set to the recovery rate. The reason for this modification is that exactly at the time of application only the parent compound can occur. If metabolites occur at DAT 0, those values are set to zero for the same reason.

If recovery rates are >110% or <90%, the values from those sampling points are excluded from the analysis as well.
The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive environmental fate modelling.
All data were equally weighted which corresponds to an absolute error model.
Four kinetic models are considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the double-first-order in parallel (DFOP) and the hockey-stick model (HS).

Conclusion
The degradation of BYI 02960 was best described with bi-phasic kinetics for trigger endpoints. The DFOP model was selected for [redacted] II and [redacted] and FOMC was used for [redacted] am [redacted] 4a. For modelling endpoints the SFO model was considered acceptable for [redacted] II and [redacted] am [redacted] 4a and the DFOP model was chosen for [redacted].

Table 7.2.3- 1: DT_{50} of BYI 02960 for modelling

Compound	Soil	Kinetic model	DT_{50} [d]	Formation fraction pa→DFA
BYI 02960	[redacted] II	SFO	38.6	n.a.)[a]
	[redacted]	DFOP	210.2)[b]	n.a.)[a]
	[redacted] am [redacted] 4a	SFO	43.0	n.a.)[a]
Geometric mean			**70.4**	

)[a] not applicable
)[b] pseudo SFO: DT_{50} calculated from slower k-rate from DFOP model

Report:	**KIIA 7.2.1/10, [redacted], [redacted], 2012**
Title:	Kinetic evaluation of the degradation rate of [Pyridine-2,6-^{14}C]BYI 02960 and its metabolite 6-chloronicotinic acid (6-CNA) for the determination of trigger and modelling endpoints
Report No & Document No:	MEF-11/838 M-422853-01-1
Guidelines:	**FOCUS kinetics 2006**
GLP:	**No**

EXECUTIVE SUMMARY
The present report is a supplement to [redacted] 2011, M-411693-01-1, summarized in KIIA 7.1.1/05. It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 and 6-CNA in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006).. The trigger endpoints of BYI 02960 have been summarized in Table 7.2.1- 13, the degradation kinetics for the metabolite is summarized in point KIIA 7.2.2

I. MATERIALS AND METHODS

A. Materials

For all residues used in the kinetic evaluation, any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 0.5% of applied radioactivity (AR) which corresponds to 0.005 mg/kg. The values of the parent compound BYI 02960 at zero days after application (DAT = 0) were set to the recovery rate. The reason for this modification is that exactly at the time of application only the parent compound can occur. If metabolites occur at DAT 0, those values are set to zero for the same reason. If recovery rates are >110% or <90%, the values from those sampling points are excluded from the analysis.
For the kinetic modelling analysis the mathematical software tool MatLab with a user shell 'KinGUI' were employed (MatLab 2005, Bramley, 2007). The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive degradation parameters for environmental fate modelling.
Four kinetic models are considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden); the double-first-order in parallel (DFOP) and the hockey-stick model (HS).

III. CONCLUSION

The degradation of BYI 02960 was best described with bi-phasic kinetics for trigger endpoints. The DFOP model was [redacted] am [redacted] 4a.

Table 7.2.3- 2: DT_{50} of BYI 02960 for modelling

Compound	Soil	Kinetic model	DT_{50} [d]
BYI 02960	[redacted] am [redacted] 4a	DFOP	90.0[a]

[a] pseudo SFO: DT_{50} calculated from slower k-rate from DFOP model
[b] not applicable

Report:	**KIIA 7.2.1/03, [redacted], 2011**
Title:	[Furanone-4-^{14}C]BYI 02960: Aerobic Soil Metabolism in Two US Soils
Report No & Document No	MERVP037 M-405497-03-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002** **US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The route of degradation and experimental design has been described under point KIIA 7.1.1/03.
The degradation kinetics was described under report KIIA 7.2.1/03 for the trigger values.
The selection of values for modeling input concluded that for both soils SFO was the appropriate model.

Table 7.2.3- 16: Synopsis of half-lives (modelling) calculated for BYI 02960

Soil	Kinetic model	Parent BYI 02960 DT_{50} [d]	Parent BYI 02960 Chi^2 error [%]
[redacted]	SFO	227.9	1.3
[redacted]	SFO	65.7	3.4

Report:	**KIIA 7.2.1/06, [redacted], 2011**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Aerobic Soil Metabolism in Two US Soils
Report No & Document No	MERVP038 M-413425-02-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002 US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The route of degradation and experimental design has been described under point KIIA 7.1.1/06.
The degradation kinetics was described under report KIIA 7.2.1/06 for the trigger values.
The selection of values for modeling input concluded that for both soils SFO was the appropriate model.

Table 7.2.1- 17 Summary of the kinetic evaluation for modeling input

Soil	Kinetic model	Parent BYI 02960	
		DT50 (d)	Chi2 value
[redacted]	SFO	215	1.6
[redacted]	SFO	62.2	3.0

Summary of Modelling Input DT_{50} values in aerobic soil studies

A summary of the DT_{50} values calculated for modeling input is given in Table 7.2.1- 18. Considering the moisture content of the soils during incubation the DT_{50} was corrected to pF2, for the studies performed on European soils no correction for soil moisture content was required, for the studies on US soils the correction factor as outlined below was employed. Details of the soil moisture correction are given in the PEC reports included in the Annex III.

Table 7.2.1- 18: Overall summary of derived DT_{50} values for degradation of BYI 02960 in aerobic soil - modeling input parameters

Soil	Study Reference	Kinetic model (best-fit)	Parent BYI 02960		
			DT50 (d) *	Moisture correction factor	DT_{50} at 20ºC and pF2
AX (Sandy loam)	KIIA 7.2.1/07	DFOP	169.1	1	169.1
AX (Sandy loam)	KIIA 7.2.1/08	DFOP	141.5	1	141.5
AX (Loamy sand)	KIIA 7.2.1/09	DFOP	210.2	1	210.2
HF (Silt loam)	KIIA 7.2.1/07	SFO	54.4	1	54.4
HF (Silt loam)	KIIA 7.2.1/08	SFO	40.5	1	40.5
HF (Silt loam)	KIIA 7.2.1/09	SFO	43.0	1	43.0
HF (Silt loam)	KIIA 7.2.1/10	SFO	90.0	1	90.0
HN (Loam)	KIIA 7.2.1/07	DFOP	157.5	1	157.5
HN (Silt loam)	KIIA 7.2.1/08	DFOP	157.5	1	157.5
DD (Clay loam)	KIIA 7.2.1/07	SFO	60.1	1	60.1
DD (Silty clay)	KIIA 7.2.1/08	SFO	55.1	1	55.1
DD (Clay loam)	KIIA 7.2.1/09	SFO	38.6	1	38.6
[redacted] (Silt loam)	KIIA 7.2.1/03	SFO	211	0.79	166.4
[redacted] (Silt loam)	KIIA 7.2.1/06	SFO	227.9	0.79	179.7
[redacted] (Sandy loam)	KIIA 7.2.1/03	SFO	62.0	0.90	55.5
[redacted] (Sandy loam)	KIIA 7.2.1/06	SFO	65.7	0.90	58.8
Overall Geomean					**94.8**

* for DFOP fits, DT_{50} from slow phase

IIA 7.2.2 Aerobic Degradation of the Active Substance in Soils at 10 °C

No study has been performed at 10ºC, degradation rates may be extrapolated from the laboratory studies performed at 20ºC applying the Arrhenius equation assuming a Q_{10} factor of 2.58.

IIA 7.2.3 Aerobic Degradation of Relevant Metabolites in Soils at 20 °C

The rate of degradation of the two major soil metabolite 6-CNA and DFA has been determined in studies with the parent and/or studies with the metabolite.

Metabolite 6-CNA

The rate of degradation of the major metabolite 6-CNA has been investigated in 3 soils. The metabolite is a common metabolite to the insecticide acetamiprid, this study has been evaluated in the context of review report for the active substance acetamiprid, SANCO/1392/2001 – Final, 16 June2004. Access to the study has been granted by the owners Nippon Soda (Nisso). Only a very brief summary of the study is presented here. Due to changes in the requirements for kinetic evaluation a new kinetic evaluation of the study was performed and is described in KIIA 7.2.3/02

Report:	**KIIA 7.2.3/01, [redacted] 1997**
Title:	NI-25: Rate of Degradation of the Acid Metabolite, [14C]-IC-O in Three Soils
Report No & Document No:	C-007660 M-196378-01-1
Guidelines:	Not specified
GLP:	No

Executive summary

The study was performed to examine the rate of degradation of [14C]-IC-O (equivalent to 6-CNA) at 20± 2°C. Three soils were selected, which were a sandy loam (RPAL reference 97/03), a silty clay loam (RPAL reference 97/04) and a clay loam (RPAL reference 97/05). Portions of soil (approximately 100 or 500 g oven dried equivalent) were placed in uniquely labelled soil flasks and treated with [14C]-IC-O solution at a rate equivalent to 130 g ha-1). The moisture content was adjusted to 45% of the maximum water holding capacity and maintained at that level throughout the study. The flasks were placed in a closed system maintained in the dark in which moist, carbon-dioxide-free air was continuously blown over the soils, then through ethylene glycol followed by potassium hydroxide. At intervals up to and including 119 days after treatment soil samples were extracted with solvent (methanol/ammonium acetate 2M (80:20 v/v)) or acetonitrile water (80:20 v/v) and the extracts containing sufficient radioactivity were examined by high performance liquid chromatography (HPLC). The unextracted radioactivity was assayed using a combustion technique. Selected extracts were further examined by liquid chromatography followed by mass spectrometry (LC-MS).

Recoveries of applied radioactivity were generally good throughout the study with all but three of the individual flasks at each time point falling within the range 90 -110%. The major metabolite detected was carbon dioxide which was trapped in the potassium hydroxide traps and accounted for approximately 84% of the applied dose in the sandy loam, about 92% in the UK silty clay loam and 84% in the clay loam at the end of the study.

The percentage of material in the solvent extract declined with time from over 90% at day 0 to less than 2% at day 119. This fall was initially concomitant with an increase in unextractable residues and with the decrease in radioactivity associated with the soil as the compound was mineralised to carbon dioxide.

Chromatographic analysis of the soil extracts showed the presence of IC-O and two very minor metabolites. Spectroscopic examination of the extracts confirmed the presence of parent IC-O. The two very minor compounds were transient metabolites and were both less than 3% maximum in all three soils.

Report:	**KIIA 7.2.3/02, [redacted] 2012**
Title:	Kinetic evaluation of the degradation rate of 6-chloronicotinic acid (6-CNA) for the determination of trigger and modelling endpoints
Report No & Document No:	MEF-11/837 M-422843-01-1
Guidelines:	**FOCUS kinetics 2006**
GLP:	**No**

EXECUTIVE SUMMARY

The aerobic degradation of 6-chloronicotinic acid (6-CNA) was kinetically evaluated based on a laboratory study with three soils ([redacted] et al. 1997, M-196378-01-1).

The evaluation followed the recommendations of the FOCUS working group on degradation kinetics and considered trigger and modelling endpoints (Table 7.2.3- 3).
Degradation of 6-CNA was best described by single first-order (SFO) kinetics in all cases.

Table 7.2.3- 3: Trigger and Modelling DT_{50} and DT_{90} of 6-CNA

Compound	Soil	Kinetic model	DT_{50} [d]	DT_{90} [d]
6-CNA	[redacted]'s Farm	SFO	2.9	9.7
	[redacted] Farm	SFO	2.2	7.4
	[redacted] Farm	SFO	5.3	17.5

I. MATERIALS AND METHODS

The biotransformation of [^{14}C]-6-CNA, labelled in the 2,6 positions of the pyridine ring was studied in three soils from the UK, sandy loam from [redacted] Farm, clay from [redacted] [redacted] Farm and loam from [redacted] Farm, for 120 days under laboratory aerobic conditions at 20±2°C in the darkness

Measured data of individual replicates were taken into account and all experimental data sets and all data points were weighted equally (weighting factor 1). For all residues used in the kinetic evaluation, any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 3.88% of applied radioactivity (AR) which corresponds to 0.005 mg/kg. The remaining values below LOD were excluded from the analysis. The values of 6-CNA at zero days after application (DAT = 0) were set to the recovery rate because exactly at the time of application only the parent compound can occur. Values from sampling points with recovery rates >110% or <90% were excluded from the analysis.

For the kinetic modelling analysis the mathematical software tool MatLab with a user shell “KinGUI” were employed (MatLab 2005, Bramley, 2007). The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive degradation parameters for comparison with triggers and for tier-1 PEC_{soil} calculation as well as for environmental fate modelling. Four kinetic models are considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the double-first-order in parallel (DFOP) and the hockey-stick model (HS). Testing of DFOP and HS models was not required.

II. KINETICS OF TEST ITEM DEGRADATION

The appropriate models for trigger and modelling endpoints were selected according to visual assessment and χ^2 error (see Table 7.2.3- 4). SFO provided the best fits for all soils.

Table 7.2.3- 4: χ^2 error, t-probability (significance of deg. rate) and visual acceptability of different kinetic models for 6-CNA; χ^2 error bold typed indicates the kinetic model chosen)

Soil	Criterion	SFO	FOMC
[redacted] Farm	χ^2 err [%]	**8.5482**	9.3384
	Visual acceptability	Yes	Yes
	t-prob	<0.001	n.a.)[a]
[redacted] Hall Farm	χ^2 err [%]	**6.9131**	7.7869
	Visual acceptability	Yes	Yes
	t-prob	<0.001	n.a.)[a]
[redacted] Farm	χ^2 err [%]	**8.5177**	9.1517
	Visual acceptability	Yes	Yes
	t-prob	<0.001	n.a.)[a]

)[a] not applicable

III. CONCLUSIONS

The current laboratory study demonstrated that 6-chloronicotinic acid is very well degradable under aerobic conditions in soils with a DT_{50} of 2.2 to 5.3 days.

Report:	**KIIA 7.2.3/03, [redacted], [redacted]; 2012**
Title:	Kinetic evaluation of the degradation rate of [Pyridine-2,6-^{14}C]BYI 02960 and its metabolite 6-chloronicotinic acid (6-CNA) for the determination of trigger and modelling endpoints
Report No & Document No:	MEF-11/838 M-422853-01-1
Guidelines:	**US EPA OPPTS 835.SUPP** **FOCUS Kinetics (2006)**
GLP:	**No**

EXECUTIVE SUMMARY

The aerobic degradation of [pyridine-2,6-^{14}C]BYI 02960 was kinetically evaluated based on a laboratory study with one soil ([redacted], 2011 M-411693-01-1, KIIA 7.1.1/05). During the study the metabolite 6-chloronicotinic acid (6-CNA) reached a maximum percentage of 2.5% of AR at DAT 7.

The present kinetics evaluation followed the recommendations of the FOCUS working group on degradation kinetics and considered trigger and modelling endpoints for the metabolite 6-CNA, the end-points for BYI 02960 were described under point 7.2.1 above.

Degradation of metabolite 6-CNA could be described with SFO kinetics. The pathway fit (FOMC parent / SFO metabolite) was considered acceptable for trigger endpoints while the pathway fit DFOP parent / SFO metabolite was used for modelling endpoints.

Table 7.2.3- 5: Trigger DT_{50} and DT_{90} of 6-CNA

Compound	Soil	Kinetic model	DT_{50} [d]	DT_{90} [d]
6-CNA	[redacted] am [redacted] 4a	SFO	3.1	10.4

Table 7.2.3- 6: DT_{50} of 6-CNA for modelling

Compound	Soil	Kinetic model	DT_{50} [d]	Formation fraction pa→6-CNA
6-CNA	[redacted] am [redacted] 4a	SFO	3.0	0.2660

I. MATERIALS AND METHODS

A. Materials

The biotransformation of [pyridine-2,6-^{14}C]BYI 02960 was studied in a one European soil (silt loam, [redacted] am [redacted] 4a) for 118 days under laboratory aerobic conditions at 20±1°C in the dark ([redacted], 2011, M-411693-01-1, see KIIA 7.1.1/05).

The test item in the extracts declined from 96.3% of AR at DAT 0 to 22.7% at the end of the study. Only minor transformation products (all mean values were ≤ 2.5% of AR) were detected in the extracts, the maximum amount was detected on DAT 7 with 2.5% of AR and was identified as 6-CNA by co-chromatography. Towards the end of the study, the amounts decreased below the detection limit. Measured data of individual replicates were taken into account and all experimental data sets and all data points were weighted equally (weighting factor 1).

For all residues used in the kinetic evaluation, any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 0.5% of applied radioactivity (AR) which corresponds to 0.005 mg/kg. The values of the parent compound BYI 02960 at zero days after application (DAT = 0) were set to the recovery rate. The reason for this modification is that exactly at the time of application only the parent compound can occur. If metabolites occur at DAT 0, those values are set to zero for the same reason. If recovery rates are >110% or <90%, the values from those sampling points are excluded from the analysis.

B. Determination of Degradation Kinetics

For the kinetic modelling analysis the mathematical software tool MatLab with a user shell “KinGUI” were employed (MatLab 2005, Bramley, 2007). The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive degradation parameters for comparison with triggers and for tier-1 PEC_{soil} calculation as well as for environmental fate modelling. Four kinetic models are considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the double-first-order in parallel (DFOP) and the hockey-stick model (HS).

II. KINETICS OF TEST ITEM DEGRADATION

The appropriate models for trigger and modelling endpoints were selected according to visual assessment and χ^2 error and are presented in in Table 7.2.3- 7 for the metabolite 6-CNA.

Table 7.2.3- 7: χ^2 error, t-probability (significance of deg. rate) and visual acceptability of different kinetic models for 6-CNA in soil [redacted] am [redacted]

Compound	Criterion	FOMC parent/ SFO metabolite	DFOP parent/ SFO metabolite
6-CNA	χ^2 err [%]	16.9529	15.3680
	Visual acceptability	Yes	Yes
	t-prob	0.0937	<0.001
	Formation fraction 6-CNA	0.2534	0.2660
	Endpoint	Trigger	Modelling

III. CONCLUSIONS

The current laboratory study demonstrated that 6-chloronicotinic acid is very well degradable (DT50, 3 days) under aerobic conditions in soils.

Report:	**KIIA 7.2.3/04, [redacted], 2011**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Aerobic Soil Metabolism in Two US Soils
Report No & Document No	MERVP038 M-413425-02-1
Guidelines:	**OECD TG 307, Aerobic and Anaerobic Transformation in Soil, 2002 US EPA, OPPTS 835.4100, Aerobic Soil Metabolism, October 2008.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The route of degradation and experimental design has been described under point KIIA 7.1.1/06.
A kinetic evaluation of the degradation and formation fraction of 6-CNA was performed

Table 7.2.3- 8: Summary of the kinetic evaluation (trigger and modeling values according to FOCUS)

Soil		6-CNA		
		DT50 (d)	DT90 (d)	Formation fractions
[redacted]	Trigger (DFOP-SFO)	36.6	121	0.5272
	Modelling (SFO-SFO)	24.8	82.4	0.6936

FOMC: First-order multi compartment

I. MATERIALS AND METHODS

A. Materials

Materials used in this study are comprehensively described within report KIIA 7.1.1/06.

B. Determination of Degradation Kinetics

In order to determine the best-fit kinetic model for the degradation of 6-CNA the models SFO parent and SFO for 6-CNA plus DFOP for parent and SFO for 6-CNA were used.. 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, the coefficient of determination (r^2) was calculated and reported by the kinetics modeling tool.

II. KINETICS OF TEST ITEM DEGRADATION

For the observed degradation of BYI 02960 and the formation and decline of 6-CNA in the [redacted] sandy loam soil, SFO-SFO and DFOP-SFO kinetic models were used. Both approaches gave an acceptable fit to the measured data. However, the visual examination of the observed data and models, as well as the $\chi 2$ scaled error statistic and the coefficient of determination (r2), showed that DFOP-SFO is the best-fit model.

The parameter values from the best-fit DFOP-SFO model should be used with care for extrapolation purposes (e.g. for environmental modeling), since the DFOP parameters are highly inter-correlated (r^2 > 0.97 between k1, k2 and g) and the parameter estimates have large standard deviations (Figure A9.12). The parameter values from the SFO –SFO model are more robust for extrapolation purposes, despite the slightly worse fit of the model to the measured data.

Table 7.2.3- 9 Summary of the kinetic evaluation formation and decline of 6-CNA

Soil	Kinetic model		6-CNA				
	Parent	6-CNA	DT50 (d)	DT90 (d)	Chi² value	Visual assessment	Formation fraction
[redacted]	SFO	SFO	24.8#	82.4	15.1	+	0.6936
	DFOP	SFO	36.6*	121	13.8	+	0.5272

modeling value

* best fit trigger value.

III. CONCLUSIONS

The current laboratory study demonstrated that 6-CNA is degraded in soil with a best fit DT_{50} of 36.6 days and a modeling DT_{50} (non-normalised) of 36.6 days.

Metabolite DFA

Report:	KIIA 7.2.3/05, [redacted], [redacted]; 2012
Title:	Kinetic evaluation of the degradation rate of [Ethyl-1-^{14}C]BYI 02960 and its metabolite difluoroacetic acid (BYI 02960-DFA) for the determination of trigger and modelling endpoints
Report No & Document No:	MEF-11/855 M-422874-01-1
Guidelines:	**US EPA OPPTS 835.SUPP**
GLP:	**No**

EXECUTIVE SUMMARY

The present report is a supplement to [redacted] 2011, M-414981-01-1, summarized in KIIA 7.1.1/04. It compiles additional raw data of the kinetic evaluation of the degradation of BYI 02960 and the major metabolite DFA in soil to determine modelling endpoints. The evaluation followed the recommendations of the FOCUS working group on degradation kinetics (FOCUS, 2006).. The degradation parameters for the metabolite are summarized below.
The present kinetics evaluation followed the recommendations of the FOCUS working group on degradation kinetics for modelling endpoints.

Table 7.2.3- 10: Trigger DT_{50} and DT_{90} of -DFA

Compound	Soil	Kinetic model	DT_{50} [d]	DT_{90} [d]
DFA	[redacted] II	SFO	44.9	149.0
	[redacted] [redacted]	SFO	73.6	244.5
	[redacted] am [redacted] 4a	SFO	67.4	223.9

n.a. = not applicable

Table 7.2.3- 11: DT_{50} of DFA for modelling

Compound	Soil	Kinetic model	DT_{50} [d]	Formation fraction pa→DFA
DFA	[redacted] II	SFO	32.0	0.9087
	[redacted] [redacted]	SFO	73.6	0.5896
	[redacted] am [redacted] 4a	SFO	37.8	1.0000
Geometric mean			44.7	
Arithmetic mean				0.8328

)[a] not applicable
)[b] pseudo SFO: DT_{50} calculated from slower k-rate from DFOP model

I. MATERIALS AND METHODS

A. Materials

The biotransformation of [ethyl-1-^{14}C]BYI 02960 was studied in three European soils: [redacted] II (DD, clay loam), [redacted] [redacted] (AX, loamy sand) and [redacted] am [redacted] 4a (HF, silt loam). One major transformation product was detected in the extracts of all three soils and was identified as difluoroacetic acid (DFA) via HPLC-MS. The metabolite reached maximum values of 30.2, 22.0 and 33.8% of AR on DAT 45 or DAT 48 in soils DD, AX and HF, respectively. Towards the end of the study, the levels of DFA declined to 17.0, 16.3 and 23.8% of AR in soils DD, AX and HF, respectively.
Measured data of individual replicates were taken into account and all experimental data sets and all data points were weighted equally (weighting factor 1).

For all residues used in the kinetic evaluation, the following procedure was applied. Any value below LOD directly before or after a value ≥ LOD was set to 0.5 LOD. The LOD was chosen as 0.5% of applied radioactivity (AR). The remaining values below LOD were excluded from the analysis. The values of the parent compound BYI 02960 at zero days after application (DAT 0) were set to the recovery rate. The reason for this modification is that exactly at the time of application only the parent compound can occur. If metabolites occur at DAT 0, those values are set to zero for the same reason. If recovery rates are >110% or <90%, the values from those sampling points were excluded from the analysis.

B. Determination of Degradation Kinetics

For the kinetic modelling analysis the mathematical software tool MatLab with a user shell "KinGUI" were employed (MatLab 2005, Bramley, 2007). The kinetic evaluation was performed following the recommendations of FOCUS 2006 to derive degradation parameters for comparison with triggers and for tier-1 PEC_{soil} calculation as well as for environmental fate modelling. Four kinetic models are

considered in the testing procedure as recommended by FOCUS 2006: the single first-order (SFO), first-order multiple-compartment (FOMC, Gustafson-Holden), the double-first-order in parallel (DFOP) and the hockey-stick model (HS).

KINETICS OF TEST ITEM DEGRADATION

The appropriate models for trigger and modelling endpoints were selected according to visual assessment and χ^2 error and are presented in the following tables.

Table 7.2.3- 12: χ^2 error, t-probability (significance of degradation rate) and visual acceptability of different kinetic models for DFA in soil

Soil	Criterion	BYI 02960-DFA	
		DFOP parent/ SFO metabolite	SFO parent/ SFO metabolite
[redacted] II	χ2 err [%]	5.412	5.169
	Visual acceptability	Yes	Yes
	t-prob	<0.001	<0.001
	Formation fraction DFA	0.7477	0.9087
	Endpoint	Trigger	Modelling
[redacted]	χ2 err [%]	8.359	
	Visual acceptability	Yes	
	t-prob	<0.001	
	Formation fraction DFA	0.5896	
	Endpoint	Trigger / Modelling	
[redacted] am	χ2 err [%]	7.394	4.033
	Visual acceptability	Yes	Yes
	t-prob	<0.001	<0.001
	Formation fraction DFA	0.7359	1.0000
	Endpoint	Trigger	Modelling

III. CONCLUSIONS

The current laboratory study demonstrated that difluoroacetic acid is degradable under aerobic conditions in soils.

IIA 7.2.4 Anaerobic Degradation of the Active Substance in Soil

The three "pathway of degradation" studies summarized under point

IIA 7.1.2 showed that the amounts of BYI 02960 remain stable under flooded, thus then anaerobic conditions in soil. Degradation would be expected to continue according to the proposed kinetics of degradation of BYI 02960 (see point IIA 7.2.1) whenever the conditions in soil turn aerobic again.

IIA 7.2.5 Anaerobic Degradation of Relevant Metabolites in Soil

The three "pathway of degradation" studies summarized under point

IIA 7.1.2 showed that the amounts of relevant metabolites of BYI 02960 remain stable under flooded, thus then anaerobic conditions in soil. Degradation would be expected to continue according to the proposed kinetics of metabolite degradation (see point IIA 7.2.3) whenever the conditions in soil turn aerobic again.

Rate of Degradation of BYI 02960 Residues in Soil - Summary

The biotransformation of BYI 02960 was studied in several EU and US soils under standardized aerobic and anaerobic laboratory conditions, as well as in a terrestrial field dissipation study performed on six different sites in Europe. BYI 02960 was found to be moderately degradable in aerobic soil under laboratory as well as under field conditions. The clear bi-phasic degradation kinetics indicates that the compound is less available for biotransformation with time, probably due to a time-depended sorption behavior in soil. The BYI 02960 residues remain stable under anaerobic conditions. The following table summarises the findings in the aerobic laboratory studies (for kinetics results of the terrestrial field dissipation studies see Table 7.3.1- 3). The kinetics data for the metabolite 6-CNA indicate that it is very rapidly degraded in soil with a mean DT_{50} of < 1 week. The metabolite DFA indicated a slightly longer DT_{50}, i.e. it was calculated to be in the range of approx. 2 months.

Soil	Dossier position of kinetic evaluation	DT50 (d) Trigger values	DT50 (d) Modelling input	Formation Fraction
Parent BYI 02960				
AX (Sandy loam)	KIIA 7.2.1/01 KIIA 7.2.1/07	63.4	169.1	-
AX (Sandy loam)	KIIA 7.2.1/02 KIIA 7.2.1/08	62.2	141.5	-
AX (Loamy sand)	KIIA 7.2.1/04 KIIA 7.2.1/09	62.0	210.2	-
HF (Silt loam)	KIIA 7.2.1/01 KIIA 7.2.1/07	52.4	54.4	-
HF (Silt loam)	KIIA 7.2.1/02 KIIA 7.2.1/08	33.2	40.5	-
HF (Silt loam)	KIIA 7.2.1/04 KIIA 7.2.1/09	34.1	43.0	-
HF (Silt loam)	KIIA 7.2.1/05 KIIA 7.2.1/10	33.0	90.0	-
HN (Loam)	KIIA 7.2.1/01 KIIA 7.2.1/07	120.0	157.5	-
HN (Silt loam)	KIIA 7.2.1/02 KIIA 7.2.1/08	98.3	157.5	-
DD (Clay loam)	KIIA 7.2.1/01 KIIA 7.2.1/07	56.4	60.1	-
DD (Silty clay)	KIIA 7.2.1/02 KIIA 7.2.1/08	49.3	55.1	-
DD (Clay loam)	KIIA 7.2.1/04 KIIA 7.2.1/09	33.9	38.6	-
[redacted] (Silt loam)	KIIA 7.2.1/03	228	211[#]	-
[redacted] (Silt loam)	KIIA 7.2.1/06	242	227.9[#]	-
[redacted] (Sandy loam)	KIIA 7.2.1/03	58.3	62.0[#]	-
[redacted] (Sandy loam)	KIIA 7.2.1/06	56.3	65.7[#]	-
Overall Geomean		**73**	**94.8**	**-**
6-CNA				
[redacted] Farm (Sandy loam)	KIIA 7.2.3/02	2.9	2.9	-
[redacted] Farm (Clay)	KIIA 7.2.3/02	2.2	2.2	-
[redacted] Farm (Loam)	KIIA 7.2.3/02	5.3	5.3	-
HF (Silt loam)	KIIA 7.2.3/03	3.1	3.0	0.266
[redacted]	KIIA 7.2.3/04	25	22.4[#]	0.69
Geomean		**4.8**	**4.7**	
Arith. mean				**0.48**
DFA				
DD (Clay loam)	KIIA 7.2.3/05	44.9	32.0	0.9087
AX (Loamy sand)	KIIA 7.2.3/05	73.6	73.6	0.5896
HF (Silt loam)	KIIA 7.2.3/05	67.4	37.8	1.000
Geomean		**60.6**	**44.7**	
Arith. mean				**0.8328**

[#] normalised to pF2

IIA 7.3 Field Studies

IIA 7.3.1 Soil Dissipation Testing in a Range of Representative soils

Report:	**KIIA 7.3.1/01, [redacted], 2011**
Title:	Determination of the Residues of BYI 02960 in/on Soil after Spraying of BYI 02960 SL 200 in the Field in Germany, Italy, Spain and the United Kingdom
Report No & Document No:	09/2702 M-414245-01-1
Guidelines:	**BBA guideline part IV, 4-1 (1986) and SETAC (1995)**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

Soil dissipation of BYI 02960 under European field conditions was investigated after application of BYI 02960 SL 200 on bare soil plots at sites in [redacted] [redacted] and [redacted] (all Germany), [redacted] (United Kingdom), , [redacted] (Italy), and [redacted] (Spain). An application was also made in [redacted] (Northern France), however the trial was aborted due to heavy rain shortly after application. The sites are located in the ecoregions Northern and Southern Europe.

BYI 02960 SL 200 was sprayed once pre-emergence onto the soil at 1.25 L/ha, corresponding to 250 g BYI 02960/ha in 180 to 320 sqm plots. The measured initial zero-time concentrations corresponded from 91% to 107% of the intended dose rate (250 g/ha). The control plots were at least 5 m away from the treated plots.

Soil samples were taken (nominally) at 0 to 540 days post-application to a maximum depth of 100 cm and analyzed for BYI 02960 and its soil metabolite DFA (difluoroacetic acid).

Soil samples of 20 g were extracted in a microwave extractor with 50 mL of acetonitrile/water (4/1, v/v). Possible matrix effects were eliminated by using an internal standard solution of isotopically labeled reference items which was added to the extracts of samples. Then a subsample was filtrated to remove fine particles of the soil. Identification and quantitation of the test items was done by high performance liquid chromatography using MS/MS detection in the Multiple Reaction Monitoring mode. The method was validated using three different soils. The limit of quantitation (LOQ) was 5.0 µg/kg, and the limit of detection (LOD) was 1.5 µg/kg for both analytes.

At [redacted] (Germany), the amount of BYI 02960 determined in 0-10 cm at day 0 was 237 g/ha, which is 95% of the nominal application rate. BYI 02960 declined from 237 g BYI 02960/ha in soil at day 0 to 35.6 g/ha at day 545, corresponding to 15% of the applied amount. BYI 02960 had a DT_{50} of 41.0 days, and a DT_{90} of 749 days.

At [redacted] (United Kingdom), the amount of BYI 02960 determined in 0-10 cm at day 0 was 236 g/ha, which is 94% of the nominal application rate. BYI 02960 declined from 236 g BYI 02960/ha in soil at day 0 to 70.2 g BYI 02960/ha in soil at day 552, corresponding to 29.8% of the applied amount. BYI 02960 had a DT_{50} of 251 days, and a DT_{90} of >1000 days.

At [redacted] (Germany), the amount of BYI 02960 determined in 0-10 cm at day 0 was 245 g/ha, which is 98% of the nominal application rate. BYI 02960 declined from 245 g BYI 02960/ha in soil at day 0 to 17.9 g/ha at day 540, corresponding to 7.3% of the applied amount. BYI 02960 had a DT_{50} of 42.8 days, and a DT_{90} value of 484 days.

At [redacted](Italy), the amount of BYI 02960 determined in 0-10 cm at day 0 was 268 g/ha, which is 107% of the nominal application rate. BYI 02960 declined from 268 g BYI 02960/ha in soil at day 0 to 7.8 g/ha at day 547, corresponding to 2.9% of the applied amount. BYI 02960 had a DT_{50} value of 8.3 days, and a DT_{90} value of 279 days.

At [redacted](Spain), the amount of BYI 02960 determined in 0-10 cm at day 0 was 240 g/ha, which is 96% of the nominal application rate. BYI 02960 declined from 240 g BYI 02960/ha in soil at day 0 to 8.8 g/ha at day 533, corresponding to 3.7% of the applied amount. BYI 02960 had a DT_{50} of 22.6 days, and a DT_{90} value of 215 days.

At [redacted] (Germany), the amount of BYI 02960 determined in 0-10 cm at day 0 was 229 g/ha, which is 91% of the nominal application rate. BYI 02960 declined from 229 g BYI 02960/ha in soil at day 0 to 20.5 g/ha at day 540, corresponding to 9.0% of the applied amount. BYI 02960 had a DT_{50} of 39.0 days, and a DT_{90} value of 579 days.

In general, BYI 02960 residues remained in the upper 0-20 cm soil layer and only very small amounts (<LOQ) were translocated to a maximum depth of 30 cm. At one site ([redacted], Spain), BYI 02960 residues just above the LOQ were found at day 13 up in the 20-30 cm soil layer. BYI 02960 dissipated from soil at all test sites with DT_{50} values ranging from 8.3 to 251 days.

The metabolite BYI 02960-DFA appeared in the upper soil layer 0-20 cm in amounts of up to 17.4 g/ha soil. However, the metabolite declined to values of max. 7.9 g/ha soil towards the end of the study (in [redacted] Germany).

An overview of the results for parent compound is given in Table 7.3.1- 1.

Table 7.3.1- 1 Results synopsis for BYI 02960

Location: Trial No.	Soil type (USDA)	Kinetic model	DT50 [d]	DT90 [d]
[redacted](Germany): 09-2702-01	Sandy loam (0-100 cm)	DFOP	41.0	749
[redacted](United Kingdom): 09-2702-02	Clay loam (0-100 cm)	DFOP	251	>1000
[redacted](Germany): 09-2702-03	Silt loam (0-75 cm), loam (75-100 cm)	DFOP	42.8	484
[redacted](Italy): 09-2702-05	Loam (0-100 cm)	DFOP	8.3	279
[redacted](Spain): 09-2702-06	Loam (0-30 cm), sandy loam (30-75 cm), sandy clay loam (75-100 cm)	DFOP	22.6	215
[redacted] (Germany): 09-2702-07	Loam(0-100 cm)	DFOP	39.0	579

I. MATERIALS AND METHODS

A. Materials

1. Test item: BYI 02960 SL 200
Content of active substance: 200 g/L (soluble concentrate)
Specification No: 102000021884, Batch No: 2009-001253

2. Trial locations: The study was conducted at six sites, typical for the ecoregions Southern and Northern Europe (Table 7.3.1- 2). At an additional site ([redacted], Northern France) was abandoned because of a heavy rain event of 25 mm occurring 7 hrs after application. The remaining six sites were neither subjected to erosion, flooding nor to run-off. A trial consisted of a treated and an untreated plot at each test site. The control plots were at least 5 m away from the treated plots.

B. Methods

1. Application: The representative formulation BYI 02960 SL 200, containing 200 g/L BYI 02960 was sprayed once with an application rate of 1.25 L/ha and 300 L water/ha, corresponding to 250 g BYI 02960 per hectare, using a knapsack sprayer.

2. Meteorological data: Air temperature, precipitation including irrigation, and sunshine data were recorded during the field tests. Temperatures were within the range of the long term average for all trial sites.
The overall precipitation was normal in all trials with some exceptions: In trial 09-2702-01 ([redacted] Germany) April and June 2010 were very dry, whereas May, July and August to September 2010 were very wet. In trial 09-2702-02 ([redacted], UK) July 2009 was wet but September and October 2009 was dry and April to July 2010 was dry but August 2010 was very wet compared to the long-term average. In trial 09-2702-03 ([redacted] Germany) May to September 2009 was dry but October to December 2009 wetter compared to the long-term average. In addition, April 2010 was very dry compared to the long-term average. In trial 09-2702-05 ([redacted], Italy) the situation was very special during the trial period: May 2009 and April 2010 was very dry and December 2009 to March 2010, May/June 2010 and August to December 2010 was wet compared to the long-term average. In trial 09-2702-06 (ソj:övt 0Kþ§fä(, Spain) the summer in 2009 and 2010 was dry compared to the long-term average, except for May 2010 which was wet compared to the long-term average. In trial 09-2702-07 ([redacted], Germany) May to September 2009 was dry but October to December 2009 wetter compared to the long-term average, but April 2010 was very dry compared to the long-term average.

3. Sampling and sample processing: Before application samples were taken from control plots to a depth of 10 cm with a soil piercer (Ø 50 mm) and immediately after application from treated plots. All samples at day 0 consisted of 20 soil cores. All subsequent samplings were performed using a "Wacker Hammer" (Ø 48 to 50.0 mm). At each sampling interval 20 cores from the treated plots were taken, randomly distributed over the plots. From control plots 10 to 20 soil cores were taken.
In all treated plots the samples were taken to a maximum depth of 100 cm on the following days: 0 (post-application; each 0-10 cm depth), 6-7 (0-30 cm depth), 12-14, 28-30, 60-68 (each 0-50 cm depth), 90-96, 111-128, 177-204, 354-394, 432-478, 533-552 (each 0-100 cm depth) after treatment. From the control plot samples were taken on day 0 before application and 354-394 and 533-552 days after application. In addition, for soil characterisation samples were taken on day -3 (i.e. prior) to day 7 after application from the treated plots to a depth of 100 cm.

Table 7.3.1- 2: Location, site description and climatic data at the study sites

Location Trial Number		[redacted] **Germany 09-2702-01**	[redacted]**, UK 09-2702-02**	[redacted] **Germany 09-2702-03**	[redacted]**, Italy 09-2702-05**	[redacted]**, Spain 09-2702-06**	[redacted]**, Germany, 09-2702-07**
Designation		[redacted] Germany, Plot 712/718, ([redacted])	[redacted] Farm Block 5.1	[redacted] Germany, Plot 4011 ([redacted])	[redacted] Marcomcini Sandro	[redacted] Plot 8, Parcel 54	[redacted] Germany [redacted], Plot 606
Plot Size [sqm]		255	320	225	180	300	225
Geographic coordinates	Latitude	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Longitude	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	Country	Germany	United Kingdom	Germany	Italy	Spain	Germany
	Ecoregion	Northern EU	Northern EU	Northern EU	Southern EU	Southern EU	Northern EU
Distance from weather station used for climatic measurements		At trial location	At trial location (sunshine hours 43 km)	At trial location	10 km (soil temp. from trial site)	At trial location (sunshine hours 13 km)	At trial location
Meteorological conditions compared to long-term average within normal levels (Yes/No)		Yes	Yes Winter 2009 colder, May and July drier, August wetter than compared to long-term average	Yes Summer 2009 drier and autumn 2009 wetter compared to the long-term average	No In 2009 dry May and August, wet winter, wet from May to December 2010 compared to the long-term average	No Dry 2009 compared to the long-term average.	Yes Dry summer 2009 and wet winter 2009 and dry April and June 2010 but wet August 2010 compared to the long-term average
Soil type (USDA)	Soil depth [cm]						
	0-30	Sandy loam	Clay loam	Silt loam	Clay loam	Loam	Loam
	30-50	Sandy loam	Clay loam	Silt loam	Loam	Sandy loam	Loam
	50-75	Loamy sand	Clay loam	Silt loam	Loam	Sandy loam	Loam
	75-100	Loamy sand	Clay loam	Loam	Sandy loam	Sandy clay loam	Loam

The soil samples were deep-frozen within 24 hours, stored and shipped to the test facility BCS, [redacted] at maximum -18°C. The cores with the frozen field samples were cut into 10 cm segments. The control samples or the treated samples of each horizon separately (laboratory samples) were milled in a hammer mill and carefully homogenized. A portion of each of the homogenized laboratory samples

was transferred into polystyrene boxes (analytical samples) and stored at or below -18 °C until preparation for analysis.

4. Analytical procedure: The modification M001 to the analytical method 01074 was developed for the determination of BYI 02960 and its metabolites in soil. Soil samples of 20 g are extracted in a microwave extractor with 50 mL of a mixture of acetonitrile/water (4/1, v/v). Possible matrix effects of BYI 02960 and its metabolite DFA are eliminated by using an internal standard solution of isotopically labeled reference items. This solution is added to the sample solutions after extraction. Then a subsample is filtrated to remove fine particles of the soil. Identification and quantitation of the test items is done by high performance liquid chromatography using MS/MS detection in the MRM mode (Multiple Reaction Monitoring mode). The method was validated using three different soils. The limit of quantitation (LOQ) for each single analyte is 5.0 µg/kg in soil. The limit of determination (LOD) for each single analyte is 1.5 µg/kg. Recoveries for each fortification level were in an acceptable range (70 - 110%).

II. RESULTS

A. Residue Concentrations

The measured initial mean concentrations (n = 4) of BYI 02960 for the test sites were 237 g/ha ([redacted] Germany), 236 g/ha ([redacted], UK), 245 g/ha ([redacted] Germany), 268 g/ha ([redacted], Italy), 240 g/ha ([redacted], Spain), and 229 g/ha ([redacted], Germany) representing 91 to 107 % of the intended dose rate.

Dissipation of BYI 02960 varied at the different test locations. At one site each a very fast and a rather slowly dissipation was observed, whereas the other four sites were similarly in a moderate range of dissipation of the residues. In general the dissipation of BYI02960 showed a biphasic behavior.

After treatment, BYI02960 dissipated in a first step very fast within one month followed by a second more slowly step until the end of the study to residue levels of 2.9 to 29.8 % of the total nominal applied amount. Mean residue levels of BYI 02960 reached 35.6 g/ha on day 545 in [redacted] Germany (15% of applied), 70.2 g/ha on day 552 in [redacted], UK (29.8% of applied), 17.9 g/ha on day 540 in [redacted] Germany (7.3% of applied), 7.8 g/ha on day 547 in [redacted], Italy (2.9% of applied), 8.8 g/ha on day 533 in [redacted], Spain (3.7% of applied), and 20.5 g/ha on day 540 in [redacted], Germany (9.0% of applied). In general, BYI 02960 residues remained in the upper 0-20 cm soil layer and only very small amounts could be detected to a maximum depth of 30 cm.

The metabolite DFA appeared in the upper soil layer 0-20 cm in amounts of up to 17.4 g/ha soil. However, the metabolite declined to values of max. 7.9 g/ha soil towards the end of the study.

B: Kinetics Analysis

Based on the chi^2 error criterion and visual assessment the best fit kinetic model was chosen for the evaluation of the dissipation time. The calculated data are based on the quantifiable residues reported for the entire soil profile in [g/ha], and results are presented in Table 7.3.1- 3.

Table 7.3.1- 3: BYI 02960 dissipation values for field studies

Location and Trial No.	Kinetic model	Parent BYI 02960 DT_{50} [d]	DT_{90} [d]	Visual Assessment	Chi^2 error [%]
[redacted] Germany 09-2702-01	SFO	105	349	--	19.3
	FOMC	46.6	>1000	+	8.6
	DFOP	**41.0**	**749**	**+**	**7.5**
[redacted], United Kingdom 09-2702-02	SFO	353	>1000	--	11.9
	FOMC	206	>1000	--	11.7
	DFOP	**251**	**>1000**	**--**	**7.5**
[redacted] Germany 09-2702-03	SFO	83.0	276	o	14.8
	FOMC	43.4	999	+	7.3
	DFOP	**42.8**	**484**	**+**	**6.3**
[redacted], Italy 09-2702-05	SFO	28.8	95.8	--	29.4
	FOMC	9.9	465	o	10.3
	DFOP	**8.3**	**279**	**+**	**7.1**
[redacted], Spain 09-2702-06	SFO	45.7	152	o	19.5
	FOMC	19.3	445	+	11.4
	DFOP	**22.6**	**215**	**+**	**6.6**
[redacted], Germany 09-2702-07	SFO	55.6	185	--	18.3
	FOMC	40.9	477	+	11.8
	DFOP	**39.0**	**579**	**+**	**11.3**

III. CONCLUSIONS

Based on the results it can be concluded that BYI 02960 shows a biphasic degradation behavior under the investigated Northern and Southern European field conditions. BYI 02960 residues remained in the upper 0-20 cm soil layer. Only small amounts below the LOQ could be detected to a maximum depth of 30 cm. At study completion, i.e. 540 days post-application, the remaining BYI 02960 residues in soil corresponded to 2.9 to 29.8% of the applied amount. The calculated DT_{50} of BYI 02960 ranged between 8.3 and 251 days.

In general the field dissipation observed for BYI 02960 residues, i.e. for BYI 02960 and its main soil metabolite DFA, was comparable to that found within the standardized laboratory studies (see point IIA 7.2.1 and IIA 7.2.3).

IIA 7.3.2 Soil Residue Testing

No further studies have been performed, this point is covered by points IIA 7.2.1 and IIA 7.3.1.

IIA 7.3.3 Soil Accumulation Testing on Relevant Soils

No field accumulation studies have been performed as the accumulation of BYI 02960 can be calculated from the degradation data obtained as described under point IIA 7.2.1 and IIA 7.3.1. The results of modeling, considering specific application rates and crops are presented in the Annex III point 9.4 for the representative uses.

IIA 7.4 Mobility Studies

IIA 7.4.1 Adsorption and Desorption of the Active Substance

Report:	**KIIA 7.4.1/01, [redacted], [redacted]; 2008**
Title:	[Pyridinylmethyl-^{14}C]BYI 02960: Adsorption to and desorption from soils
Report No & Document No:	MEF-08/261 M-327492-01-2
Guidelines:	**OECD Guideline No. 106; US EPA Subdivision N, Section 163-1; Canada PMRA DACO Number 8.2.4.2; OPPTS 835.1230**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

Freundlich adsorption and desorption constants K_F and K_{OC} of BYI 02960 have been determined in batch equilibrium experiments with four different soils using radiolabeled test substance ([PYM-^{14}C]BYI 02960). The adsorption phase of the study was carried out using pre-equilibrated air-dried soils in 0.01 M aqueous $CaCl_2$ solution with soil/solution ratios of 1:4 for all soils. BYI 02960 was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L. Desorption phase was performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution for one desorption cycle. Adsorption and desorption took place in the dark at 20 ± 1 °C for 24 hours, each. The test item was stable throughout the study, and the parental mass balance determined for all soils at the highest concentration was in the range of 92.9 - 94.7%.
For key data and results of study see Table 7.4.1- 1.
The calculated adsorption constants $K_{F(ads)}$ of the Freundlich isotherms for the four test soils ranged from 2.08 to 3.82 mL/g, and the mean $K_{OC(ads)}$ was 93.3 mL/g. The Freundlich exponent 1/n was in the range of 0.8445 to 0.8682, and the mean 1/n was 0.86.
The desorption $K_{F(des)}$ and the normalized $K_{OC(des)}$ values were significantly higher (i.e. 2 times higher) than those obtained for the adsorption phase, indicating that the test item once adsorbed to soil is not readily desorbed.

Table 7.4.1- 1 Key data and results of study

Soil origin **Soil type (USDA)**	[redacted] **Sandy loam**	[redacted] **am** [redacted] [redacted] **4a** **Loam**	**Hanscheider Hof** **Loam**	[redacted] **II** **Loam**
pH (aqueous $CaCl_2$ solution)	6.2	6.6	5.3	7.2
Organic carbon [%]	2.1	2.4	2.2	5.1
$K_{F(ads)}$ [mL/g]	2.077	2.213	2.354	3.822
1/n	0.8445	0.8682	0.8643	0.8648
$K_{oc(ads)}$ [mL/g]	98.9	92.2	107.0	74.9
Mean $K_{OC(ads)}$ [mL/g]	**93.3**			

I. MATERIAL AND METHODS

A. Materials

1. Test Item: Code BYI 02960, CAS no. 951659-40-8 (unlabeled substance)
Label position = [Pyridinylmethyl-^{14}C]BYI 02960, sample ID BECH 2135
Specific activity: 118.1 µCi/mg (4.37 MBq/mg)
Radiochemical and chemical purity: >99% (at beginning of study)
The test item was identified by LC-MS/MS)

The test item was dissolved in acetonitrile / Milli-Q-water 1:1 (v/v) (approx. 1 mg/mL) after arrival at the testing facility and stored in a freezer in the dark. Radiopurity of the labeled test item was checked by HPLC analysis before application.

2. Soils: Four soils originating from Germany were used in the batch equilibrium experiments. 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 Table 7.4.1- 2.

Table 7.4.1- 2 Physico-chemical characteristics of test soils used

Parameter Soil Batch ID	Results/Units [redacted] (AX) 20061129	[redacted] am [redacted] 4a (HF) 20061130	[redacted] (HN) 20061130	[redacted] II (DD) 20061130
Geographic Location (City/ State/ Country)	[redacted] Germany	[redacted] / Germany	[redacted] / Germany	[redacted] / Germany
Texture Class[A]	Sandy loam	Loam	Loam	Loam
Sand[A] Silt[A] Clay[A]	73% 18% 9%	39% 46% 15%	35% 50% 15%	33% 42% 25%
pH ($CaCl_2$) pH (Water) pH (KCl)	6.2 6.6 6.1	6.6 7.0 6.4	5.3 5.7 4.9	7.2 7.4 7.0
Organic Matter[B]	3.6%	4.1%	3.8%	8.8%
Organic Carbon[C]	2.1%	2.4%	2.2%	5.1%
Cation Exchange Capacity (meq/100 g)	9.4	12.3	8.8	20.3
Moisture at 0.33 bar	12.5%	24.3%	23.9%	36.6%
Bulk Density	1.22 g/mL	1.04 g/mL	1.05 g/mL	0.98 g/mL
Soil Taxonomic Classification (USDA)	Sandy, mixed, mesic Typic Cambudolls	Loamy, mixed, mesic Typic Argudalfs	Not available	Not available
Soil Mapping Unit	[redacted]	[redacted]	[redacted]	[redacted]

[A]) According to USDA classification
[B]) Calculated: organic matter = organic carbon * 1.724
[C]) Determination method: LECO

B. Methods

1. Experimental conditions: Adsorption and desorption constants of BYI 02960 were determined by batch equilibrium experiments. 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. Following the preliminary tests the adsorption and desorption phases were carried out for 24 hours using a soil: solution ratio of 1:4.

The adsorption phase of the study was carried out using pre-equilibrated air-dried soils (each 5 g dry weight) in 0.01 M aqueous $CaCl_2$ solution with soil/solution ratios of 1:4 for all soils. [PYM-^{14}C]BYI 02960 was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L. Desorption was performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution for one desorption cycle. The samples were incubated at constant temperature of 20 ± 1 °C for 24 hours in the dark and continuously agitated using an overhead shaker. Thereafter, the suspensions were centrifuged and the supernatants were analyzed by LSC. Additionally, the pH values of the supernatants were determined.

The experiments were performed in duplicate. The adsorption parameters were calculated using the Freundlich adsorption isotherm.

2. Analytical procedures: In the pre-test the supernatant was analyzed by HPLC. The recovery in HPLC was 97.6% of the injected RA. The limit of quantification (LOQ) of the HPLC analysis was calculated by considering the applied amount of radioactivity and the lowest amount of radioactivity which could be quantified (i.e. LOD = 3 Bq). As an example, for the supernatant phase in case of 0.1 mg/L application rate at soil [redacted] a limit of quantification of 1.15% of AR was calculated, corresponding to a LOQ of approx. 1.2 µg/kg.

No degradation product was detected.

In the definitive test the BYI 02960 residue in the supernatant was analyzed by liquid scintillation counting (LSC). After the desorption step, for the calculation of the mass balance, the remaining soil was freeze-dried and combusted. The trapped $^{14}CO_2$ after combustion was measured by LSC. Due to the stability of the test item, the partition of the test item was determined based on the amount of radioactivity measured in the supernatant by LSC.

II. RESULTS

A. Mass Balance

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, a summary of the recovery after adsorption is presented in Table 7.4.1- 3

Table 7.4.1- 3: Preliminary study - Recovery of Test Item in Soil after Preliminary Equilibrium Test: Adsorption after incubation for 96 hours calculated as percentage of test item in solution and soil extract.

Soil ID	[redacted]	[redacted] am [redacted] 4a	[redacted]	[redacted] II
Supernatant [%AR]	51.4	49.4	46.1	34.9
Test item [% of injected]	97.3	97.5	97.7	96.7
Solid phase (ACN extract) [%AR]	45.0	47.5	50.0	60.6
Test item [% of injected]	97.9	98.0	97.7	97.7
Non-extractable residues	N/A	N/A	N/A	N/A
Total recovery of test item [% AR]	94.0	94.7	93.9	92.9

For the definitive study he overall material balance for all concentrations was in the range of 95.7 to 98.6% (overall mean: 97.4%) of the applied radioactivity.

Table 7.4.1- 4: Recovery of Total Radioactivity of BYI 02960 after Adsorption and Desorption Expressed as percentage of applied radioactivity

Soil ID Conc. ID	AX Recovery (% of AR)	HF Recovery (% of AR)	HN Recovery (% of AR)	DD Recovery (% of AR)
1.0 mg/L	98.4	98.2	97.6	97.2
0.30 mg/L	97.9	97.9	97.6	95.7
0.10 mg/L	97.8	97.1	97.4	96.3
0.030 mg/L	97.4	97.4	97.6	95.9
0.010 mg/L	98.1	98.6	98.3	95.7
Mean	97.9	97.8	97.7	96.2
sd	± 0.32	± 0.53	± 0.30	± 0.56

Transformation of Test Item

The stability of the BYI 02960 in the test system used was confirmed by performing HPLC analyses prior to the definitive test.

C. Findings

After 24 hours of equilibration, 34.9 - 52.9%, 36.6 - 52.3%, 38.1- 54.3%, and 50.2 - 66.4% of the applied test item were adsorbed in soils [redacted] [redacted], [redacted] am [redacted] 4a, [redacted], and [redacted] II, respectively. The adsorption behavior of BYI 02960 in the concentration range of two orders of magnitude (i.e. from 0.01 to 1.0 mg/L) was accurately described for all soils with the Freundlich equation (see Table 7.4.1- 8). The correlation coefficient of the individual isotherms was 0.9988 to 0.9999. The calculated adsorption constants $K_{f\,(ads)}$ of the Freundlich isotherms ranged from 2.077 mL/g to 3.822 mL/g. The Freundlich exponents 1/n were in the range of 0.8445 to 0.8682, indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range

In general, the organic matter in soil, determined as organic carbon content, is the most important part to bind organic chemicals. Therefore, the adsorption coefficients $K_{f\,(ads)}$ are correlated with the organic carbon content of the soil, in order to get a comparability of the adsorption behavior in different soils. For BYI 02960 the calculated $K_{OC(ads)}$ values varied between 74.9 and 107.0 mL/g (mean: 93.3 mL/g.

At the end of the desorption phase, 31.5 - 44.4%, 33.8 - 43.4%, 31.0 - 39.9%, and 22.0 - 31.9% of the initially adsorbed amount was desorbed in soils [redacted] [redacted], [redacted] am [redacted] 4a,

[redacted], and [redacted] II, respectively. The calculated desorption constants $K_{f\ (des)}$ of the Freundlich isotherms for the four test soils ranged from 4.115 - 7.056 mL/g. The $K_{OC(des)}$ values of the soils ranged from 138.4 - 236.7 mL/g (mean: 188.9 mL/g). Thus, the $K_{OC(des)}$ values were significantly (1.8 to 2.2 times) higher than the $K_{OC(ads)}$ values, indicating a strengthened binding of the test item once adsorbed to the soil.

Table 7.4.1- 5: Concentration of BYI 02960 in the Solid and Liquid Phases at the End of the Adsorption Period

Description	**Soil**			
Soil ID	**AX**			
Concentration	Soil	Solution	Percentage	
	(mg/kg)	(mg/L)	adsorbed	
Control	N/A	N/A		
0.010 mg/L	0.022	0.005	52.9	± 0.03
0.030 mg/L	0.062	0.015	50.7	± 0.57
0.10 mg/L	0.187	0.053	47.0	± 1.10
0.30 mg/L	0.480	0.181	39.9	± 0.12
1.01 mg/L	1.417	0.660	34.9	± 0.52
Soil ID	**HF**			
Control	N/A	N/A		
0.010 mg/L	0.021	0.005	52.3	± 0.29
0.030 mg/L	0.060	0.016	49.1	± 0.35
0.10 mg/L	0.181	0.054	45.4	± 0.04
0.30 mg/L	0.494	0.177	41.1	± 0.58
1.01 mg/L	1.485	0.643	36.6	± 0.34
Soil ID	**HN**			
Control	N/A	N/A		
0.010 mg/L	0.022	0.005	54.3	± 0.00
0.030 mg/L	0.063	0.015	51.5	± 0.33
0.10 mg/L	0.187	0.053	47.1	± 0.48
0.30 mg/L	0.517	0.171	43.0	± 0.32
1.01 mg/L	1.544	0.628	38.1	± 0.05
Soil ID		DD		
Control	**N/A**	**N/A**		
0.010 mg/L	0.027	0.003	66.4	± 0.41
0.030 mg/L	0.078	0.011	64.5	± 0.30
0.10 mg/L	0.239	0.040	60.1	± 0.08
0.30 mg/L	0.679	0.131	56.5	± 0.46
1.01 mg/L	2.037	0.505	50.2	± 0.19

Table 7.4.1- 6: Concentration of BYI 02960 in the Solid and Liquid Phase at the End of Desorption Phases

Description	**Soil**			
Soil ID	**AX**			
Concentration of a.i.	Soil (mg/kg)	Solution (mg/L)	Percentage Desorbed*	
Control	N/A	N/A		
0.010 mg/L	0.015	0.002	31.5	± 0.33
0.030 mg/L	0.041	0.005	34.0	± 0.40
0.10 mg/L	0.120	0.017	35.7	± 0.45
0.30 mg/L	0.286	0.048	40.4	± 0.74
1.01 mg/L	0.789	0.157	44.4	± 0.19
Soil ID	**HF**			
Control	N/A	N/A		
0.010 mg/L	0.014	0.002	33.8	± 0.88
0.030 mg/L	0.038	0.005	35.8	± 0.67
0.10 mg/L	0.112	0.017	38.1	± 0.05
0.30 mg/L	0.292	0.050	40.8	± 0.32
1.01 mg/L	0.840	0.161	43.4	± 0.31
Soil ID	**HN**			
Control	N/A	N/A		
0.010 mg/L	0.015	0.002	31.0	± 0.15
0.030 mg/L	0.043	0.005	31.3	± 0.18
0.10 mg/L	0.123	0.016	34.4	± 0.49
0.30 mg/L	0.328	0.047	36.6	± 0.00
1.01 mg/L	0.929	0.154	39.9	± 0.16
Soil ID	**DD**			
Control	N/A	N/A		
0.010 mg/L	0.021	0.001	22.0	± 0.81
0.030 mg/L	0.059	0.005	24.0	± 0.30
0.10 mg/L	0.175	0.016	27.0	± 0.21
0.30 mg/L	0.484	0.049	28.8	± 0.42
1.01 mg/L	1.387	0.162	31.9	± 0.46

* = expressed as a percentage of the initially adsorbed material
Desorption steps: One desorption step for all concentrations

Table 7.4.1- 7: Adsorption and Desorption Constants of BYI 02960 in the Soils

Soil Type	**Adsorption** K_F (mL/g)	**1/n**	**R^2**	**Koc (mL/g)**	**Desorption** K_F (mL/g)	**1/n**	**R^2**	**Koc (mL/g)**
LH [redacted]	2.077	0.8445	0.9988	98.9	4.115	0.8786	0.9994	196.0
[redacted] am [redacted] 4a	2.213	0.8682	0.9999	92.2	4.431	0.9086	0.9999	184.6
[redacted]	2.354	0.8643	0.9998	107.0	5.208	0.9099	0.9996	236.7
[redacted] II	3.822	0.8648	0.9995	74.9	7.056	0.8923	0.9998	138.4
Mean	2.616	0.8604	0.9995	93.3	5.202	0.8973	0.9997	188.9

III. CONCLUSIONS

BYI 02960 can be classified as intermediate mobile for adsorption and low mobile for desorption. For a compilation of results see Table 7.4.1- 8.

Table 7.4.1- 8: Adsorption and desorption of [^{14}C]BYI 02960 on four different soils

Soil (Soil type)	Adsorption				Desorption			
	K_f [mL/g]	1/n	R^2	K_{oc} [mL/g]	K_f [mL/g]	1/n	R^2	K_{oc} [mL/g]
AX (sandy loam)	2.077	0.8445	0.9988	98.9	4.115	0.8786	0.9994	196.0
HF (loam)	2.213	0.8682	0.9999	92.2	4.431	0.9086	0.9999	184.6
HN (loam)	2.354	0.8643	0.9998	107.0	5.208	0.9099	0.9996	236.7
DD (loam)	3.822	0.8648	0.9995	74.9	7.056	0.8923	0.9998	138.4
Arithmetic mean	**2.616**	**0.8604**	**0.9995**	**93.3**	**5.202**	**0.8973**	**0.9997**	**188.9**

Report:	**KIIA 7.4.1/02, [redacted]; 2011**
Title:	[Pyridinylmethyl-^{14}C]BYI 02960: Adsorption/desorption on two soils
Report No & Document No:	MERVP017 M-363541-01-1
Guidelines:	**OECD TG No. 106, 2000** **US EPA Fate Guidelines, OPPTS 835.1230, October 2008** **Canada PMRA DACO Number 8.2.4.2, 1987**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

Freundlich adsorption and desorption constants K_f and K_{OC} of BYI 02960 have been determined in batch equilibrium experiments with two different soils using radiolabeled test substance ([PYMl-^{14}C]BYI 02960). The adsorption phase of the study was carried out using pre-equilibrated air-dried soils in 0.01 M aqueous $CaCl_2$ solution with soil/solution ratios of 1:1 soil [redacted] and 1/2 for soil [redacted]. BYI 02960 was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L. Desorption phase was performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution for one desorption cycle. Adsorption and desorption took place in the dark at 20 ± 1 °C for 24 hours, each. For the highest concentration, two additional desorption cycles were performed with 24 hours equilibration time each. The test item was stable throughout the study, and the parental mass balance determined for all soils at the highest concentration was in the range of 93.4 - 94.4%. For key data and results of study see Table 7.4.1- 9.

The calculated adsorption constants $K_{f\ (ads)}$ of the Freundlich isotherms for the two test soils were 0.597 and 2.512 mL/g, and the mean $K_{OC(ads)}$ was 1.554 mL/g. The Freundlich exponent 1/n was 0.8505 and 0.9021 (mean 0.8763).
The desorption $K_{f\ (des)}$ and the normalized $K_{OC(des)}$ values were significantly (i.e. 2.0 and 3.6 times) than those obtained for the adsorption phase, indicating that the test item once adsorbed to soil is not readily desorbed.

Table 7.4.1- 9: Key data and results of study

Soil origin	**[redacted], [redacted], USA**	**[redacted], [redacted], USA**
Soil type (USDA)	Sandy loam	Silt loam
pH (aqueous $CaCl_2$ solution)	6.8	6.5
Organic carbon [%]	0.7	1.9
$K_{f\,(ads)}$ [mL/g]	0.597	2.512
1/n	0.9021	0.8505
$K_{oc(ads)}$ [mL/g]	**85.2**	**132.2**

(Mean $K_{OC(ads)}$: 108.7 mL/g)

I. MATERIAL AND METHODS

A. Materials

1. Test Item: Code BYI 02960, CAS no. 951659-40-8 (unlabelled substance)
Label position = [Pyridinylmethyl-^{14}C]BYI 02960, sample ID MERVP017-SS
The test item was identified by HPLC-MS/MS
Specific activity: 118.1 µCi/mg (4.37 MBq/mg)
Radiochemical and chemical purity: >99% (at beginning of study)

The test item was dissolved in acetonitrile after arrival at the testing facility and stored in a freezer in the dark. Radiopurity of the labeled test item was checked by HPLC analysis before application.

2. Soils: Two soils originating from the USA were used in the batch equilibrium experiments. 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 Table 7.4.1- 10.

B. Methods

1. Experimental conditions: Adsorption and desorption constants K_{OC} of BYI 02960 were determined for two soils (see Table 7.4.1- 10) with batch equilibrium experiments using [PYM-^{14}C]BYI 02960. Preliminary tests were performed prior to the definitive test in order to optimize the test conditions.
The adsorption phase of the study was carried out using pre-equilibrated air-dried soils (each 5 g dry weight) in 0.01 M aqueous $CaCl_2$ solution with soil/solution ratios of 1:4, 1:2 and 1:1 for the soils. BYI 02960 was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L. Desorption phase was performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution for one desorption cycle. The samples were incubated at constant temperature of 20 ± 1 °C for 24 hours in the dark. Thereafter, the suspensions were centrifuged and the supernatants were analyzed by LSC. Additionally, the pH values of the supernatants were determined.

The experiments were performed in duplicate. The adsorption parameters were calculated using the FREUNDLICH adsorption isotherm.

2. Analytical procedures: In the pre-test the supernatant was analyzed by HPLC. The recovery in HPLC was 97.4% of the injected radioactivity. The limit of quantification (LOQ) of the HPLC analysis was calculated by considering the applied amount of radioactivity and the lowest amount of radioactivity which could be quantified (LOD). The limit of detection (LOD) was set to 150 dpm, i.e. 0.3% of applied radioactivity, deduced from the chromatograms of the parental mass balance samples (supernatant, 96 hours equilibration time, highest concentration nominal 1.0 mg/L). The LOQ (limit of quantification) was set to three times the LOD, i.e. 450 dpm or 0.9% of applied radioactivity.

In the definitive test the BYI 02960 residue in the supernatant was analyzed by liquid scintillation counting (LSC). After the desorption step, for the calculation of the mass balance, the remaining soil was freeze-dried and combusted. The trapped $^{14}CO_2$ after combustion was measured by LSC.

Table 7.4.1- 10: Physico-chemical characteristics of test soils used

Parameter	Results/Units [C]	
Soil (Soil ID) Batch ID	[redacted] ([redacted]) 091609-S	[redacted] ([redacted]) 072209-S
Geographic Location (City / State / Country)	[redacted]/ California/ USA	[redacted]/ Nebraska/ USA
Soil Series	Hanford	Marshall
Texture Class [A]	Sandy Loam	Silt Loam
Sand [A] Silt [A] Clay [A]	64.3% 28.1% 7.6%	11.1% 62.2% 26.7%
pH (0.01 M $CaCl_2$, 1/1) pH (Water, 1/1) pH (Saturated Paste)	6.8 7.3 7.3	6.5 7.1 6.9
Organic Matter [B]	1.2%	3.3%
Organic Carbon	0.7%	1.9%
Cation Exchange Capacity (CEC)	6.7 meq/100 g	19.0 meq/100 g
Water Holding Capacity 0.1 bar Water Holding Capacity 0.33 bar	22.1% 11.5%	33.3% 25.0%
Maximum Water Holding Capacity	28.9 g/100 g	37.9 g/100 g
Bulk Density Particle Density	1.30 g/cm³ N/A	0.96 g/cm³ N/A
Biomass	N/A	N/A
Soil Taxonomic Classification (USDA)	Coarse-loamy, mixed, superactive, nonacid, thermic Typic Xerorthents	Fine-silty, mixed, superactive, mesic Typic Hapludolls
Soil Mapping Unit	[redacted]	[redacted]

[A]) According to USDA classification
[B]) Calculated: organic matter = organic carbon * 1.724
[C]) Analyses performed at [redacted]

II. RESULTS

A. Mass Balance

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, a summary of the recovery after adsorption is presented in Table 7.4.1- 11.

Table 7.4.1- 11: Preliminary study - Recovery of Test Item in Soil after Preliminary Equilibrium Test: Adsorption after incubation for 96 hours calculated as percentage of test item in solution and soil extract.

Soil ID	SA	SP
Supernatant [% AR]	36.8	26.7
a.i. in supernatant [area %]*	97.1	96.6
Recovery a.i. in supernatant [% AR]	**35.7**	**25.8**
Solid phase (organic extract) [% AR]	60.0	69.1
a.i. in solid phase (organic extract) [area %]*	97.8	97.8
Recovery a.i. in solid phase (organic extract) [% AR]	**58.7**	**67.6**
Non-extractable residues	N/A	N/A
Total recovery of AR [%]	96.8	95.9
Total recovery of a.i. [% AR]	**94.4**	**93.4**

The overall material balance for all concentrations was in the range of 96.0 - 98.7% (mean: 97.1%) of the applied radioactivity. The complete material balance found at all sampling intervals demonstrated that no significant RA dissipated from the test vessels or was lost during processing.

Table 7.4.1- 12: Recovery of Total Radioactivity of BYI 02960 after Adsorption and Desorption Expressed as percentage of applied radioactivity

Soil ID	SA Recovery	SP Recovery
Conc. ID	(% of AR)	(% of AR)
1.0 mg/L	97.6	97.4
0.30 mg/L	97.1	96.0
0.10 mg/L	96.5	97.6
0.030 mg/L	98.7	96.9
0.010 mg/L	97.3	96.0
Mean	97.5	96.8
sd	±0.7	±0.7

B. Transformation of Test Item

The stability of the BYI 02960 in the test system used was confirmed by performing HPLC analyses prior to the definitive test.

C. Findings

The adsorption behavior of BYI 02960 in the concentration range of two orders of magnitude (i.e. from 0.01 - 1.0 mg/L) was accurately described for all soils with the Freundlich equation (for summary of results see Table 7.4.1- 15). The correlation coefficient of the individual isotherms was 0.9993 and 0.9995. The calculated adsorption constants $K_{f\,(ads)}$ of the Freundlich isotherms were 0.597 and 2.512 mL/g (mean: 1.554 mL/g). The Freundlich exponents 1/n were 0.8505 and 0.9021 (mean: 0.8763), indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range.

In general, the organic matter in soil, determined as organic carbon content, is the most important part to bind organic chemicals. Therefore, the adsorption coefficients $K_{f\,(ads)}$ were correlated with the organic carbon content of the soil, in order to get a comparability of the adsorption behavior in different soils. For BYI 02960 the calculated $K_{OC(ads)}$ values were 85.2 and 132.2 mL/g (mean: 108.7 mL/g).

At the end of desorption phase, 23.7 - 29.5 % and 13.2 - 23.1 % of the initially adsorbed amount was desorbed in soils [redacted] and [redacted], respectively. The calculated desorption constants $K_{f\ (des)}$ of the FREUNDLICH isotherms for the two test soils were 2.143 and 5.096 mL/g (mean: 3.620 mL/g). The $K_{OC(des)}$ values of the soils were 268.2 and 306.2 mL/g (mean: 287.2 mL/g).

The $K_{OC(des)}$ values were significantly (2.0 and 3.6 times) higher than the $K_{OC(ads)}$ values, indicating a strengthened binding of the test item once adsorbed to the soil.

Table 7.4.1- 13: Concentration of Test Substance in the Solid and Liquid Phases at the End of Adsorption Equilibration (Mean ± SD)

Concentration of a.i.	Soil (mg/kg)	Solution (mg/L)	Percentage adsorbed	
Soil	(Soil ID: SA)			
Control	N/A	N/A		
0.010 mg/L	0.005	0.005	50.1	± 0.1
0.030 mg/L	0.014	0.016	47.2	± 1.5
0.10 mg/L	0.044	0.057	43.6	± 1.0
0.30 mg/L	0.128	0.175	42.3	± 0.2
1.01 mg/L	0.384	0.622	38.2	± 0.8
Soil	(Soil ID: SP)			
Control	N/A	N/A		
0.010 mg/L	0.015	0.003	74.9	± 0.5
0.030 mg/L	0.043	0.008	72.4	± 0.6
0.10 mg/L	0.136	0.032	67.9	± 0.4
0.30 mg/L	0.391	0.108	64.5	± 0.5
1.01 mg/L	1.166	0.422	58.0	± 0.6

Table 7.4.1- 14: Concentration of Test Substance in the Solid and Liquid Phases at the End of Desorption Equilibration (Mean ± SD)

Concentration of a.i.	Soil (mg/kg)	Solution (mg/L)	Percentage desorbed*	
Soil	SA			
Control	N/A	N/A		
0.010 mg/L	0.004	0.001	23.7	± 0.4
0.030 mg/L	0.011	0.004	25.4	± 1.2
0.10 mg/L	0.032	0.012	26.5	± 0.9
0.30 mg/L	0.094	0.035	26.9	± 0.6
1.01 mg/L	0.270	0.113	29.5	± 0.2
Soil	SP			
Control	N/A	N/A		
0.010 mg/L	0.013	0.001	13.2	± 0.3
0.030 mg/L	0.037	0.003	14.7	± 0.0
0.10 mg/L	0.113	0.012	17.3	± 0.0
0.30 mg/L	0.314	0.039	19.9	± 0.1
1.01 mg/L	0.897	0.135	23.1	± 0.0

* Expressed as a percentage of the initially adsorbed material, one desorption step for all concentrations, two additional desorption steps for highest concentration only (not calculated in this evaluation).

III. CONCLUSIONS

BYI 02960 can be classified as intermediate mobile for adsorption and low mobile for desorption. For a compilation of results see Table 7.4.1- 15.

Table 7.4.1- 15: Adsorption and desorption of [^{14}C]BYI 02960 in tested soils

Soil	Adsorption				Desorption			
	K_f [mL/g]	1/n	R^2	K_{OC} [mL/g]	K_f [mL/g]	1/n	R^2	K_{OC} [mL/g]
SA	0.597	0.9021	0.9993	85.2	2.143	0.9407	0.9992	306.2
SP	2.512	0.8505	0.9995	132.2	5.096	0.8603	0.9999	268.2
Mean	1.554	0.8763	0.9994	108.7	3.620	0.9005	0.9995	287.2

Report:	**KIIA 7.4.1/03, [redacted], 2011**
Title:	[Pyridinylmethyl-^{14}C]BYI 02960: Aerobic soil metabolism/degradation and time-dependent sorption in soils
Report No & Document No:	MEF-07/334 M-414615-01-2
Guidelines:	**OECD TG 307** **US EPA, OPPTS 835.4100, October 2008.** **OECD TG 106: 2001 (only in parts)**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The biotransformation and time dependent sorption of [pyridinylmethyl-^{14}C]BYI 02960 was studied in four European soils: [redacted] (AX), [redacted] am [redacted] (HF), [redacted] Plot 611 (HN), and [redacted] II (DD) for a maximum period of 120 days under aerobic conditions in the dark at ca. 20 °C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 0.53 mg/kg soil, which is equivalent to 200 g/ha field application rate.

At each sampling date the soil samples were shaken for 24 hours with 400 mL $CaCl_2$-solution in order to measure the time-dependent desorption of the test item. Subsequently they were extracted by shaking at ambient temperature and in a microwave at 70 °C with acetonitrile/water mixtures, and the BYI 02960 residues were analyzed and quantified by TLC with HPLC as the confirmatory method.

The degradation and metabolism part of the study was summarized earlier (see KIIA 7.1.1/01), this summary only considers results relevant to the assessment of time dependent sorption.

At the beginning of the study (DAT-0; equivalent to approximately two hours) the distribution coefficient values K_{TDS} were 1.46, 1.91, 3.35 and 4.66 mL/g for soils AX, HF, HN and DD, respectively. Depending on the ageing time, these values increased with time until the end of the study to 6.50, 5.64, 10.78 and 12.16 mL/g for the four soils indicating a significant increase of sorption with time. Based on results from the ageing time of 120 days, the R_{TDS} values increased by a factor of 4.4, 2.9, 3.2 and 2.6 (mean of all four soils = 3.3).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Flupyradifurone: Code = BYI 02960;
Label PYM = [Pyridinyl-methyl-^{14}C]BYI 02960 (sample ID: BECH 2123)
Specific activity 4.37 MBq/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 confirmed.

2. Soil: The time-dependent sorption of BYI 2960 was studied in four different soils (for physico-chemical properties see KIIA 7.1.1/01. These soils are representative for agricultural use areas as required by the guidelines and cover a representative range of physico-chemical properties. Other details were summarized earlier (see KIIA 7.1.1/01.

B. Methods

See for summary of KIIA 7.1.1/01.

In addition the changes of the sorption parameter R_{TDS} (equivalent to K_d) of BYI 02960 affected by a preceding ageing period in soil were investigated by time-dependent desorption experiments. This additional test was designed in analogy to a "batch equilibrium" study. The soils incubated with the test item were shaken for 24 hours with 400 mL 0.1 M $CaCl_2$ solution, i.e. a soil to solution ratio of 1:4 was applied, which was derived from the respective batch equilibrium adsorption/desorption study (see report KIIA 7.1.1/01).

After centrifugation, the distribution of the test item between supernatant and soil was determined by means of LSC. For TLC analysis, the extracts were not processed further. About 20 Bq of the extracts were spotted per cm lane onto silica plates. For HPLC, the desorption solution ($CaCl_2$ extract) was analyzed directly as well (designation: D).

The R_{TDS} values (ratio time-dependent sorption, equivalent to K_d values) were calculated by:

$$R_{TDS} = \frac{concentration\ of\ test\ item\ in\ soil\ extract\,[\frac{\mu g}{g}]}{concentration\ of\ test\ item\ in\ CaCl_2\ solution\,[\frac{\mu g}{mL}]} = \frac{c_{extr}}{c_{des}}$$

II. RESULTS

A. Data

For basic data including recovery and degradation data see summary under report KIIA 7.1.1/01.

B. Findings

The results of desorption measurements affected by the ageing period are presented in Table 7.4.1- 16.

The R_{TDS} values were 1.46, 1.91, 3.35 and 4.66 mL/g for soils AX, HF, HN and DD, respectively, at the beginning of the study (DAT-0; equivalent to approximately two hours). With time of ageing in soil, these values increased until the end of the study to 6.50, 5.64, 10.78 and 12.16 mL/g for the four soils. Based on these results during an ageing time of 120 days, the mean R_{TDS} value increased by a factor of 4.4, 2.9, 3.2 and 2.6 (mean of all four soils = 3.3).

III CONCLUSIONS

A. Major Outcomes of Study

During the ageing period of 120 days a clear increase of the distribution coefficient R_{TDS} became apparent. Based on the results of an ageing time of 120 days, the R_{TDS} values increased by a factor of 2.6 to 4.4 (mean of all four soils = 3.3).

Table 7.4.1- 16: **R_{TDS} values [mL/g] of time-dependent sorption of [[14]C]BYI 02960 on four different soils; mean of duplicates**

DAT	LH [redacted] (sandy loam)	[redacted] am [redacted] (loam)	[redacted] (loam)	[redacted] II (loam)
0	1.46	1.91	3.35	4.66
1	2.09	2.78	4.58	5.29
3	1.88	2.32	4.32	5.23
7	2.40	2.57	5.07	5.90
14	2.91	3.03	6.04	6.47
21	3.38	3.38	6.95	7.21
30	3.71	3.72	7.38	7.78
59	4.98	4.84	9.35	9.73
120	6.50	5.64	10.78	12.16
Factor DAT-120/DAT-0	**4.4**	**2.9**	**3.2**	**2.6**
Mean factor DAT-120/DAT-0	**3.3**			

B. Significance of Results to Environmental Behavior of BYI 02960

The current laboratory study demonstrated that whenever BYI 02960 has time to age in soil it is much less susceptible to leaching. Parametesr for exposure modeling can be derived from the study as decribed in report KIIA 7.4.1/04.

Report:	**KIIA 7.4.1/04, [redacted], [redacted]; 2012**
Title:	Evaluation of the time-dependent sorption of flupyradifurone based on laboratory time-dependent sorption experiments in four soils
Report No & Document No:	MEF-11/723 M-422824-01-1
Guidelines:	**US EPA OPPTS 835.SUPP**
GLP:	**no**

This report is a supplement to KIIA 7.4.1/03 and evaluates the study to derive input parameters for implementation in environmental modeling.

EXECUTIVE SUMMARY

Experimental sorption data of BYI 02960 on four soils (see report KIIA 7.1.1/01) were used to derive kinetic sorption parameters via curve fitting. These parameters constitute the prerequisite to adequately address time-dependent sorption processes in regulatory exposure modelling. The kinetic sorption was evaluated according to the model of [redacted] et al. (1989) implemented in the groundwater leaching models PEARL and FOCUS-PELMO (FOCUS, 2009).

BYI 02960 was incubated at initial concentrations of about 0.5 mg/kg for 120 d at constant soil moisture (55% MWHC) and 20°C in a number of four soils with varying properties. After the respective incubation period, BYI 02960 was first desorbed with aqueous $CaCl_2$ and subsequently extracted with organic solvent in multiple steps. Up to the end of the incubation period (120 days) the (desorption) $K_{d,oc}$ (defined as $R_{TDS,OC}$: ratio of concentration adsorbed to soil and in solution) increased by a factor of 4.4 at the maximum (see report KIIA 7.4.1/03 and Table 7.4.1- 16) indicating kinetically controlled sorption processes.

The experimental raw data were pre-processed to calculate the concentration in the $CaCl_2$ desorption solution, and the total mass. These data were used to fit the kinetic-sorption model using PEARLNEQ.

The evaluation showed that the experimental data could be well described by the kinetic-sorption model, with excellent fits and reliable parameter estimates. The inferred parameters were the desorption rate constant $k_{\_des}$ [1/d], the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium compartment $f_{\_ne}$ [-], the degradation half-life in the equilibrium compartment DT_{50_eq} [d], the organic matter normalized distribution coefficient in the equilibrium compartment K_{om_eq} [L/kg], and the initial total mass in the system $M_{\_ini}$ [µg]. The corresponding values for all four soils are shown in Table 7.4.1- 17.

The kinetic desorption rate constant $k_{\_des}$ indicates a time scale of about 22 d, calculated as "pseudo half-life" for the exchange between equilibrium and non-equilibrium domain.
Values for $f_{\_ne}$ in the range of 0.387 – 0.779 indicate that the kinetically controlled "sorption capacity" is in the range of 39% - 78%, and accounts on average for about 58% of the instantaneous "sorption capacity". The DT_{50_eq} representing degradation in the equilibrium domain was on average (geomean) 58 d suggesting moderate degradation during the laboratory study.

Table 7.4.1- 17: Estimated parameters of the kinetic-sorption model for all soils

Soil	M_ini [µg]	Kom_eq [L/kg]	DT50_eq [d]	K_des [1/d]	F_ne [-]
[redacted] (AX)	44.5	40.3	57.3	0.031	0.779
[redacted] II (DD)	46.0	39.3	50.1	0.029	0.470
[redacted] (HN)	46.0	62.8	86.6	0.032	0.683
[redacted] am [redacted] (HF)	47.7	43.6	45.7	0.033	0.387
Arithm. mean	46.1	46.5			0.580
Geom. mean			58.0	0.031	

I. MATERIAL AND METHODS

A. Materials

1. Test Item: See report KIIA 7.1.1/01.

2. Data: For basic data see report KIIA 7.1.1/01. In the present study the Freundlich exponent 1/n of the four soils was taken from report KIIA 7.4.1/01, see Table 7.4.1- 8.

B. Methods

Experimental sorption data of BYI 02960 (Flupyradifurone) on four soils were used to derive kinetic sorption parameters via curve fitting. These parameters constitute the prerequisite to adequately address time-dependent sorption processes in regulatory exposure modelling. The kinetic sorption was evaluated according to the model of [redacted] ET AL. (1989) implemented in the groundwater leaching models PEARL and FOCUS-PELMO (FOCUS, 2009).
The sorption is described as rate limited between an instantaneous equilibrium and a non-equilibrium domain. Degradation of the compound occurs only in the equilibrium domain including a dissolved and an equilibrium sorbed phase. If kinetic sorption is relevant a part of the substance is sorbed in the non-equilibrium domain where no degradation is assumed. Therefore, considering both domains the apparent degradation of the substance is lower than the observed degradation in the equilibrium domain only. Thus, the degradation curve of the total substance may show a bi-phasic behaviour as a

slowdown of total degradation results from the increasing fraction of substance being sorbed in the non-equilibrium domain.

The experimental raw data were pre-processed to calculate the concentration in the $CaCl_2$ desorption solution, and the total mass. These data were used to fit the kinetic-sorption model using PEARLNEQ program (Version 5; [redacted] et al. 2007).

II. RESULTS

The evaluation showed that the experimental data could be well described by the kinetic-sorption model, with excellent fits and reliable parameter estimates. The inferred parameters were the desorption rate constant $k_{\_des}$ [1/d], the ratio between the Freundlich coefficients in the non-equilibrium and in the equilibrium compartment $f_{\_ne}$ [-], the degradation half-life in the equilibrium compartment DT_{50_eq} [d], the organic matter normalized distribution coefficient in the equilibrium compartment K_{om_eq} [L/kg], and the initial total mass in the system $M_{\_ini}$ [µg].

Results of the curve fitting procedure used to derive optimized kinetic sorption parameters for BYI 02960 are shown in Table 7.4.1- 17. The kinetic desorption rate constant $k_{\_des}$ indicates a time scale of about 22 days, calculated as "pseudo half-life" for the exchange between equilibrium and non-equilibrium domain. Values for $f_{\_ne}$ in the range of 0.387 - 0.779 indicate that the kinetically controlled "sorption capacity" is in the range of 39 - 78%, and accounts on average for about 58% of the instantaneous "sorption capacity". The DT_{50_eq} representing degradation in the equilibrium domain was on average (geomean) 58 days suggesting moderate degradation during the laboratory study.

The fits between experimental and modelled data were visually good. The statistical parameters also indicate a sufficient goodness of fit. The χ^2 statistics indicate a very good fit with values ranging from 3.1 for the [redacted] soil to 1.6 for the [redacted] soil (Table 7.4.1- 18). RSE values were generally small and never exceeded 0.25 implying parameter estimates with a high confidence.

Table 7.4.1- 18: χ^2 statistics and RSE values for the parameter estimates of the kinetic sorption model

Soil	χ^2	RSE				
		M_ini	Kom_eq	DT50_eq	k_des	f_ne
AX	3.12	0.014	0.121	0.041	0.133	0.105
DD	1.74	0.008	0.026	0.018	0.090	0.057
HN	1.93	0.009	0.038	0.031	0.090	0.061
HF	1.62	0.007	0.042	0.017	0.099	0.064

III CONCLUSIONS

The time-dependent sorption data received for BYI 02960 constitute the prerequisite to adequately address the obvious TDS process in higher tier regulatory modeling, e.g. related to a potential groundwater contamination.

IIA 7.4.2 Adsorption & Desorption of Rel. Metabolites, Degr. & React. Products

Metabolite 6-CNA

6-CNA is a common metabolite to the active substance acetamiprid. The following study has been performed in the context of the acetamiprid registration and is owned by Nippon Soda (NISSO), access to the study has been granted by the owner.: This study was already evaluated in the context of

review report for the active substance acetamiprid, SANCO/1392/2001 – Final, 16 June2004. Therefore, just a short summary is included in this dossier.

Report:	**KIIA 7.4.2/01, [redacted], 1997**
Title:	[6-Chloronicotinic Acid (Acetamiprid Metabolite): Soil Adsorption/Desorption Study
Report No & Document No	C007666 M-196394-01-1
Guidelines:	**U. S. EPA-FIFRA, 40 CFR, Section 158.290, Subdivision N, Guideline 163-1 EU Commission Directive 95/3 6/EC, Annex I, Section 7.1.2 (14 July 1995) Canada PMRA DACO Number 8.2.4.2, 1987**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The soil adsorption/desorption of 6-chloronicotinic acid [pyridyl-2,6-^{14}C], was studied in equilibrium experiments using five soils (loamy sand I, loamy sand II, silt loam, clay, and clay loam) as well as in an aquatic pond sediment. The study was conducted at 20± 1°C in the dark at five different concentrations (0.035, 0.070, 0.349, 1.170 and 2.319 ppm) in a 0.01 M CaCl$_2$ solution (equivalent to 0.174, 0.349, 1.745, 5.847, and 11.586 ppm in the soil, respectively). The preliminary range-finding study conducted using loamy sand II, silt loam and pond sediment, showed that both adsorption equilibrium and desorption equilibrium were reached at 16 hours. Therefore, the adsorption cycle and each desorption cycle were carried out for 24 hours each in the definitive study. No adsorption of 6-chloronicotinic acid to glass was observed.
Total accountability for all the soils averaged 99% and ranged from 94% to 110% of the applied dose activity with the exception of 0.07 ppm clay (87%). The average % of applied 6-chloronicotinic acid remaining in the supernatants of each phase was as follows:

The Freundlich adsorption (K_f) and desorption (K_{des}) constants as well as adsorption K_{oc} are summarised in Table 7.4.2- 1.
The Freundlich adsorption (K_f) constants ranged from 0.569 for silt loam to 2.121 for pond sediment, averaging 0.981. Adsorption Koc values ranged from 70 for loamy sand II and clay to 258 for loamy sand I, averaging 116. Similar K_f and K_{des} values for all of the soils and pond sediment shows the reversible equilibration between adsorption and first desorption phases.

Table 7.4.2- 1: Average % of applied 6-CNA remaining in the supernatants

Soil type	OC	Adsorption			Desorption		
	[%]	1/n	K_f	Koc	K_{des1}	K_{des2}	K_{des3}
Loamy Sand I *	*0.25*	*0.967*	*0.643*	*258*	0.762	2.394	6.789
Loamy Sand II	1.5	1.007	1.027	70	1.391	1.243	2.551
Silt Loam	0.44		0.569	129	0.472	0.989	6.789
Clay	1.2		0.833	70	0.393	3.522	2.551
Clay Loam	0.82		0.690	84	0.505	2.394	6.789
Pond sediment (Sandy Loam) **	2.5		*2.121*	*85*	3.144	1.243	2.551
Average soils		0.949	0.780	88			

*: not considered for calculation of mean since the OC was regarded as too low

**: pond sediment not considered for calculation of mean in soils

Metabolite DFA

Report:	**KIIA 7.4.2/02, [redacted], [redacted], 2011**
Title:	[1-[14]C]BYI 02960-DFA (BCS-AB60481): Adsorption to and desorption from five soils
Report No & Document No	MEF-10/538 M-413836-01-2
Guidelines:	**OECD TG No. 106 Adsorption/Desorption, 2000** **US EPA Fate, Transport and Transformation Test Guidelines, OPPTS 835.1230, Adsorption/Desorption (Batch Equilibrium), October 2008** **Canada PMRA DACO Number 8.2.4.2, 1987**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The adsorption/desorption behavior of BYI 02960-DFA was studied in batch equilibrium experiments in five soils originating from Germany and the USA.

The adsorption phase of the study was carried out using pre-equilibrated air-dried soils in 0.01 M aqueous $CaCl_2$ solution with a soil to solution of 1:1.25 for soil [redacted] II and soil to solution ratios of 1:1 for soils [redacted] am [redacted] 4a, [redacted], [redacted] and [redacted]. BYI 02960-DFA was applied at concentrations of nominal 1.0, 0.3, 0.1, 0.03, and 0.01 mg/L supernatant.

Desorption experiments were performed by supplying pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution. One desorption cycle was performed for all concentrations and three desorption cycles were performed for the highest concentration only.

Adsorption and desorption took place in the dark at 20 ± 1 °C for 24 hours with triplicate samples, respectively. The aqueous supernatant after adsorption and desorption was separated by centrifugation, and the BYI 02960-DFA residue in the supernatant was analyzed by liquid scintillation counting (LSC). The adsorption and desorption parameters were calculated using the Freundlich equation.

The test item was stable throughout the study. The mass balance of the parent compound determined for all soils at the highest concentration was >90%. In the definitive adsorption test 16.9 - 25.3%, 16.6 - 34.9%, 22.3 - 26.6%, 2.6 - 10.7% and 2.4 - 5.7% of the applied test item were adsorbed to soils [redacted] am [redacted] 4a, [redacted], [redacted] II, [redacted] and [redacted], respectively.

The calculated adsorption constant $K_{F(ads)}$ of the Freundlich isotherms for the five test soils ranged from 0.028 - 0.368 mL/g, the $K_{oc(ads)}$ from 1.7 – 9.5 mL/g. The Freundlich exponent 1/n was in the range of 0.6902 to 0.9579, indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range.

The $K_{oc(des)}$ values were 12.7 - 57.1 times higher than the $K_{oc(ads)}$ values, indicating a strong binding of the test item to the soil. Soils [redacted] II and [redacted] did not show an acceptable linearity of the Freundlich desorption isotherms and therefore desorption constants could not be calculated. For the highest concentration of each soil, a second and a third desorption step were performed. In these steps, the desorbed amounts of BYI 02960-DFA range from 13.4 to 45.2% of the adsorbed amount, indicating that the binding is partly reversible. The following table summarizes the key data of this study.

BYI 02960-DFA can be classified as very mobile for adsorption and low mobile for desorption.

Table 7.4.2- 2: Key data and results of study

Soil origin	[redacted] am [redacted] 4a, Germany	[redacted], Germany	[redacted] II, Germany	[redacted], USA	[redacted], USA
Soil type (USDA)	Silt Loam	Loam	Clay loam	Sandy Loam	Silty Clay loam
pH (aqueous $CaCl_2$ solution)	6.5	5.8	7.4	6.0	6.5
Organic carbon [%]	2.4	2.9	4.5	0.5	1.7
$K_{F(ads)}$ [mL/g]	0.228	0.226	0.368	0.033	0.028
1/n	0.9053	0.8013	0.9579	0.6902	0.8194
$K_{oc(ads)}$ [mL/g]	9.5	7.8	8.2	6.7	1.7
$K_{oc(des)}$ [mL/g]	121.3	155.5	*	380.7 **	*
Mean $K_{oc(ads)}$: mL/6.8 g; Mean $K_{oc(des)}$: 219.1 mL/g					

* The correlation coefficient determined for the Freundlich desorption isotherms was not significant
** For soil [redacted] (SA) the highest concentration (1 mg/L) was not included in the calculation of the Freundlich desorption isotherm due to a negative supernatant concentration calculated for one replicate

I. MATERIAL AND METHODS

A. Materials

1. Test Item: BYI 02960-DFA Code BCS-AB60481,
CAS no. 2218-52-2 [sodium salt], 381-73-7 [free acid]
[1-[14]C]BYI 02960-difluoro-acetic acid, sample ID KATH 6450
Specific activity: 76.68 µCi/mg (2.84 MBq/mg)
Radiochemical and chemical purity: >98% / 99.5% (at beginning of study)
The test item was identified by LC-MS, LC-MS/MS and NMR)

The ^{14}C-test item was dissolved in TKA-water (approx. 1.6 mg/mL) after arrival at the testing facility and stored in a freezer in the dark. Radiopurity of the labeled test item was checked by TLC analysis.

2. Soils: The adsorption/desorption behavior of DFA was studied in batch equilibrium experiments in five soils originating from Germany and the USA: [redacted] am [redacted] 4a, silt loam, pH 6.5, 2.4% organic carbon; [redacted], loam, pH 5.8, 2.9% organic carbon; [redacted] II, clay loam, pH 7.4, 4.5% organic carbon; [redacted], sandy loam, pH 6.0, 0.5% organic carbon; [redacted], silty clay loam, pH 6.5, 1.7% organic carbon (pH values derived from aqueous CaCl2 suspension). 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) and stored in a climate chamber at 5 °C. The detailed parameters of soils are shown in Table 7.4.2- 3.

B. METHODS

1. Experimental conditions: In the definitive test, 16 g of soil [redacted] II and 20 g of soils [redacted] am [redacted] 4a, [redacted], [redacted] and [redacted] were weighed into the centrifuge tubes and aqueous 0.01 M $CaCl_2$ solution was added to a solution volume of 18 mL (corrected for soil humidity). After pre-equilibration by shaking for about three days, 2 mL of the respective application

solution were added. The adsorption/desorption measurements were performed over a range of five BYI 02960-DFA concentrations (0.01 mg/L to 1.0 mg/L), covering two orders of magnitude. The samples were incubated at constant temperature in the dark and continuously agitated using an overhead shaker. After a certain time the suspensions were centrifuged and the supernatants were analyzed by LSC. Additionally, the pH values of the supernatants were determined.

For the desorption experiments the supernatants were removed, measured, and replaced by an equivalent volume of aqueous 0.01 M $CaCl_2$ solution. After agitation (for e.g. 24 h in the definitive test) and centrifugation, the supernatant was decanted, measured, and analyzed by LSC. One desorption step was performed for the samples of all five concentration levels whereas three desorption steps were performed for the samples of the highest concentration level.
Mass balances were calculated for the one sample of each concentration level. For this purpose, the remaining soil was freeze-dried, grounded, combusted, and analyzed by LSC.
Due to the stability of the test item, the partition of the test item was determined based on the amount of RA measured in the supernatant by LSC. The adsorption and desorption experiments were performed in triplicate.
2. Application procedures: The total amount of [1-^{14}C]BYI 02960-DFA delivered (6.5 mg) was dissolved in 4 mL TKA-water resulting in a solution containing approximately 1.6 mg test item/mL. In the definitive test, the soil to solution ratio for soils [redacted] an [redacted]4a, [redacted], [redacted] and [redacted] was 20 g soil to 20 mL of 0.01 M aqueous $CaCl_2$ solution. For soil [redacted] II, the soil to solution ratio was 16 g soil to 20 mL 0.01 M aqueous $CaCl_2$ solution. The nominal concentrations of radiolabeled BYI 02960-DFA were 0.01, 0.03, 0.1, 0.3, and 1.0 mg/L. Using pipette aliquots of 2 mL, the radiolabeled BYI 02960-DFA application solutions were added to the pre-equilibrated suspensions consisting of 16 or 20 g soil and 18 mL 0.01 M aqueous $CaCl_2$ solution.

3. Analytical procedures: The stability of the test item was determined by TLC of the control samples and of supernatants and extracts of the soil samples (highest concentration) in the pre-tests. The test item was considered to be stable (parental mass balance >90). The limit of quantification (LOQ) was set to three times the background radioactivity, i.e. about 0.96 Bq (0.33 ng). The lowest amount measured was about 5.5 Bq, i.e. about 5.7 times higher than the LOQ.
In the definitive test supernatants were analyzed by liquid scintillation counting (LSC). After the desorption step, for the calculation of the mass balance, the remaining soil was freeze-dried and combusted. The trapped 14CO2 after combustion was measured by LSC. Due to the stability of the test item, the partition of the test item was determined based on the amount of RA measured in the supernatant by LSC.

Table 7.4.2- 3: Physico-chemical characteristics of test soils

Soil ID / Batch #	**[redacted] am [redacted] 4a (HF) 20100308**	**[redacted] (HN) 20100308**	**[redacted] II (DD) 20100308**	**[redacted] (SA) 20090626**	**[redacted] (SF) 20090629**
Geographic Location (City / State / Country)	[redacted] / [redacted] / Germany	[redacted] / Germany	[redacted] / [redacted] / Germany	[redacted] / California / USA	[redacted] / Nebraska / USA
Texture Class[A]	Silt Loam	Loam	Clay Loam	Sandy Loam	Silty Clay loam
Sand[A] Silt[A] Clay[A]	23% 60% 17%	37% 42% 21%	33% 36% 31%	63% 26% 11%	19% 50% 31%
pH ($CaCl_2$) pH (Water) pH (KCl)	6.5 6.8 6.3	5.8 6.1 5.4	7.4 7.5 7.1	6.0 6.4 5.7	6.5 6.6 6.0
Organic Matter[B]	4.1%	5.0%	7.8%	0.9%	2.9%
Organic Carbon[C]	2.4%	2.9%	4.5%	0.5%	1.7%
Cation Exchange Capacity (CEC)	13.4 meq/100 g	10.1 meq/100 g	20.6 meq/100 g	7.4 meq/100 g	19.4 meq/100 g
Moisture at 0.33 bar	22.3%	22.5%	32.7%	9.4%	23.0%
Bulk Density Particle Density	1.05 g/mL	1.08 g/mL	1.00 g/mL	1.39 g/mL	1.03 g/mL
Soil Mapping Unit	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[A]) According to USDA classification
[B]) Calculated: Organic matter = organic carbon * 1.724
[C]) Determination method: Combustion

II. RESULTS AND DISCUSSION

A. Mass Balance

The overall material balance for all soils was in the range of 95.1-100.3% (mean: 97.2%) of the applied radioactivity. The complete material balances found at all sampling intervals demonstrated that no significant RA dissipated from the test vessels or was lost during processing.

Table 7.4.2- 4: Recovery of Total Radioactivity of BYI 02960 after Adsorption and Desorption Steps (Expressed as percentage of applied radioactivity)

Description Soil ID Conc. ID	Soil 1 HF (% of AR)	Soil 2 HN (% of AR)	Soil 3 DD (% of AR)	Soil 4 SA (% of AR)	Soil 5 SF (% of AR)
1.0 mg/L	98.2	97.6	97.7	100.3	98.7
0.30 mg/L	96.0	95.3	95.3	99.2	97.3
0.10 mg/L	96.2	94.8	95.1	98.9	97.0
0.030 mg/L	97.0	94.3	94.7	97.8	97.1
0.010 mg/L	96.2	93.6	95.4	105.2	101.2
Mean	96.7	95.1	95.6	100.3	98.3
SD	± 0.8	± 1.4	± 1.1	± 2.6	± 1.6

B. Stability and Recovery of Test Item

The stability of DFA in the test system used was confirmed by performing HPLC analyses prior to the definitive test. Recoveries of radioactivity in the 0.01 M $CaCl_2$ solutions of control samples without soil range from 99.8 to 100.7% of applied radioactivity (0 - 96 h). The test item was stable in control samples without soil.

C. Findings

In the definitive adsorption test 16.9 - 25.3%, 16.6 - 34.9%, 22.2 - 26.6%, 2.6 - 10.7% and 2.4 - 5.7% of the applied test item were adsorbed to soils [redacted]am [redacted] 4a, [redacted], [redacted] II, [redacted] and [redacted], respectively. The adsorption behavior of BYI 02960-DFA in the concentration range of two orders of magnitude (i.e. from 0.01 - 1.0 mg/L) was accurately described for all soils with the Freundlich equation. The correlation coefficient of the individual isotherms was in the range of 0.9699 - 0.9984. The calculated adsorption constants $K_{F(ads)}$ of the Freundlich isotherms for the five test soils ranged from 0.028 - 0.368 mL/g. The Freundlich exponents 1/n were in the range of 0.6902 - 0.9579, indicating that the concentration of the test item affected the adsorption behavior in the examined concentration range.

Table 7.4.2- 5: Concentration of BYI 02960 in the Solid and Liquid Phases at the End of the Adsorption Period

Concentration	Soil (mg/kg)	Solution (mg/L)	Percentage adsorbed	
Soil ID		HF		
Control	N/A	N/A		
0.010 mg/L	0.002	0.008	24.4	± 1.3
0.030 mg/L	0.008	0.022	25.3	± 0.5
0.10 mg/L	0.025	0.075	24.8	± 0.3
0.30 mg/L	0.067	0.234	22.2	± 0.3
0.99 mg/L	0.167	0.825	16.9	± 0.2
Soil ID		HN		
Control	N/A	N/A		
0.010 mg/L	0.003	0.006	34.9	± 1.3
0.030 mg/L	0.010	0.020	34.4	± 0.7
0.10 mg/L	0.030	0.070	30.0	± 1.2
0.30 mg/L	0.078	0.222	26.0	± 0.3
0.99 mg/L	0.165	0.828	16.6	± 0.8
Soil ID		DD		
Control	N/A	N/A		
0.010 mg/L	0.003	0.007	25.4	± 1.5
0.030 mg/L	0.010	0.022	26.6	± 0.4
0.10 mg/L	0.032	0.075	25.5	± 0.3
0.30 mg/L	0.091	0.228	24.1	± 1.0
0.99 mg/L	0.276	0.772	22.3	± 0.4
Soil ID		SA		
Control	N/A	N/A		
0.010 mg/L	0.001	0.009	10.7	± 0.6
0.030 mg/L	0.003	0.027	10.0	± 0.1
0.10 mg/L	0.008	0.092	7.5	± 0.1
0.30 mg/L	0.017	0.284	5.5	± 0.3
0.99 mg/L	0.026	0.966	2.6	± 0.2
Soil ID		SF		
Control	N/A	N/A		
0.010 mg/L	0.001	0.009	5.5	± 1.4
0.030 mg/L	0.002	0.028	5.7	± 1.1
0.10 mg/L	0.005	0.095	4.9	± 0.8
0.30 mg/L	0.011	0.289	3.7	± 0.3
0.99 mg/L	0.024	0.968	2.4	± 0.4

Table 7.4.2- 6: Concentration of BYI 02960 in the Solid and Liquid Phase at the End of First Desorption Phase

Concentration	Soil (mg/kg)	Solution (mg/L)	Percentage desorbed*	
Soil ID		HF		
Control	N/A	N/A		
0.010 mg/L	0.002	0.000	19.8	± 3.6
0.030 mg/L	0.006	0.002	26.3	± 2.3
0.10 mg/L	0.019	0.006	25.3	± 2.5
0.30 mg/L	0.050	0.017	25.1	± 3.5
0.99 mg/L	0.133	0.034	20.5	± 1.7
Soil ID		HN		
Control	N/A	N/A		
0.010 mg/L	0.003	0.001	18.9	± 1.6
0.030 mg/L	0.008	0.002	19.4	± 1.7
0.10 mg/L	0.024	0.006	21.2	± 1.6
0.30 mg/L	0.062	0.016	21.0	± 1.5
0.99 mg/L	0.138	0.027	16.2	± 0.4
Soil ID		DD		
Control	N/A	N/A		
0.010 mg/L	0.003	**	**	**
0.030 mg/L	0.010	0.000	3.8	± 0.8
0.10 mg/L	0.030	0.001	5.2	± 1.1
0.30 mg/L	0.085	0.004	5.5	± 2.6
0.99 mg/L	0.281	**	**	**
Soil ID		SA		
Control	N/A	N/A		
0.010 mg/L	0.001	0.000	42.8	± 4.1
0.030 mg/L	0.002	0.001	44.2	± 2.7
0.10 mg/L	0.004	0.003	43.0	± 2.8
0.30 mg/L	0.010	0.006	38.5	± 2.6
0.99 mg/L	0.025	**	**	**
Soil ID		SF		
Control	N/A	N/A		
0.010 mg/L	0.000	0.0001	25.7	± 13.16
0.030 mg/L	0.001	0.0005	24.9	± 8.82
0.10 mg/L	0.004	**	**	**
0.30 mg/L	0.011	**	**	**
0.99 mg/L	0.033	**	**	**

* Expressed as a percentage of the initially adsorbed material, one desorption step for all concentrations
** The subtraction of the remaining radioactivity in solution from the measured concentrations resulted in negative liquid concentrations for some samples. Therefore the mean concentration was not presented and the percentage of desorbed test item was not calculated for this concentration level

The adsorption coefficients $K_{F(ads)}$ were correlated with the organic carbon content of the soil, in order to get a comparability of the adsorption behavior in different soils. For DFA the calculated $K_{oc(ads)}$ values varied between 1.7 and 9.5 mL/g (mean: 6.8 mL/g).

One desorption step was performed for all concentrations. For soils [redacted] am [redacted] 4a and [redacted] this first desorption step resulted in highly significant correlation coefficients for the Freundlich isotherm with values of 0.9788 and 0.9884. When omitting the highest concentration, the first desorption step of soil [redacted] had a significant correlation coefficient as well (0.9801). At the end of the first desorption phase, 19.8 - 26.3%, 16.2 - 21.2% and 38.5 - 44.2% of the initially adsorbed

amount was desorbed in soils [redacted] am [redacted] 4a, [redacted] and [redacted], respectively. The other soils ([redacted] II and [redacted]) did not show an acceptable linearity of the Freundlich desorption isotherms and therefore, desorption constants could not be calculated.

The calculated desorption constants $K_{F(des)}$ of the Freundlich isotherms for the three evaluated test soils ranged from 1.903 - 4.509 mL/g. The respective mean $K_{oc(des)}$ values was 219.1 mL/g). The $K_{oc(des)}$ values were 12.7 to 57.1 times higher than the $K_{oc(ads)}$ values, indicating a strong binding of the test item to the soil. For the summary of results see Table 7.4.2- 7.

Table 7.4.2- 7: Adsorption and desorption constants of BYI 02960-DFA in five soils

Soil	Adsorption				Desorption			
	K_F (mL/g)	1/n	R^2	Koc (mL/g)	K_F (mL/g)	1/n	R^2	Koc (mL/g]
HF	0.228	0.9053	0.9945	9.5	2.911	0.975	0.9788	121.3
HN	0.226	0.8013	0.9903	7.8	4.509	1.013	0.9884	155.5
DD	0.368	0.9579	0.9984	8.2	*	*	*	*
SA	0.033	0.6902	0.9769	6.7	1.903**	1.052**	0.9801**	380.7**
SF	0.028	0.8194	0.9699	1.7	*	*	*	*
Mean	0.177	0.8348	0.9860	6.8	3.1	1.0	1.0	219.1

* The correlation coefficient determined for the Freundlich desorption isotherms was not significant
** For soil [redacted] (SA) the highest concentration (1 mg/L) was not included in the calculation of the Freundlich desorption isotherm due to a negative supernatant concentration calculated for one replicate

III. CONCLUSIONS

DFA can be classified as very mobile for adsorption and low mobile for desorption.

IIA 7.4.3 Column Leaching Studies with the Active Substance

In the EU and NAFTA this requirement is covered by the adsorption/desorption studies with the parent compound as presented in chapter IIA 7.4.1.
However, a soil column leaching study for the active substance was performed in order to fulfill a requirement of Brazilian registration authorities. A short summary of that study is given as follows.

Report:	**KIIA 7.4.3/01, [redacted], 2012**
Title:	Mobility of [Pyridine-2,6-^{14}C]-BYI 02960 in Brazilian Soils – Soil columns leaching method
Report No & Document No	2301-LIX-344-11 M-424966-01-2
Guidelines:	**OECD - Guideline for the Testing of Chemicals. Method 312 "Leaching in Soil Columns" (Adopted: 13 April 2004)**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The leaching behaviour of [pyridine-2,6-^{14}C]-BYI 02960 was studied in four Brazilian soils under laboratory conditions according to OECD Guideline Method 312 (2004). The four soil types were Argissolo (clay), Latossolo vermelho (clay), Neossolo (loamy sand/sandy loam) and Gleissolo humico (loam). Duplicate glass columns per soil type were filled with the respective soil to a height of 30 cm.

After conditioning with 0.01 M $CaCl_2$ solution [PYR-^{14}C]-BYI 02960 was applied to the top of the columns at a dosage approximating a maximum application rate of 1500 g a.i./ha. Monuron was used as a reference substance and was applied at a rate of 5 kg a.i./ha. After the application of test and reference substance each column was irrigated with 200 mm of artificial rain over a 48-hrs period, and the respective leachate samples were collected. After percolation, the soil columns were divided into six layers of ca. 5 cm each, and the soil samples were extracted with acetonitrile:water (80:20 v/v). Layers containing more than 10% of applied radioactivity (AR) were sequentially extracted until the last extract represented less than 10% of AR. Extracted radioactivity was analyzed by high-performance liquid chromatography (HPLC) with radiometric detection. The results of the HPLC analysis did not show degradation of the parent compound during the timescale of the study.

The average mass balance ranged from 96.6 - 106.3% in the four tested soils. Extractable radioactivity ranged for all soils from 73.75 - 95.26%, and non-extractable radioactivity ranged from 3.64 - 25.22%.

No radioactivity (<LOQ) was detected in the leachate of Argissolo and Gleissolo soils. Thus the AR was completely retained in Argissolo and Gleissolo soils, however, percolated to the leachate in Latossolo and Neossolo soils after application of the artificial rain. In the leachates of Latossolo and Neossolo mean 14.93 and 23.50% of AR were detected, respectively. Analysis of the leachates showed that there was detectable Monuron in the leachate of Argissolo soil (one replicate), Latossolo and Neossolo soils.

The maximum (mean) depth penetration of the radioactivity in the soil profiles was < 10 cm for Argissolo soil, > 30 cm for Latossolo, ≥ 30 cm for Neossolo, and ≤ 7.5 cm for Gleissolo soil, whereas the leaching depth of Monuron was ≤ 27.5, ≥ 30, > 30 and ≤ 7.5 cm in the respectively listed soils.

In order to compare leaching data from different experiments, a relative mobility factor (RMF) to a reference chemical such as Monuron was used. Monuron is known to be moderately mobile in the field. Knowing the RMF values of crop protecting compounds allows their classification into mobility classes. The relative mobility factor (RMF) for BYI 02960 was 0.45 for Argissolo soil, 1.0 for Latossolo, 1.0 for Neossolo soil and 1.0 for Gleissolo soil. Based on these values, BYI 02960 can be classified as little mobile in Argissolo and moderately mobile in Latossolo, Neossolo and Gleissolo soils.

IIA 7.4.4 Column Leaching Studies Rel. Metabolites, Degr. & React. Products

Column leaching studies were not performed for metabolites. This requirement is covered by the adsorption/desorption studies with the parent compound as presented in chapter IIA 7.4.2.

IIA 7.4.5 Aged Residue Column Leaching

Studies are not required under Regulation (EC) 1107/2009.

IIA 7.4.6 Leaching (TLC)

Studies are not required under Regulation (EC) 1107/2009.

IIA 7.4.7 Lysimeter Studies

Not required as potential leaching to groundwater can be predicted from the available data.

IIA 7.4.8 Field Leaching Studies

Based on the results of laboratory and modeling studies mentioned in the chapters before it is concluded that the mobility of BYI 02960 residues in soil is sufficiently understood after its intended use, and no concern with regard to groundwater contamination is indicated. This is supported by the results of terrestrial field dissipation study (see IIA 7.3.1). BYI 02960 residues remained in the upper 0-20 cm soil layer. Only small amounts below the LOQ could be detected to a maximum depth of 30 cm.

Mobility of BYI 02960 Residues in Soil - Summary

The equilibrium sorption BYI 02960 was studied in two batch equilibrium studies in the laboratory at 20°C with six different soils. The data were evaluated to derive FREUNDLICH isotherms. The resulting arithmetic mean value for 1/n, $K_{f\ (ads)}$ and Koc was 0.8657, 2.263 (L/kg) and 98.4 (L/kg. The desorption $K_{f\ (des)}$ and the normalized $K_{OC(des)}$ values were significantly higher (i.e. approx. 2 times) than those obtained for the adsorption phase, indicating that the test item once adsorbed to soil is not readily desorbed.
BYI 02960 can be classified as intermediate mobile for adsorption and low mobile for desorption.

Time dependent sorption (TDS) of BYI 02960 was studied in four soils a maximum period of 120 days under aerobic conditions in the dark at ca. 20 °C and 55% WHC_{max}. At the beginning of the study the distribution coefficient values R_{TDS} were 1.46, 1.91, 3.35 and 4.66 mL/g for soils AX, HF, HN and DD, respectively. Based on results from the ageing time of 120 days, the R_{TDS} values increased by a factor of 4.4, 2.9, 3.2 and 2.6 (mean of all four soils = 3.3).
A more detailed evaluation showed that the before-mentioned experimental data can be well described by a kinetic-sorption model, with excellent fits and reliable parameter estimates. Such time-dependent sorption data received for BYI 02960 constitute the prerequisite to adequately address the obvious TDS process in higher tier regulatory modeling, e.g. related to a potential groundwater contamination. For BYI 02960 a fitted mean Koc of 80.2 L/kg and the Freundlich exponent 1/n of 0.866 can be used in higher tier simulation runs.

The equilibrium sorption of 6-chloronicotinic was studied in experiments using five soils and an aquatic pond sediment. The arithmetic mean 1/n value for soil was 0.949, adsorption Koc values in soils ranged from 70 to 258, averaging 88.0 (L/kg). Similar K_f and K_{des} values for all soils and the pond sediment indicated a reversible equilibration between adsorption and first desorption phases.
BYI 02960-6-CNA can be classified as medium to high mobile for adsorption and desorption.

The equilibrium sorption of difluoroacetic acid was studied in experiments using five soils. The arithmetic mean 1/n value for soil was 0.8348, adsorption Koc values ranged from 1.7 to 9.5, averaging 6.8 (L/kg). The $K_{oc(des)}$ values were 12.7 to 57.1 times higher than the $K_{oc(ads)}$ values, indicating a strong binding of the test item to the soil. BYI 02960-DFA can be classified as very mobile for adsorption and low mobile for desorption.

IIA 7.4.9 Volatility - Laboratory Studies

Based on the results of vapor pressure and Henry's law constant determination it is concluded that significant volatilization of BYI 02960 in the environment is not expected. Therefore, no further laboratory experiments were considered necessary.

The result of the studies on vapor pressure and Henry’s law constant, determined in accordance with dossier chapters IIA 2.3.1 and IIA 2.3.2), are given below.

The vapor pressure of BYI 02960 is low, extrapolated to be 9.1 x 10^{-7} Pa for 20 °C ([redacted] 2008; M-309853-01-1).
Henry's law constant at 20 °C in distilled water of pH 4 to 9 is given with 8.2 x 10^{-8} Pa x m^3 x mol^{-1} ([redacted] 2011; M-414341-01-1).

IIA 7.5 Hydrolysis in Sterile Buffers of pH 4, 7 and 9

In accordance with Point IIA 2.9.1, tests on hydrolysis of BYI 02960 using radiolabelled test substance in sterile buffer solutions at pH 4.0, 7.0, and 9.0 in the absence of light is submitted. A summary of this study is repeated here, focusing on formation and hydrolytic degradation of metabolites.

Report:	**KIIA 7.5/01, [redacted] and [redacted], 2011**
Title:	BYI 02960: Hydrolytic Degradation
Report No & Document No	MERVP019 M-398952-01-1
Guidelines:	**US EPA subdivision N, Section 161-1** **Canada PMRA DACO Number 8.2.3.2** **OECD 111, , proposal October 2002.** **JAPAN: MAFF Guideline, 12 Nousan 8147**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

Hydrolysis of radiolabeled [furanone-4-^{14}C]BYI 02960 at 1.0 mg a.i./L was studied in the dark at 50 °C in sterile aqueous buffers at pH 4 [acetate buffer], pH 7 [tris buffer], and pH 9 [borate buffer] for 5 days. Samples were analyzed at 0, 1, 2, 3, 4 and 5 days. The samples were analyzed directly by HPLC without extraction.
The results showed that BYI 02960 is hydrolytically stable at ambient temperature. Most of the applied radioactivity was associated with the parent compound at test termination in the pH 4, 7 and 9 buffer solutions. There were three minor components which accounted for a total of 4.9% of AR, none was more than 2.7% of AR in any of the pH.
A preliminary study was conducted at 50 °C to determine if volatiles would be formed. It demonstrated that no volatiles were formed during hydrolysis. This result was further confirmed by the acceptable recoveries observed in the definitive experiments.

I. MATERIAL AND METHODS

A. Materials

1. Test Item: Code BYI 02960, CAS no. 951659-40-8 (unlabelled substance)
Label position = [Furanone-4-^{14}C]BYI 02960 (vial No. C-1116)
Specific activity: 30.74 mCi/mMole (3.94 MBq/mg)
Radiochemical purity: 99% (at beginning of study)

Identity and purity of test item in the application solutions were checked. The radiochemical purity of the dosing solution (test item in85 µL acetonitrile, each) was determined by HPLC and found to be 96%.

2. Test matrix: The test matrices for this study were a 0.01 M sodium acetate/acetic acid solution (pH 4), a 0.01 M tris(hydroxymethyl)aminomethane/HCI solution (pH 7), and a 0.01 M boric acid/NaOH solution (pH 9). The acetate, tris, and borate buffers were used since they are unlikely to affect the rate of hydrolysis at their respective pH.

B. Methods

1. Experimental conditions: The test system consisted of a 30 mL amber vial containing 10 mL of buffer and capped with a septum lined crimp cap. It was autoclaved for 60 minutes at >93 °C to sterilize the system. Forty-five test systems were prepared consisting of three groups of 15 vials, one group for each pH (4, 7, and 9). This included 6 time points, 2 replicates per interval and 3 extra vials. Each vial was filled with 10 mL of the appropriate buffer and sealed with a crimp cap equipped with a Teflon™-lined septum. The test systems were autoclaved for 60 minutes at >93 °C to sterilize the system. Test systems were allowed to cool to room temperature before the addition of the test material. No volatiles were observed in the preliminary experiment. Therefore, no trapping system was used.
The test systems were maintained in a covered water bath. The temperature was held at 50 ±0.5 °C.
The pH and sterility were measured at each sampling interval.

2. Application Procedures: 100-µL Hamilton syringe was used to deliver 85 µL of application solution to each test containing 10 ml of buffer. The septum of the each vial was pierced by the syringe, and the application solution was added. To check the DPM applied to the system, using the same syringe, 85 µl of application solution was first put into two vials containing 10 ml of water. Then all the buffers were treated with 85 µl of application solution. After this another aliquot of 85 µl was put into two vials containing 10 ml of water. Three aliquots were taken from each vial for a total of 12 samples and analyzed by LSC which gave average of 240,371 dpm /mL equivalent to 1 ppm

3. Sampling: The sampling intervals for all three pH values were 0, 1, 2, 3, 4 and 5 days post treatment. Samples were typically analyzed the day of sampling with the maximum storage duration before analysis of less than 24 hrs. The samples were stored under refrigerated conditions before analysis if analysis was not conducted on the sampling date. No storage stability data was generated because samples were analyzed within one day of collection.

4. Analytical procedures: At each interval, two replicate test systems for each pH were removed from the water bath, three 1-mL aliquots were counted on the liquid scintillation counter (LSC) and 1 mL of each sample was directly analyzed by HPLC.
The retention time for [^{14}C]BYI 02960 using the HPLC system was 22.0 minutes. Recovery of radioactivity from the HPLC column ranged from 94.2 - 99.2% with a mean recovery of 97.1%.
The linearity of the detector's response to ^{14}C was confirmed. The limit of quantitation (LOQ) was determined empirically by a series of injections at decreasing concentrations. The lowest concentration that resulted in a peak 2 to 3 times the background level was determined to be the LOQ. The limit of quantitation used in this experiment was 890 dpm. Defining 445 dpm as the limit of detection (LOD) of the HPLC radio detector a minimum of approximately 1.1% or 1.6 ppb of AR was detected by HPLC analysis.
No attempt was made to identify the minor transformation products observed in the study since they comprised <2.7% of the applied radioactivity, at any interval.

The rate of degradation was not determined for BYI 02960 since minimal degradation was observed in the pH 4, 7 and 9 buffer solutions.

II. RESULTS

A. Data

All buffer solutions (pH 4 = acetate, pH 7 = tris, pH 9 = borate) had a concentration of < 0.01 M. The measured average pH of the buffers was 3.94, 6.89 and 8.99, respectively.
Sterility was maintained throughout the study with the exception of a single time point in a single pH. The Day 4, pH 9.0, replicate 1 and 2 samples gave a positive result (sterility was lost) on the aerobic count plate. The samples were analyzed by HPLC to determine if a lack of sterility was an issue. The results showed comparable results to the other test system (stable), thus the lack of sterility did not impact results.
The test systems were maintained in the dark, in a covered water bath held at 50.1 ±0.13 °C (minimum = 49.7 °C, maximum = 50.3 °C).
The resulting data based on LSC and analyses are shown in Table 7.5- 1 to Table 7.5- 3.

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 tables.
The mean material balance for pH 4 buffer ranged from 95.8 - 100.9% of AR, with a mean of 98.8% (Table 7.5- 1). The mean material balance for pH 7 ranged from 95.0 - 102.7% of AR, with a mean of 99.9% (7.5- 2). The mean material balance for pH 9 ranged from 100.3 - 101.2% of AR, with a mean of 100.7% (Table 7.5- 3). Individual values are provided for pH 4, 7 and 9 in the report. Adsorption of the test compound to the test vessels was not indicated based on the material balances. 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.

Table 7.5- 1: Transformation of BYI 02960 @ 50 °C, expressed as percentage of applied radioactivity (mean ± S.D.), in pH 4 buffer solution

Sampling times [days]	0	1	2	3	4	5
BYI 02960	96.2 ± 1.2	96.1 ± 1.9	91.2 ± 0.2	93.7 + 1.6	96.3 ± 0.5	92.1 ± 3.6
Unknown A	2.7 ± 0.1	2.2 ± 0.0	1.9 ± 0.1	1.7 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
Unknown B	0.0 ± 0.0	1.0 ± 0.3	1.4 ± 0.0	1.5 ± 0.0	1.8 ± 0.2	1.7 ± 0.1
Unknown C	1.6 ± 0.2	1.5 ± 0.0	1.3 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.2
Total% recovery	100.6 ± 0.9	100.9 + 1.6	95.8 + 0.1	98.4 + 1.6	100.7 ± 0.7	96.5 + 3.8

Table 7.5- 2: Transformation of BYI 02960 @ 50 °C, expressed as percentage of applied radioactivity (mean ± S.D.), in pH 7 buffer solution

Sampling times [days]	0	1	2	3	4	5
BYI 02960	94.5 ± 1.0	90.7 ± 6.0	94.3 ± n/a	95.1 ± 2.1	97.8 ± 0.1	96.6 ± 0.4
Unknown A	2.6 ± 0.1	1.8 ± 0.2	1.8 ± n/a	1.6 ± 0.0	1.6 ± 0.0	1.5 ± 0.2
Unknown B	0.4 + 0.6	1.1 ± 0.1	1.6 ± n/a	1.8 ± 0.0	1.8 + 0.3	1.8 + 0.1
Unknown C	1.4 ± 0.1	1.4 ± 0.1	1.4 ± n/a	1.2 ± 0.0	1.5 ± 0.1	1.3 ± 0.1
Total% recovery	98.8 ± 1.6	95.0 ± 5.9	99.0 ± n/a	99.7 ± 2.2	102.7 ± 0.2	101.3 ± 0.3

Table 7.5- 3: Transformation of BYI 02960 @ 50 °C, expressed as percentage of applied radioactivity (mean ± S.D.), in pH 9 buffer solution

Sampling times [days]	0	1	2	3	4	5
BYI 02960	95.8 ± 0.7	97.5 ± 1.1	96.2 ± 0.2	95.8 ± 0.1	96.9 ± 0.2	95.8 ± 1.1
Unknown A	1.8 + 0.0	1.4 + 0.3	1.5 ± 0.3	1.5 + 0.1	1.2 ± 0.0	1.4 + 0.1
Unknown B	1.5 ± 0.2	0.9 ± 1.3	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.0	1.9 ± 0.1
Unknown C	1.4 ± 0.0	1.4 ± 0.2	1.2 ± 0.1	1.1 ± 0.0	1.2 ± 0.1	1.1 ± 0.1
Total% recovery	100.6 ± 0.9	101.2 ± 0.2	100.5 ± 0.4	100.3 ± 0.1	101.2 ± 0.1	100.3 ± 1.2

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

The amounts of BYI 02960 and degradates were quantified by HPLC. The results for all tests, expressed as percent AR, are given in tables above. Throughout, the concentration of parent compound remained almost constant from day 0 to day 5, with 96.2 - 92.1% of AR at pH 4, 94.5 - 96.6% of AR at pH 7, and 95.8 - 95.8% of AR at pH 9, respectively.
Minimal degradation of the parent compound occurred at all three pH values. No major transformation products were formed at any pH. Three minor degradates “Unknown A, B and C” at maximum amounts of 2.7% of AR.

F. Kinetics of Test Item Degradation

The rate of degradation was not determined for BYI 02960 since minimal degradation was observed in the pH 4, 7 and 9 buffer solutions. Thus, BYI 02960 can be considered to be hydrolytically stable under environmental conditions.

III. CONCLUSIONS

From the current laboratory study it is concluded that hydrolysis is not relevant for the degradation of BYI 02960 in the environment, and that a further study with another radiolabel is not needed.

IIA 7.6 Phototransformation in water

In accordance with Points IIA 2.9.2 and IIA 2.9.3, tests on photolysis (direct photo-transformation) of BYI 02960 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 IIA 2.9.2) radiolabeled BYI 02960 was investigated in pure buffers. 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.

Further a supplementary study on the phototransformation of BYI 02960 in natural water is submitted.

Report:	**KIIA 7.6/01, [redacted] 2011**
Title:	Phototransformation of [^{14}C]BYI 02960 in aqueous pH 7 buffer
Report No & Document No	MERVP042 M-418426-02-1
Guidelines:	**OPPTS 835.2240, 2008** **Japanese JMAFF New Test Guidelines, 2000** **Canada PMRA DACO Number 8.2.3.3.2**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The aqueous phototransformation of [FUR-^{14}C]BYI 02960 was studied at 25 °C in sterile buffer (pH 7) at an initial concentration of approx. 1.0 mg a.i./L under artificial irradiation (xenon lamp, >290 nm, quartz and Suprax® filter) for 35 hours. At this timepoint the DT_{75} for BYI 02960 was exceeded.

Duplicate irradiated test systems and single dark control test systems were analyzed at 0, 4, 8, 12, 16, 22, 28, and 35 hours by direct injection of samples into HPLC. Identification of parent and major transformation products was accomplished by co-elution with authentic reference standards, LC/MS and/or NMR analysis.

The anticipated test conditions (temperature, sterility and pH 7) were maintained, and material balances for the irradiated and dark test systems were complete throughout the study. In the dark test systems (i.e. the controls), BYI 02960 was found to be stable.

In the irradiated test systems, BYI 02960 decreased from 98.1% of AR at time 0 (mean of replicates) to 8.4% of AR at 35 hours of irradiation. The major degradates included BYI 02960-succinamide (max. 39.6% of AR at 28 hours) and BYI 02960-azabicyclosuccinamide (max. 25.9% of AR at 35 hours). A minor degradate was identified as BYI 02960-deschlorohydroxysuccinamide (DCHS, max. of 2.5% at 35 hours) which is considered as intermediate between the two major photodegradates. No other single detected component exceeded 3% of AR at 35 hours.

The first-order half-life for photolytic degradation of BYI 02960 in sterile buffer of pH 7 was 13.8 experimental hours. Based on this experimental half-life of BYI 02960, the direct phototransformation half-life in top layer of surface water is calculated to be 1.75 days under environmental sunlight conditions in Phoenix, AZ, USA (latitude 33.3°N) and 2.7 days in Athens, Greece (latitude 38.03N).. Based on this finding, BYI 02960 should rapidly degrade in the aqueous environment if exposed to sunlight.

Table 7.6- 1: Half-life, DT_{75} and DT_{90}

Test System	Kinetic Model	Equation	χ^2 test error (%)	Experimental hours DT_{50}	DT_{75}	DT_{90}
Irradiated	SFO	$M_{(t)} = 103.3^{-0.0503\,t}$	10.4	13.8	27.6	45.8
Dark	SFO	$M_{(t)} = 99.7e^{-(1.2e-10)\,t}$	0.50	>>1000	nc	nc

nc = not calculated since compound is stable

I. MATERIAL AND METHODS

A. Materials

1. Test Item: Code BYI 02960, CAS no. 951659-40-8 (unlabelled substance)
Label position = [Furanone-4-^{14}C]BYI 02960 (vial No. C-1116)
Specific activity: 30.74 mCi/mMole (3.94 MBq/mg)
Radiochemical purity: 99.3% (at beginning of study)
Identity and purity of test item in the application solutions were checked.

2. Test system: The irradiated test system consisted of 20 mL of filter-sterilized sterile buffer (pH 7) treated with BYI 02960 at ~1.0 µg/mL and sealed in a quartz glass vessel. The dark control test systems consisted of 20 mL of filter-sterilized buffer (pH 7) treated with BYI 02960 at ~1.0 µg/mL and crimp-sealed in a 20-mL amber bottle.

3. Test matrix: The test matrix for this study was comprised of sterile 10 mM potassium phosphate buffer (pH 7). Buffer was prepared by dissolving 2.72 g of potassium dihydrogen phosphate in ~1.9 L of water. The pH was adjusted to 7 by the addition of 1 M potassium hydroxide. After adjustment to pH 7, the buffer was diluted to 2.0 L and thoroughly mixed. The buffer was stored at room temperature overnight and used immediately the following morning.

B. Methods

1. Experimental conditions: Twenty four test systems were prepared for irradiation. Duplicate test systems were prepared for each of the nine planned sampling intervals as well as six spare test systems for additional sampling intervals or for unforeseen losses. Each quartz glass vessel was filled with 20 mL of filter-sterilized sterile buffer (pH 7) containing approximately 1.0 µg/mL of test substance. Ten control test systems were prepared and were maintained in the dark at 25 ± 1°C in an incubator.

2. Application procedures: Treated buffer (pH 7) was sterilized by passing it through a 0.22-µm sterile filter into each autoclaved test vessel using aseptic techniques in a laminar flow hood. The amount of water added to each vessel was determined by the weight (approximately 20 g) added to a tared vessel. The irradiated test vessels were closed with ground glass stoppers. The quartz photolysis vessels were placed in a Suntest CPS unit containing a Heraeus xenon-arc lamp. Light emission was filtered using a Suprax® filter that eliminated wavelengths <290 nm. For the dark control test systems, the same amount of treated sterile buffer (pH 7) was filter-sterilized and transferred into sterile amber bottles which were sealed with crimp caps.
The test substance concentration was 1.11 µg/mL which is much less than half of the water solubility of BYI 02960 at pH 7 (water solubility is 3.2 mg/mL).

3. Sampling: Duplicate irradiated test systems and individual dark control test systems were analyzed at 0, 4, 8, 12, 16, 22, 28, and 35 hours post treatment. All test systems were checked for pH and sterility. Samples were immediately analyzed after removal from either the incubator or the Suntest.

4. Analytical procedures: Triplicate aliquots (100 µL) from each test system were radioassayed. For all samples, a 1-mL aliquot was removed and used for sterility testing. An aliquot (approximately 4 mL) was transferred to an HPLC autosampler vial, and the remaining test solution was transferred to a glass vial. All samples were directly analyzed by HPLC (1 mL injection) without any concentration or extraction.

Major degradates, including the parent compound, were either isolated by HPLC from an MID sample or a kinetic sample was analyzed directly by LC/MS. Collected fractions were dissolved in water and analyzed by LC/MS. Some degradates were isolated and analyzed by high resolution MS and NMR.

II. RESULTS

A. Data

The target temperature of 25 ± 1 °C was maintained during the study, and the mean pH of the water was 6.99 in the irradiated systems and 7.01 in the dark test systems. Sterile conditions were maintained in all test systems. The irradiated systems were continuously exposed to artificial sunlight with an intensity of 680 W/m^2 for a period of 35 hours. The analytical results of the study are presented in Table 7.6- 2.

B. Mass Balance

The mean material balance of the irradiated [^{14}C]BYI 02960 test systems was 100.6% and ranged from 99.3 to 101.6% in individual systems. The mean material balance for the dark control test systems was 101.1% and ranged from 99.6 to 101.4% in individual test systems.

C. Bound and Extractable Residues

N/A.

D. Volatilization

No attempt was made to trap volatiles. This was justified since the mass balances were complete throughout the study.

E. Transformation of Test Item

Four radioactive components exceeded 10% of AR at any interval, one of these was [^{14}C]BYI 02960. Two major degradates were identified as BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide. The fourth region that exceeded 10% of AR was a group of components ("polar mixture") which eluted early in the chromatogram in reverse-phase HPLC. By TLC it was shown to consist of multiple components, and no single component exceeded 3% of the applied radioactivity at 35 hours. One minor degradate was identified as BYI 02960-dechlorohydroxy-succinamide (DCHS) by comparison to a synthetic standard.

Table 7.6- 2: Phototransformation of [^{14}C]BYI 02960 in irradiated test systems (mean ± SD) as a percentage of applied radioactivity (1)

Compound	Sampling times [days]			
	0	**4**	**8**	**12**
BYI 02960	98.1 ± 0.2	86.8 ± 0.1	71.8 ± 2.2	55.3 ± 2.5
BYI 02960-succinamide	0.0 ± 0.0	9.40 ± 0.1	17.30 ± 0.8	26.0 0 ± 4.2
BYI 02960-azabicyclosuccinamide	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.1	2.4 ± 0.8
DCHS (2)	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1	0.9 ± 0.3
Polar Region 2 (3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.7
Polar Region 1 (3)	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 0.6	3.8 ± 1.4
Minor others (4)	1.4 ± 0.3	4.6 ± 0.6	7.3 ± 0.3	11.4 ± 0.3
Total volatile organic	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total% recovery	99.5 ± 0.0	100.8 ± 0.6	100.3 ± 1.0	101.2 ± 0.4
	16	**22**	**28**	**35**
BYI 02960	55.7 ± 1.8	41.3 ± 4.7	14.1 ± 2.2	8.4 ± 1.4
BYI 02960 - succinamide	26.6 ± 0.7	32.7 ± 1.6	39.6 ± 0.1	37.6 ± 1.2
BYI 02960-azabicyclosuccinamide	2.8 ± 0.3	4.9 ± 1.1	19.4 ± 3.2	25.9 ± 1.9
DCHS (2)	0.8 ± 0.2	1.2 ± 0.3	2.1 ± 0.3	2.5 ± 0.4
Polar Region 2 (3)	1.5 ± 0.4	1.9 ± 0.1	3.0 ± 0.9	3.5 ± 0.3
Polar Region 1 (3)	2.5 ± 0.4	4.8 ± 0.2	6.9 ± 0.0	8.2 ± 1.5
Minor others (4)	10.3 ± 0.3	13.1 ± 1.2	16.4 ± 0.0	15.1 ± 0.9
Total volatile organic	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total% recovery	100.3 ± 1.4	100.1 ± 0.7	101.5 ± 0.2	101.2 ± 0.2

(1) Ppb analyte can be calculated as follows: Ppb analyte = Analyte as % of applied x (ppb parent applied ÷ 100%) x (MW analyte/MW parent); MW of BYI 02960 = 288.7
(2) DCHS – BYI 02960-deschlorohydroxysuccinamide - intermediate between BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide
(3) Polar regions combined and analyzed by TLC. No single component comprised >3% of the applied radioactivity
(4) Minor others is comprised of several compounds, none of which comprised >7% of the applied radioactivity at any interval

F. KINETICS OF TEST ITEM DEGRADATION

Half-life, DT_{75} and DT_{90} of [^{14}C]BYI 02960 are presented in Table 7.6- 3. The first-order half-life for photolytic degradation of BYI 02960 in sterile buffer of pH 7 was 13.8 experimental hours. Based on this experimental half-life of BYI 02960, the direct phototransformation half-life in top layer of surface water is calculated to be 1.75 days under environmental sunlight conditions, e.g. in Phoenix, AZ, USA (latitude 33.3°N) and 2.7 days in Athens, Greece (latitude 38.03N).. Based on this finding, BYI 02960 should rapidly degrade in the aqueous environment if exposed to sunlight.

Table 7.6- 3: Half-life, DT_{75} and DT_{90}

Test System	Kinetic Model	Equation	χ^2 test error (%)	Experimental hours			Equivalent days in Phoenix, AZ; USA		
				DT_{50}	DT_{75}	DT_{90}	DT_{50}	DT_{75}	DT_{90}
Irradiated	SFO	$M_{(t)} = 103.3^{-0.0503\ t}$	10.4	13.8	27.6	45.8	1.8	3.5	5.8
Dark	SFO	$M_{(t)} = 99.7e^{-(1.2e-10)\ t}$	0.50	>>1000	nc	nc	nc	nc	nc

nc = not calculated since compound is stable

III. CONCLUSIONS

[^{14}C]BYI 02960 photolytically degraded in sterile buffer (pH 7) with a half-life of 13.8 experimental hours. Based on this finding, BYI 02960 should degrade within a few days in the aqueous environment if exposed to sunlight. The major degradates (>10% of applied radioactivity) were identified as [^{14}C]BYI 02960-succinamide and [^{14}C]BYI 02960-azabicyclosuccinamide. The findings were included in the proposal for the pathway of degradation of BYI 02960 in an aqueous environment (see Figure 7.8- 1).

Report:	**KIIA 7.6/02, [redacted]; 2011**
Title:	BYI02960: Determination of the Quantum Yield and Assessment of the Environmental Half-life of the Direct Photodegradation in Water
Report No & Document No	MEF-11/554 M-414756-01-2
Guidelines:	**Phototransformation of Chemicals in Water, Part A: Direct Phototransformation, UBA, Berlin, Germany (Dec 1992); Test Method: ECETOC (Polychromatic Light Source)**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The quantum yield of direct phototransformation of BYI 02960 was determined in aqueous solutions using polychromatic light according to the ECETOC method. Degradation of BYI 02960 in aqueous solution of ≤10% was measured by HPLC-UV after a maximum irradiation period of 500 minutes. This indicated moderate degradability of BYI 02960 via direct phototransformation in aqueous and buffered solutions. A mean quantum yield of Φ = 0.000138 was calculated on the basis of UV absorption data and the degradation kinetics determined from both experiments.

The estimates based on the two modelling concepts (Zepp & Cline or Frank & Kloepffer) are comparable. Both estimates consider the quantum yield Φ and the absorption in the UV-VIS spectrum being in the range of wavelengths relevant for the environment (see tables below). Environmental direct phototransformation half-lives of BYI 02960 in sunlight exposed surface water layers are estimated to in a range of 10 to 14 days during periods of main use, i.e. in spring to summer.

Thus, direct phototransformation in aqueous solution may contribute to the dissipation of BYI 02960 from the environment. This assessment does not consider other potential mechanisms which may enhance the degradation in natural water, e.g. by indirect photolytic processes.

Table 7.6- 4: Zepp and Cline modelling using GC Solar program

Season	Environmental DT_{50} of Direct Phototransformation of BYI 02960 [days]			
	30th degree lat.	40th degree lat.	50th degree lat.	60th degree lat.
Spring	11.4	12.2	13.6	16.2
Summer	10.3	10.3	10.7	11.3
Fall	15.9	20.2	29.5	54.2
Winter	21.2	32.2	63.2	195

Marginal conditions: pure surface water at 0-5 cm depth, 10th degree longitude, clear sky, typical ozone concentrations in the atmosphere, half-lives integrated over the entire day.
The columns of the 40-50th degree of latitude is more or less relevant to the conditions of Europe.

Table 7.6- 5: Frank and Kloepffer Modeling (MEF-11/554)

Month	Photolysis Constant [1/sec]	Environmental DT_{50} of Direct Phototransformation of BYI 02960 [days]		
		Minimum	Mean	Maximum
January	0.501 x 10-7	76	160	730
February	0.104 x 10-6	37	77	340
March	0.203 x 10-6	21	40	160
April	0.340 x 10-6	13	24	94
May	0.434 x 10-6	12	18	74
June	0.486 x 10-6	11	17	66
July	0.433 x 10-6	12	19	62
August	0.423 x 10-6	13	19	63
September	0.246 x 10-6	19	33	120
October	0.135 x 10-6	31	59	270
November	0.601 x 10-7	58	130	670
December	0.316 x 10-7	120	250	1300

Marginal conditions: pure stagnant surface water at 0-5 cm depth, geographic and climatic conditions of Germany (50th degree latitude); no contribution of another mono- or bimolecular elimination process.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: Common name: Flupyradifurone (CAS no. 951659-40-8)
Chem. Code: BYI 02960
Sample ID: AZ16895, Batch NLL7780-47-4
Certified assay: 99.4% w/w

2. Test Solutions: The study was conducted using highly pure water (taken from a TKA-Genpure unit): conductivity = 0.055 µS; TOC = 2 ppb. UV-VIS spectra were measured in 0.01 M aqueous buffer solutions (acetate pH 4, phosphate pH 7 and borate pH 9). A solution containing 5.03 mg BYI 02960 (1.74 x 10^{-5} mol)/L pure water was prepared for irradiation experiments.

B. Methods

The quantum yield for direct phototransformation of BYI 02960 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 merry-go-round irradiation apparatus is warmed up for at least 15 minutes prior to the exposure of the samples in order to guarantee a constant radiation of the light source as well as the projected sample temperature of 25°C ± 1°C at the beginning of the experiment. Subsequently, only the merry-go-round but not the lamp or the cycle cooling is switched off for adding or removing samples.
After equilibration, two measuring cells with 3.0 mL of actinometer solution (A) are first exposed to the light in the system for 10 minutes. The measuring cells containing 3.0 mL of test solution are swiftly placed onto the 10 positions of the merry-go-round apparatus. At the respective sampling interval a single sample is removed. Finally, *i.e.* after both degradation experiments were finished, two samples with solution (A) were irradiated and used for actinometry.
The results of BYI 02960 analysis (usually means of duplicates) were evaluated on the basis of linear regression in order to receive the time (in minutes) after which 10% of the molecules of the test item have been degraded. That value is necessary to calculate the quantum yield of phototransformation

II. RESULTS

A. Data

Intensity of irradiation was calculated to 6.023 x 10^{16} (experiment #1) and 6.1599 x 10^{16} (experiment #2) photons absorbed per second for the 3 mL actinometry solution in the range of wavelength from 295 to 490 nm. Phototransformation results are presented in Table 7.6- 6.

Table 7.6- 6: Phototransformation of BYI 02960 in water

Duration of Irradiation [min]	Experiment #1 BYI 02960 [mg/L]	Experiment #2 BYI 02960 [mg/L]
0	5.03 (= 100%)	5.03 (= 100%)
50	4.90	4.97
100	4.89	4.91
150	4.93	4.87
200	4.89	4.83
250	4.81	4.83
300	4.71	4.77
350	4.72	4.79
400	4.64	4.70
450	4.58	4.61
500	4.56 (= 90.7%)	4.69 (= 93.2%)

A degradation of BYI 02960 of 7 and 10% was determined by HPLC-UV after a maximum irradiation period of 500 minutes. This indicated moderate photostability of BYI 02960 in the aqueous buffered solution.
Based on both degradation experiments quantum yields Φ of 1.5538 x 10^{-4} (experiment #1) and 1.1983 x 10^{-4} (experiment #2) were calculated.
Environmental half-lives were calculated according to Zepp & Cline and Frank & Kloepffer (see Table 7.6- 4 and Table 7.6- 5).

III CONCLUSIONS

A mean quantum yield of direct phototransformation of Φ = 0.000138 was calculated for BYI 02960 on the basis of UV absorption data and the degradation kinetics determined from both experiments.

Thus, direct phototransformation in aqueous solution may contribute to the dissipation of BYI 02960 from the environment. A comparison of the estimates derived from models of Zepp & Cline and Frank & Kloepffer shows that both approaches are well comparable. Environmental direct phototransformation half-lives of BYI 02960 in sunlight exposed surface water layers are estimated to in a range of 10 to 14 days during periods of main use, i.e. in spring to summer.
However, this assessment does not consider any other potential mechanisms which may enhance the degradation in unpurified water, i.e. caused by indirect photolytic processes. May be such was the difference to the kinetics result of the earlier study not performed in pure water but in buffer (report KIIA 7.6/01). Thereby, BYI 02960 degraded faster, i.e. with an estimated DT_{50} of just a few days in an aqueous environment if exposed to sunlight.

Report:	**KIIA 7.6/03, [redacted] 2011**
Title:	Phototransformation of [^{14}C]BYI 02960 in Natural Water
Report No & Document No	MERVP020 M-415368-01-1
Guidelines:	**Japanese Test Guidelines for Supporting Registration of Chemical Pesticides 12 Nousan 8147 (adopted in November 24, 2000), J-MAFF 2-6-2 (amended June 26, 2001 and March 31, 2008) concerning Photolysis Studies in Water and Guidance Notification 13, Seisan No. 3986, October 10, 2001.**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY
The aqueous phototransformation of [FUR-^{14}C]BYI 02960 was studied at 25 °C in sterile natural water at an initial concentration of 1 mg a.i./L under artificial irradiation (xenon lamp, >290 nm, quartz and Suprax® filter) for max. 28 hours, equivalent to max 7.5 environmental days at [redacted], Japan. This duration of irradiation exceeded the DT_{50} for BYI 02960 in the experiment. Duplicate irradiated and single dark test systems were analyzed at 0, 4, 8, 12, 16, 22 and 28 hours by direct injection of samples into HPLC. Identification of parent and major transformation products was accomplished by co-elution with authentic reference standards, LC/MS and/or NMR analysis.

The anticipated test conditions (temperature and sterility) were maintained, and material balances for the irradiated and dark test systems were complete throughout the study. In the dark test systems (i.e. the controls), BYI 02960 was found to be stable, showed very little degradation at pH 8 and 25 °C.

In the irradiated test systems, [^{14}C]BYI 02960 degraded from 95.1% of AR at time 0 (mean of replicates) to 17.2% of AR after 28 hours of irradiation. The major degradates included BYI 02960-succinamide (max. 38.2% at 28 hours) and BYI 02960-azabicyclosuccinamide (max. 14.3% at 28 hours). A minor degradate was identified as BYI 02960-deschlorohydroxysuccinamide (maximum of 2.2% at 22 hours) and is considered an intermediate leading to the formation of BYI 02960-azabicyclosuccinamide. A group of components (“polar mixture”) that eluted near the HPLC column’s void volume was shown by TLC to consist of multiple components, and none of these components exceeded 7.3% of the applied radioactivity.

The first-order half-life for photolytic degradation of BYI 02960 in the natural water was 14.0 experimental hours. Based on this value the half-life of BYI 02960 under environmental conditions is calculated to be, e.g., 3.8 days at Tokyo, Japan (latitude 35°N). Thus, phototransformation in aqueous

solution contributes very well to the dissipation of BYI 02960 from the environment. Therefore, the findings were included in the proposal for the pathway of degradation of BYI 02960 in an aqueous environment (see Figure 7.8- 1).

I. MATERIALS AND METHODS

A. Materials

1. Test Item: Flupyradifurone: Code = BYI 02960; CAS no. 951659-40-8 (unlabeled)
Designation of label: [furanone-4-^{14}C]BYI 02960 or [FUR-^{14}C]BYI 02960
Specific Activity: 30.74 mCi/mmol (~236,400 dpm/µg; 3.94 MBq/mg)
Radiochemical Purity: 99.3%

2. Test System: The test matrix for this study was comprised of natural water collected on March 29, 2010 from the lake at [redacted]) Kansas. Water was collected at the surface, 0-6 inches deep. The natural water was stored at room temperature overnight and used immediately the following morning.

Table 7.6- 7: Physicochemical characteristics of unfiltered natural water

Parameter	Results/Units [(a)]
pH	8.2
Dissolved oxygen	8.43 mg/L [(b)]
Calcium	67 mg/L (ppm)
Magnesium	12 mg/L (ppm)
Hardness ($CaCO_3$ equivalent)	217 mg/L
Electrical conductivity	0.88 mmho/cm
Total Dissolved Solids	486 mg/L (ppm)
Turbidity	3.53 NTU
Alkalinity	158 mg $CaCO_3$/L
Total Organic Carbon	4.3 mg/L (ppm)
Dissolved Organic Carbon	3.7 mg/L (ppm)
Carbonates	0.58 meq/L
Bicarbonates	2.16 meq/L
Total Nitrogen	1.1 mg/L (ppm)
Total Phosphorus	0.9 mg/L (ppm)

B. Methods

1. Experimental conditions: The tests were performed using individual static test systems held at aerobic conditions at 25 ± 1 °C for a period of 6 experimental days. They consisted of 24 quartz glass vessels [50 mm x 25 mm x 15 mm (height)] each containing 20 mL of the test solution (buffer solution + 1.0 µg/mL 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. 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). The Suntest unit was operated at approximately 680 W/m^2 (290-800 nm) to simulate highly photoreactive conditions. At this intensity it would take 3.71 hours in the Suntest unit to equal one solar day in Tokyo, Japan. Since 3.71 hours represents 1 environmental day, 111.4 hours (4.64 days) of continuous irradiation at 680 W/m^2 is equivalent to 30 environmental days.

2. Sampling: Duplicate irradiated test systems and individual dark control test systems were analyzed at 0, 4, 8, 12, 16, 22, and 28 hours post treatment. All samples were immediately analyzed after removal from either the incubator or the Suntest, with analyzes completed within 3 hrs. Volatile traps were not used.

3. Description of analytical procedures: The radioactivity of the test solutions was radio-assayed by triplicate 100-µL aliquots. All samples were directly analyzed by HPLC without any concentration or extraction. Identification and confirmation of the parent compound and transformation products was done by co-chromatography (LC-MS/MS and NMR techniques).

C. Determination of Degradation Kinetics

The photolytic degradation of BYI 02960 in the natural water was characterized using kinetics modeling. A simple first order (SFO) model was used for determination of rate constants. The mean percentage (two replicates) of applied radioactivity as BYI 02960 of two replicates at all sampling times was used to determine the degradation rate and kinetic endpoints (modelling tool MATLAB (Ver. 7.0.4))

II. RESULTS AND DISCUSSION

A. Data

The pH of the test solutions was about pH 8 at the beginning irradiated and dark test systems. The test water was maintained sterile throughout the test period. The 22-hr dark control (sample 5C) showed the presence of seven colony forming units. However, this contamination did not have a significant impact on the degradation of BYI 02960 since the percentage of parent in the dark control sample at 22 hours was slightly higher than the percentage of parent in either the 16 or 28-hour samples.

B. Mass Balance

The mean material balance of the irradiated test systems was 98.1% and ranged from 95.7 - 99.7% in individual test systems (see Table 7.6- 8). The mean material balance for the dark control test systems was 98.5% and ranged from 97.1 - 99.8% in individual test systems.

C. Transformation of Test Item

Irradiated samples: During the study, the concentration of [^{14}C]BYI 02960 in irradiated test systems decreased from 95.1 to 17.2% of the theoretical applied amount of radioactivity (mean of replicates) after 28 hours (see Table 7.6- 8). Only two degradates exceeded 10% of AR in 28 hours in the irradiated test systems. The major degradate was identified as [^{14}C]BYI 02960-succinamide which reached a max. of 38.2% of AR after 28 hours. [^{14}C]BYI 02960-azabicyclosuccinamide reached a max. of 14.3% of AR after 28 hours. A group of radioactive components eluted near the solvent front by HPLC in the irradiated samples (polar regions 1 and 2). These two regions were collected together and reanalyzed by TLC which showed that the polar region was comprised of many components present at low levels. No single component in the polar region comprised more than 7.3% of AR at 22 hours or 4.5% of AR at 28 hours. A minor degradate, [^{14}C]BYI 02960-deschlorohydroxysuccinamide, was identified by LC/MS comparison to a synthetic standard. The max. concentration of BYI 02960-deschloro-hydroxysuccinamide was 2.2% of AR at 22 hours.

Dark control samples: In the dark test systems, [^{14}C]BYI 02960 was more or less constant (98.0 and 96.0% (single values) after 28 hours. No single degradate exceeded 0.8% of the applied radioactivity at any sampling interval.

Table 7.6- 8: Phototransformation of [^{14}C]BYI 02960 in natural water test systems (mean ± SD) as a percentage of applied radioactivity (1)

Compound	Sampling times [days]						
	0	4	8	12	16	22	28
BYI 02960	95.1 ± 0.3	85.0 ± 1.0	72.7 ± 2.3	64.0 ± 1.0	55.7 ± 1.2	21.8 ± 3.1	17.2 ± 1.5
A	0.2 ± 0.2	0.2 ± 0.3	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.4 ± 0.5	0.0 ± 0.0
B	0.2 ± 0.3	1.2 ± 0.0	2.2 ± 0.2	2.8 ± 0.1	3.2 ± 0.1	4.6± 0.8	5.3 ± 0.1
C	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.3	0.0 ± 0.0	0.6 ± 0.0
D	0.2 ± 0.2	1.3 ± 0.0	2.6 ± 0.1	3.4 ± 0.2	4.0 ± 0.1	3.6 ± 0.0	4.0 ± 0.2
E	0.0 ± 0.0	0.6 ± 0.2	1.4 ± 0.0	1.6 ± 0.2	2.2 ± 0.0	2.8 ± 0.2	2.8 ± 00
F	0.4 ± 0.1	1.0 ± 0.1	1.2 ± 0.0	1.0 ± 0.3	1.0 ± 0.4	1.3 ± 1.1	0.0 ± 0.0
-Succinamide	0.0 ± 0.0	8.4 ± 0.6	15.6 ± 1.7	20.9 ± 0.3	22.7 ± 0.5	35.2 ± 1.2	38.2 ± 0.1
H	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.4	0.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.1
I	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.2	0.6 ± 0.0
-Azabicyclo succinamide	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	1.4 ± 0.3	1.9 ± 0.2	10.4 ± 0.6	14.3 ± 0.8
DCHS (2)	0.0 ± 0.0	0.5 ± 0.1	0.5 ± 0.2	0.8 ± 0.3	0.9 ± 0.2	2.2 ± 0.1	2.0 ± 0.0
Polar Region2 (3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.7	1.4 ± 0.2	2.2 ± 0.6	2.8 ± 0.6
Polar Region1 (3)	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 0.2	2.9 ± 0.4	3.7 ± 0.2	10.5 ± 2.7	8.8 ± 0.8
Total extractable residues	96.1 ± 0.6	98.3 ± 0.6	99.0 ± 0.1	99.7 ± 0.0	97.6 ± 0.2	96.9 ± 1.2	98.9 ± 0.7
CO_2	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
Bound to walls	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0	n/a ± 0.0
Total% recovery	96.1 ± 0.6	98.3 ± 0.6	99.0 ± 0.1	99.7 ± 0.0	97.6 ± 0.0	96.9 ± 1.2	98.9 ± 0.7

(1) Ppb analyte can be calculated as follows: Ppb analyte = Analyte as % of applied x (ppb parent applied ÷ 100%) x (MW analyte/MW parent). MW of BYI 02960 = 288.7
(2) DCHS – BYI 02960-deschlorohydroxysuccinamide - intermediate between BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide
(3) Polar regions combined and analyzed by TLC. No single component comprised >3% of the applied radioactivity

D. Kinetics of Test Item Degradation

Degradation kinetics of [^{14}C]BYI 02960 (DT_{50}, DT_{75}, and DT_{90}) is summarised in Table 7.6- 9.

Table 7.6- 9: BYI 02960 degradation kinetics

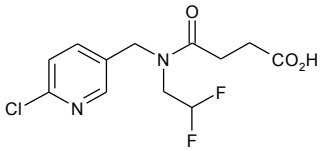
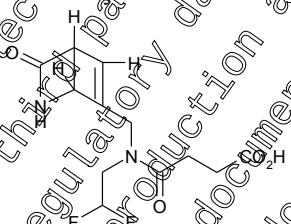
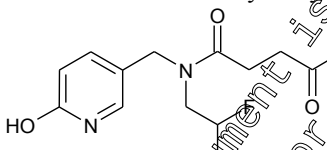
Test System	Kinetic Model	Equation	χ^2test error (%)	Experimental hours			Equivalent days, at Tokyo, JAP		
				DT_{50}	DT_{75}	DT_{90}	DT_{50}	DT_{75}	DT_{90}
Irradiated	SFO	$M_{(t)} = 101.7e^{-0.0495\ t}$	10.7	14.0	28.0	46.5	3.8	7.5	12.5
Dark	SFO	$M_{(t)} = 98.2e^{-0.00062\ t}$	0.50	1118	>2200	>3700	46.6	>92	n/a

III CONCLUSIONS

BYI 02960 photolytically degraded in sterile natural water with a half-life of 14.0 experimental hours. The major degradates (>10% of AR) were identified as BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide. A minor degradate was identified as BYI 02960-deschlorohydroxy-succinamide. In the dark controls BYI 02960 was stable. Based on the experimental half-life of 14.0

hours, the half-life of BYI 02960 under environmental conditions in Tokyo, Japan was calculated to be 3.8 days. The phototransformation study in natural water is regarded more relevant for the aqueous environment as the pure buffer study (compare KIIA 7.6/01). The following data represent the synopsis for phototransformation of BYI 02960, and the findings were included in the proposal for the pathway of degradation of BYI 02960 in an aqueous environment (see Figure 7.8- 1).

Table 7.6- 10: Result Synopsis

Test substance	**[Furanone-4-^{14}C]BYI 02960**
Test medium	Sterile natural water from lake in [redacted] Park, [redacted] Kansas
Source of irradiation	Xenon lamp with cut-off filter to remove light of waves <290 nm
Experimental DT_{50} in Suntest	14.0 hours
Environmental DT_{50}, e.g. in Tokyo, Japan	3.8 days
Dark control DT_{50} in Tokyo, Japan	>46 days, Very little degradation observed
Major transformation products after irradiation: BYI 02960-succinamide  BYI02960-azabicyclosuccinamide 	
Minor transformation products after irradiation: BYI 02960-deschlorohydroxysuccinamide 	
Total number of photodegradates formed by irradiation	At least 18 compounds formed, but only two products exceeded 10% of applied radioactivity
Total number of degradates observed in dark control samples	Maximum of 3 degradates observed, and all were <1%.

IIA 7.7 Ready biodegradability of the active substance

A respective study was not performed. The parent compound BYI 02960 is considered to be "**Not Readily Biodegradable** (as shown by the following studies).

IIA 7.8 Degradation in aquatic systems

Degradation of BYI02960 in aquatic systems was investigated in studies on anaerobic and aerobic biodegradation under dark laboratory conditions using natural water-sediment systems (see points

IIA 7.8.2 and IIA 7.8.3). Results also relevant for the behavior of BYI 02960 in natural water, i.e. those of the abiotic hydrolysis and aqueous photolysis study in water, were shown in points IIA 7.5 and

IIA 7.6 IIA 7.6, already. All those findings are considered for the overall pathway of BYI 02960 in water shown by Figure 7.8- 1.

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

Report:	**KIIA 7.8.2/01, [redacted]; 2012**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Anaerobic Aquatic Metabolism in two water/sediment systems
Report No & Document No:	MERVP027 M-422616-01-1
Guidelines:	**OECD TG No. 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, adopted April 24, 2002** **US EPA Fate, Transport and Transformation Test Guidelines, OPPTS 835.4300 and OPPTS 835.4400, Aerobic and Anaerobic Aquatic Metabolism, 2008** **Guidelines for determining environmental chemistry and fate of pesticides. Agriculture Canada Food Protection and Inspection Branch, Oct 30 1987, T-1-255. DACO No. 8.2.3.5.6**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The anaerobic biotransformation of [pyridine-2,6-^{14}C]BYI 02960 was studied in two pond water/sediment systems. One system was collected from a pond near [redacted], KS, USA and the other was taken from a pond in [redacted], NC, USA. The study was conducted for 102 days in the dark at 24 ± 2 °C. BYI 02960 was applied at a rate of 0.233 and 0.203 mg a.i./L for [redacted], KS and [redacted], NC. The treatment rate was based an application rate of 410 g a.i./ha. The test systems consisted of an Erlenmeyer flask containing 50 g (dry weight) sediment and 150 mL pond water, i.e. a sediment/water ratio of 1:3. Eight sampling intervals were conducted and included 0, 7, 14, 21, 29, 43, 70 (71 for [redacted], NC), and 102 days post treatment. The water samples were filtered and analyzed by direct sample injection into HPLC. The sediment was extracted sequentially at ambient temperature, followed by two aggressive extractions using a microwave extractor at 70 ºC. Appropriate volumes of both the ambient and aggressive extracts were concentrated and analyzed by HPLC coupled to a ^{14}C detector to characterize ^{14}C-BYI 02960 residues. Identification of the BYI 02960 was achieved by co-chromatography and liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS).

The anticipated test conditions (incubation temperature, anaerobicity and microbial viability) were maintained, and material balances for both test systems were complete throughout the study.

[redacted] <u>test system</u>: The radioactive residues in the water phase decreased from an average of 99.1% at Day 0 to 48.6% at Day 7 and declined to 25.1% at the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 8.8% on Day 0 to 46.8% at Day 7 and increased to 70.4% at Day 43, and 68.9% at the end of the study. Unextractable residues were increasing from 0.2% at Day 0 to 4.9% at the end of the study. Volatile compounds remained low with $^{14}CO_2$ ≤0.1% and organic volatiles below the LOQ. No major degradates were formed in the test systems with [redacted], KS sediment. Total minor unidentified ranged from 1.3 - 5.3% throughout the study.

[redacted] <u>test system</u>: The radioactive residues in the water phase decreased from an average of 87.3% at Day 0 to 48.4% at Day 7 and further declined to 18.5% by the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 11.4% on Day 0 to 49.2% at Day 7 and continued to increase to 78.7% at Day 71 and 64.8% at the end of the study. Unextractable

residues were increase from 0% at Day 0 to 12% at the end of the study. Volatile compounds remained low with $^{14}CO_2$ ≤0.1% and organic volatiles below the LOQ. No major degradates were formed in the test systems. Total minor unidentified ranged from 0.0 -. 0.7% throughout the study.

Kinetics: BYI 02960 dissipated from the water phase to the sediment with a half-life of 7.2 and 9.6 days for [redacted] and [redacted] test system, respectively. The half-lives of BYI 02960 in the sediment under anaerobic conditions were 415 days for [redacted] sediment and greater than 1000 days for [redacted] sediment. Thus the compound is regarded as stable under anaerobic conditions.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: [Pyridine-2,6-^{14}C]BYI02960; Sample ID: C-1135
Specific activity = 269,387 dpm/µg (35.03 mCi/mMole)
Radiochemical purity: 99%

2. Test system: The test matrices used in this study were a water/sediment collected from pond near [redacted], KS, USA (water: pH 8.3, sediment: texture silty clay, pH 7.7 (saturated paste), OC% 1.1) and the other was taken from a pond in [redacted], NC, USA (water: pH 7.4, sediment: texture loamy sand, pH 5.1 (saturated paste), OC% 1.5). The top 6 inches of sediment were collected using a shovel.

Description of the test matrix collection and storage data is given in Table 7.8.2- 1.

Selected physico-chemical characteristics of water are presented in Table 7.8.2- 2, and of sediment in Table 7.8.2- 3.

B. Methods

1. Experimental conditions: Each twenty-two kinetics test systems were treated. In addition, 6 test systems were dosed at 4 times the kinetic rate for the NC sediment and 5 for KS sediment to be used for identification of metabolites. Seven additional test systems for each sediment/water system were used as controls for determining biomass at the beginning and end of the study. The experimental design is depicted in Table 7.8.2- 4.

Table 7.8.2- 1: Description of water and sediment collection and storage

Parameter	[redacted], KS	[redacted], NC
Geographic location	[redacted]	[redacted]
Site description	Research farm pond	Pond in mix land used area
Latitude and longitude	35.050283 N; 95.1935 W	35.484401 N ; 78.043249 W
Pesticide use history at the collection site	No pesticide used	Roundup® spot treatments on slope and water edge Reglone® (diquat) spot treatment at water's edge, more than two years ago.
Collection procedures for Water	Collected water and placed over sediment in 5-gallon bucket	
Sediment	Collected ca. top 15 cm of sediment with shovel device; placed in 5-gallon buckets	
Collection date	05/21/2010	07/07/2010
Shipping conditions and date	Vehicle transport on the same day of sampling	FedEx at ambient temperature, received on 07/08/2010
Sampling depth (cm)	Water 0-20 cm Sediment 0-15 cm	
Storage conditions	Ambient conditions during transport; stored at 4 °C upon arrival at BRP until used for study. Acclimated to ambient temperature for 4 days prior to start of pre-incubation period	Ambient conditions during shipment; stored at 4 °C upon arrival at BRP until used for study. Acclimated to ambient temperature for 4 days prior to start of pre-incubation period
Storage length	7 days to pre-incubation	7 days to pre-incubation
Preparation of water and sediment samples (e.g., water –filtered/not filtered; sediment sieved/not sieved)	Water and sediment were filtered or wet sieved under water with a 2-mm sieve	

Table 7.8.2- 2: Physico-chemical characteristics of water

Property	[redacted], KS		[redacted], NC	
pH	8.3		7.4	
Hardness (mg $CaCO_3$/L)	65		24	
Conductivity (mmhos/cm)	0.18		0.09	
Total Organic Carbon (ppm)	7.8		11.9	
Dissolved organic carbon (ppm)	6.3		9	
Total nitrogen (ppm)	<LOQ of 0.2		0.6	
Total phosphorus (ppm)	0.7		1.0	
Temperature (°C) [b]	24 ± 2			
Redox potential E_h (mV)[c]	Initial	Final	Initial	Final
	112	82	115	158
Dissolved Oxygen (mg/L) [c]	Initial	Final	Initial	Final
	0.04	0.19	0.03	0.08
Biomass[d] (cells / mL water)	Initial	Final	Initial	Final
	5.75E+06	8.66E+05	5.71E+06	7.95E+06

Table 7.8.2- 3: Physico-chemical characteristics of sediment

Property		[redacted], KS		[redacted], NC	
Textural classification		Silty Clay		Loamy Sand	
	% Sand	7.0		80.7	
	% Silt	47.9		16.3	
	% Clay	45.1		3.0	
pH	1:1 soil:water	7.9		4.9	
	saturated paste	7.7		5.1	
	0.01 M $CaCl_2$	7.6		4.7	
Bulk Density (gram/cc)		1.09		1.15	
Organic carbon (%)		1.1		1.5	
Organic matter (%)		1.8		2.6	
CEC (meq/100 g)		26.8		4.3	
Total nitrogen (%)		0.08		0.08	
Total phosphorus (ppm)		163		95	
Soluble Salts (mmhos/cm)		0.46		0.18	
Redox potential E_h (mV)[b]		Initial	Final	Initial	Final
		85	95	109	155
Biomass[c] (cells / g soil)		Initial	Final	Initial	Final
		1.22E+08	1.07E+08	5.76E+07	8.21E+07

Table 7.8.2- 4: Experimental design

Parameter		Description
Duration of test		102 days
Water		2-mm sieved
Sediment		2-mm sieved
Sample Size	Water: Sediment:	150 mL 50 g (dry weight)
Water:Sediment ratio		3:1
Test concentrations	mg a.i./L water mg a.i./kg sediment mg a.i./kg total system	0.233 (KS), 0.203 (NC) 0.700 (KS), 0.608 (NC) 0.175 (KS), 0.152 (NC)
Control conditions (if used)		24 ± 2 °C, dark
Number of replications per interval	Treatments	2
	Control (untreated)	0
Test apparatus		250-mL Pyrex Erlenmeyer flask with side-arm and double-valve sealable top (or mineral oil bubblers during pre-incubation)
Traps for CO_2 & organic volatiles		2 N KOH, ethylene glycol, 1 M H_2SO_4
Test material application	Identity of solvent	Methanol (< 0.1%)
	Volume of test solution used per test system	0.230 mL (KS) 0.190 mL (NC)
	Application method	250-µL Hamilton syringe to water surface
	Evaporation of application of solvent	No
Indication of test material adsorbing to walls of test apparatus		None
Experimental conditions	Temperature (ºC)	24 ± 2 °C
	Continuous darkness (Yes/No)	Yes
Other details		None

2. Sampling:

Sampling intervals	Kinetics interval, pH, redox, and dissolved oxygen	0, 7, 14, 21, 29, 43, 70 (71 for NC), and 102 days post-application
	Sterility Checks	None
Sampling method	Water	Aqueous Phase: Water decanted through filter paper.
	Sediment	Ambient Shake: Sediment shaken at ambient temperature 3X with ACN:water (70:30 v:v), 1X ACN, centrifuged, supernatant filtered and combined. Microwave extraction: Sediment extracted 1X with ACN:water (70:30 v: v) and 1X methanol:water (70:30 v:v)
Method of collection of CO2 and volatile organics		Test systems flushed with nitrogen through volatile traps containing 2 N KOH, ethylene glycol, and 1 M H2SO4
Sample storage before analysis		Stored in freezer below -15 ºC. (LIMS ID: BLDG2WF)
Other observations, if any		None

3. Description of analytical procedures:

At each sampling interval the water and sediment were separated by decantation. The sediment was extracted sequentially three times with 30-minute ambient shakes using 50 mL 70:30 (v/v) ACN:water and one 30-minute shake with 50 mL ACN. The sediment/solvent mixture was centrifuged after each shake extraction, and the supernatants of all ambient extracts were filtered into the same graduated cylinder. The sediment then underwent two aggressive extractions using a microwave extractor at 70 ºC for 10 minutes with first 70:30 ACN: water and one more microwave extract with 70:30 methanol/water was added after Day 14. The two aggressive extracts were combined.

Appropriate volumes of both the ambient and aggressive extracts were concentrated and analyzed by HPLC coupled to a ^{14}C detector to characterize ^{14}C-BYI 02960 residues. Water was analysed by radio-HPLC directly without concentration. Identification of the BYI 02960 was achieved by co-chromatography and liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS). Identification of metabolites was confirmed by mass spectrometry at day 102.

The LOD was determined empirically to be the lowest concentration resulting in a peak height of approximately 3 times the background level in the chromatogram was approximately 500 dpm. T Assuming 500 dpm as the detection limit, the calculated limit of detection for the radioactivity detector would be 500 dpm/specific activity (dpm/µg) = 0.002 µg.

Triplicate 1-mL aliquots of the aqueous and the sediment extracts of the sample were radioassayed by LSC. The extracted sediment samples (soil cakes) were air dried, weighed and homogenized thoroughly with coffee grinder. Triplicate aliquots of the sediment were analyzed by combustion.

C. Determination of Degradation Kinetics

Dissipation rates from the water phase and rates of degradation for the total system were calculated by use of the software KinGui, version 1.1, which was built within the frame-work of MATLAB (Ver.7.0.4). The kinetic evaluation included the fitting of data with kinetic models. The bi-phasic model DFOP was chosen.

II. RESULTS

A. Findings

The redox potential and dissolved oxygen content of the test systems indicated an anaerobic and reducing environment throughout the study (Table 7.8.2- 5) .

Table 7.8.2- 5: Dissolved oxygen, pH, Eh and temperature measurements taken throughout the study period (average of replicates)

Sediment	Interval (days)	Temperature (°C)[a]	Dissolved Oxygen (mg/L)	pH	E_h[c] Water (mV)	E_h[c] Sediment (mV)
[redacted], KS	0	19.9	0.04	7.1	112	85
	7	20.2	0.04	7.1	56	67
	14	20.0	0.04	6.7	85	104
	21	-[b]	0.71	6.7	66	55
	29	20.5	0.06	7.3	29	41
	43	20.4	0.05	6.8	62	62
	70	21.3	0.04	6.8	39	44
	102	19.7	0.19	6.8	82	95
	Mean	20.3	0.1	6.9	66	69
	Min	19.7	0.04	6.7	29	41
	Max	21.3	0.71	7.3	112	104
[redacted], NC	0	20.2	0.0	6.4	115	109
	7	21.2	0.1	6.5	137	146
	14	20.0	0.0	6.0	147	151
	21	21.3	0.0	5.9	128	123
	29	21.4	0.6	5.7	184	146
	43	20.7	0.1	5.7	162	140
	71	21.8	0.3	5.4	173	167
	102	19.5	0.1	6.0	158	155
	Mean	20.7	0.17	5.9	151	142
	Min	19.5	0.03	5.4	115	109
	Max	21.8	0.63	6.5	184	167

[a]: Temperature was taken after the flasks had been taken out of incubator for measurement during the intervals.

[b]: No temperature reading was taken for this interval

[c]: $Eh = E_{obs} + 197$ mv (Ag/AgCl reference electrode)

A. Data

The distribution of radioactive residues for the test system of [redacted] during the course of the study is summarized in Table 7.8.2- 6. The distribution of radioactive residues for the test system [redacted] during the course of the study is summarized in Table 7.8.2- 7.

Table 7.8.2- 6: **Distribution of [pyridine-2,6-^{14}C]BYI02960 residues, expressed as percentage of AR (mean ± s.d.) in [redacted] sediment/water system under anaerobic aquatic conditions**

Compound	Sample		DAT 0	7	14	21	29	43	70	102
BYI 02960	Water Layer	Mean	94.0	47.4	34.9	24.2	31.1	24.2	28.9	25.1
		SD ±	5.3	0.2	1.4	0.3	1.1	0.4	0.9	2.7
	Sediment	Mean	8.8	45.4	54.7	64.7	61	65.2	65.9	66.5
		SD ±	5.2	0.7	0.8	0.4	0.8	0.5	0	0.7
	Entire System	Mean	103	92.8	89.5	88.9	92.0	89.4	94.8	91.6
		SD ±	0.1	0.5	0.6	0.1	1.9	0.1	1.0	2.0
Sum of Unidentified Minor Radio-activity[a]	Water Layer	Mean	5.1	1.2	1.2	1.2	0	0	0	0
		SD ±	1.4	1.7	0.1	0.1	0	0	0	0
	Sediment	Mean	0	1.4	2.9	3.4	2.8	5.3	1.3	2.4
		SD ±	0	0.7	0.1	0.4	0.1	0.7	1.9	3.4
	Entire System	Mean	5.1	2.6	4.1	4.5	2.8	5.3	1.3	2.4
		SD ±	1.4	2.4	0.3	0.3	0.1	0.7	1.9	3.4
Total Extractable Radio-activity	Water Layer	Mean	99.1	48.6	36	25.4	31.1	24.2	28.9	25.1
		SD ±	6.8	1.4	1.3	0.4	1.1	0.4	0.9	2.7
	Sediment	Mean	8.8	46.8	57.6	68.1	63.7	70.4	67.3	68.9
		SD ±	5.1	1.4	0.9	0.8	0.8	1.0	1.8	4.1
	Entire System	Mean	108	95.5	93.6	93.4	94.8	94.6	96.1	94.0
		SD ±	1.7	2.8	0.3	0.4	2.0	0.6	0.9	1.4
CO_2		Mean	0	0	0.1	0	0.1	0.1	0.1	0.1
		SD ±	0	0	0	0	0	0	0	0
Volatile organics		Mean	0	0	0	0	0	0	0	0
		SD ±	0	0	0	0	0	0	0	0
Total volatiles		Mean	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
		SD ±	0	0	0	0	0	0	0	0
Bound residues		Mean	0.2	1.4	3.0	3.0	3.3	3.5	3.5	4.9
		SD ±	0.1	1.7	0.2	0.1	0.2	0.1	0	0.6
Total, % recovery		Mean	108	96.9	96.6	95.5	98.1	98.2	99.8	99.0
		SD ±	1.6	1.1	0.2	0.3	1.7	0.5	1.0	2.0

[a]: individual minor degradates did not exceed a mean of 3.1 %

Table 7.8.2- 7: Distribution of [Pyridine-2,6-14C]BYI02960 residues, expressed as percentage of AR (mean ± s.d.) in [redacted] sediment/water system under anaerobic aquatic conditions

Compound	Sample		DAT 0	7	14	21	29	43	70	102
BYI 02960	Water Layer	Mean	86.6	48.4	33	25.6	20.7	13.6	7.7	18.5
		SD ±	3.4	0.2	1.4	0.8	2.2	0.7	0.4	3.1
	Sediment	Mean	11.3	49.0	62.5	70.6	72.4	78.2	78.7	64.8
		SD ±	1.9	0.2	0.6	0.1	0.3	0.7	1.1	1.2
	Entire System	Mean	97.9	97.4	95.5	96.2	93.1	91.8	86.4	83.3
		SD ±	1.4	0.1	0.8	0.8	1.8	0	1.6	1.9
Sum of Unidentified Minor Radio-activity[a]	Water Layer	Mean	0.7	0	0	0	0.3	0	0.2	0
		SD ±	0.9	0	0	0	0.4	0	0.3	0
	Sediment	Mean	0	0.2	0.1	0	0.4	0	0	0
		SD ±	0	0	0.2	0	0.5	0	0	0
	Entire System	Mean	0.7	0.2	0.1	0	0.7	0	0.2	0
		SD ±	0.9	0	0.2	0	1.0	0	0.3	0
Total Extractable Radio-activity	Water Layer	Mean	87.3	48.4	33.0	25.6	21.0	13.6	7.9	18.5
		SD ±	2.4	0.2	1.4	0.8	1.7	0.7	0.1	3.1
	Sediment	Mean	11.4	49.2	62.6	70.6	72.8	78.2	78.7	64.8
		SD ±	2.0	0.2	0.8	0.1	0.9	0.7	1.1	1.2
	Entire System	Mean	98.6	97.6	95.6	96.2	93.8	91.8	86.6	83.3
		SD ±	0.5	0.1	0.6	0.8	0.8	0	1.2	1.9
CO_2		Mean	0	0.1	0	0.1	0	0	0.1	0.1
		SD ±	0	0	0	0	0	0	0	0
Volatile organics		Mean	0	0	0	0	0	0	0	0
		SD ±	0	0	0	0	0	0	0	0
Total volatile		Mean	0	0.1	0	0.1	0	0	0.1	0.1
		SD ±	0	0	0	0	0	0	0	0
Bound residues		Mean	0	1.5	3.0	3.6	4.8	7.4	11.6	12.0
		SD ±	0	0	0.2	0.1	0.1	0.2	0.6	0.1
Total % recovery		Mean	98.7	99.2	98.7	99.9	98.7	99.2	98.4	95.4
		SD ±	0.5	0.1	0.5	0.9	0.9	0.2	1.8	1.8

[a]: individual minor degradates did not exceed a mean of 0.7 %

B. Mass balance

The material balances per sampling interval for the test systems of [redacted], KS ranged from 95.5 to 108% of the applied radioactivity, with an overall mean material balance of 99.1 ± 3.9%.
The material balances per sampling interval for the test systems of [redacted], NC ranged from 95.4 to 99.9% of the applied radioactivity, with an overall mean material balance of 98.5 ± 1.3%.

C. Residues in water, bound and extractable residues in sediment

In the [redacted] system the radioactive residues in the water phase decreased from an average of 99.1% at Day 0 to 48.6% at Day 7 and declined to 25.1% at the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 8.8% on Day 0 to 46.8% at Day 7 and increased to 70.4 at Day 43 and 68.9% at the end of the study. Unextractable residues increased from 0.2% at Day 0 to 4.9% at the end of the study. Volatile compounds remained low with 14CO2 ≤0.1% and organic volatiles below the LOQ. BYI 02960 decreased from 103% at Day 0 to 91.6% at the end of the study in the total [redacted] water/sediment system. No major degradates were formed in the test systems with [redacted] sediment; total minor unidentified ranged from 1.3 to 5.3% throughout the study.

In the Kansas system radioactive residues in the water phase decreased from an average of 87.3% at Day 0 to 48.4% at Day 7 and declined to 18.5% at the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 11.4% on Day 0 to 49.2% at Day 7 and increased to 78.7% at Day 71 and 64.8% at the end of the study. Unextractable residues increased from 0% at Day 0 to 12% at the end of the study. Volatile compounds remained low with $^{14}CO_2$ ≤0.1% and organic volatiles below the LOQ. BYI 02960 decreased from 97.9% at Day 0 to 83.3% at the end of the study in the total water/sediment system. No major degradates were formed; the total minor unidentified ranged from 0 to 0.7% throughout the study.

D. Volatilization

No volatile compounds were detected in the study.

E. Transformation of parent compound

BYI 02960 dissipates to the sediment from aqueous phase with a half-life of 7.2 and 9.6 for [redacted] and [redacted], respectively. BYI 02960 is stable in the sediment under anaerobic condition with a half-life 415 days for [redacted] and greater than 1000 days for [redacted] sediment.

Table 7.8.2- 8: Kinetic analysis

Sediment Origination	Test System	DT_{50} (days)	Double First Order Parallel k_1 day^{-1}	k_2 day^{-1}	f2 %	DT_{90} (days)
[redacted], KS	Water	7.2	0.1672	2.0E-4	6.0403	>1000
	Total	>1000	2.3E-14	3.5E-4	4.1304	>1000
[redacted], NC	Water	9.6	0.0947	2.6E-14	9.0418	>1000
	Total	415	0.0026	0.0017	0.6588	>1000

III. CONCLUSIONS

BYI 02960 dissipated from aqueous phase to sediment with a half-life of 7.2 and 9.0 for [redacted] and [redacted] test system, respectively. With respect to the entire test system, BYI 02960 is regarded stable under anaerobic condition, no major metabolite were formed.

IIA 7.8.3 Water/sediment studies

Report:	**KIIA 7.8.3/01, [redacted], E., [redacted], 2012**
Title:	[Pyridine-2,6-^{14}C]BYI 02960: Aerobic Aquatic Metabolism
Report No & Document No:	MEF-11/907 M-422359-01-1
Guidelines:	**OECD TG No. 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, adopted April 24, 2002** **US EPA Fate, Transport and Transformation Test Guidelines, OPPTS 835.4300 and OPPTS 835.4400, Aerobic and Anaerobic Aquatic Metabolism, 2008**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The aerobic transformation of [pyridine-2,6-^{14}C]BYI 02960 was studied in two different water/sediment systems ([redacted] and [redacted]) for a maximum of 119 days in the dark at 20 ± 1 °C. In a supplementary test, the degradation of [PYR-^{14}C]BYI 02960 was studied in samples which were sterilized by gamma radiation and (separate vessels) by steam pressure. The test systems

consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. Individual flasks filled a volume ratio of water to sediment of 3:1 were treated with [PYR-^{14}C]BYI 02960 with an application rate of 20.9 µg/batch corresponding to approx. 40 µg/L water assuming a maximum field application rate of 400 g BYI 02960/ha. During incubation the supernatant water was agitated gently.

Duplicate test systems were processed and analyzed after 0, 3, 7, 14, 28, 63, 91, and 119 days of incubation. The water samples were analyzed for radioactivity after centrifugation. Prior to radio-HPLC analysis, a concentration step was performed. The sediment samples were extracted at ambient temperature three times with acetonitrile/water (70/30, v/v), followed by one extraction step with pure acetonitrile. Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v). The combined extracts from the ambient extractions and the aggressive extraction were analyzed by LSC and, after a concentration step, via radio-HPLC. Identification of BYI 02960 residues was by HPLC-MS, HPLC-MS/MS, NMR and HPLC co-chromatography.

The non-extractable residues (NER) in sediment samples were determined, and those from [redacted] in case of the last sampling date were separated into humin, humic acid and fulvic acid fractions.

The test conditions outlined in the study protocol were maintained throughout the study, and the material balance in the two test series was complete (on average 98.9% for HW and 98.0% for AW test system). The complete material balance demonstrates that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

The radioactivity in the water phase of HW test systems decreased to 12.9% of AR, and in the water phase of AW test systems to 24.6% of AR at study termination. Extractable ^{14}C sediment residues in test systems from HW increased from 1.9% of the applied radioactivity at DAT-0 to 59.8% at DAT-63 and declined to 53.0% towards the end of the study. Extractable ^{14}C residues in the sediments from AW increased from 1.1% of the applied radioactivity at day 0 to 49.7% at study termination. The maximum amount of non-extractable ^{14}C residues (NER) in the sediment was 25.0% of AR for test systems from HW and 13.6% of AR for AW (DAT-119). The fractionation of NER of HW resulted in portions of 4.5 and 5.4% of AR within the humic acids, whereas similar amounts of radioactivity were associated with the fulvic acids (9.1 and 9.3% of AR) or strongly integrated into the insoluble humin of the soil matrix (10.7 and 9.8% of AR). At the end of the study, 6.8% and 8.5% of AR were present as $^{14}CO_2$ in the test systems from HW and AW, respectively. Organic volatile compounds were not detected in significant amounts (≤ 0.1% of AR in all test systems).

In the water phase of HW and AW, the amount of [PYR-^{14}C]BYI 02960 decreased from 96.7 and 96.5% of AR at day 0 to 11.4 and 22.3% at study termination, respectively. In the sediment phase of HW, the amounts of BYI 02960 increased from 1.8% of AR at day 0 to a maximum of 59.4% at DAT-63, followed by a decrease to 52.6% towards the end of the study. In the AW sediment the amounts of BYI 02960 increased from 1.0% of AR at day 0 to 49.5% at study termination. Not any major transformation products were to be detected in the water phase and the sediment of both test systems.

The dissipation of BYI 02960 from the water phases was mainly characterized by rapid partitioning into the sediment. The best-fit kinetic models for the determination of trigger values were the FOMC kinetic model for HW and the DFOP kinetic model for AW with DT_{50} values of 8.5 and 34.5 days, respectively. The corresponding modeling endpoints were calculated using the DFOP kinetic model. The DT_{50} values for the slow degradation phase were 63.0 and 63.6 days for the water phase of HW

and AW, respectively. In the entire water/sediment systems, BYI 02960 was degraded slowly which was best described using single first-order kinetics (SFO). The estimated DT_{50} values were 193.1 and 246.9 days for test systems from HW and AW, respectively.

In the supplemental test, i.e. the test systems sterilized by gamma radiation or steam pressure and then incubated for 0, 60 or 120 days, no CO_2 was formed (≤ 0.1% AR). The amount of radioactivity in the water phase, predominantly represented by parent compound, was about two times higher than in that from the microbial active samples. Further, there was a clear trend that NER formation in sediment was lower than in the microbial active test flasks, especially seen for both test systems HW and AW sterilized by steam pressure. This shows that the NER were at least partly formed by microbial processes.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: BYI02960, CAS No: 951659-40-8
[Pyridine-2,6-^{14}C]BYI02960, sample ID: KML 9090
Specific activity = 4.49 MBq/mg (121.35 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV-detection at 210 nm)

2. Test System: The study was carried out using the natural water/sediment systems [redacted] (HW), near [redacted] Germany and [redacted] (AW), [redacted], Germany.

[redacted] is an artificially dammed pond in the course of the [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 chosen systems are well characterized, physical-chemical characteristics of the water/sediment systems are summarized in Table 7.8.3- 1.

Water and sediment samples were taken separately and poured into plastic containers. The collected sediments were sieved down to 2 mm mesh-size to remove parts of e.g. plants and stones. The collected water phases were filtered through a 0.06 mm sieve.

Table 7.8.3- 1: Physico-chemical characteristics of water and sediment

Parameter	[redacted] (HW)	[redacted] (AW)
Geographic Location	[redacted] Germany [redacted]	[redacted] Germany [redacted]
Properties of Water		
Temperature [°C][1]	5.2	6.8
pH[1]	7.4	7.7
Hardness [°dH] [,2]	3.1 / 2.6 / 4.8	9.8 / 12.5 / 14.2
Oxygen Conc. (saturation) [mg/L][1]	13.33	13.48
Total Org. Carbon (TOC) [mg/L][,2]	<2 / 3 / 9	<2 / 4 / 16
Dissolved Org. Carbon (DOC) [mg/L]*[,2]	<2 / 3 / 5	< 2 / 4 / 11
Total Nitrogen [mg/L] [,2]	4.7 / < 1.0 / 3.4	4.7 / 2.8 / 1.3
Total Phosphorus [mg/L] [,2]	< 0.03 / < 0.03 / < 0.03	< 0.03 / 0.14 / 0.20
Redox Potential E_h [mV][1,4]	436.7	505.1
Properties of Sediment		
Soil Taxonomic Classification (USDA)[#]	Sandy Loam	Loamy Sand
Sand (2000 – 50 µm) [%][#]	65	85
Silt (< 50 – 2 µm) [%][#]	30	12
Clay (< µm) [%][#]	5	3
pH[1]	7.1	7.0
pH[#]	5.2 ($CaCl_2$); 5.4 (H_2O)	6.7 ($CaCl_2$); 7.1 (H_2O)
Temperature [°C][1]	3.9	6.2
Organic Matter [%][2,3]	6.19 / 6.34 / 6.14	2.07 / 2.34 / 2.53
Organic Carbon [%][*,2]	3.59 / 3.68 / 3.56	1.20 / 1.36 / 1.47
% $CaCO_3$ [#]	n.a.	n.a.
Sediment Microbial Activity [mg CO_2 /hr/kg sediment (dry wt)][2]	16.25 / 12.25 / 7.50	15.42 / 14.17 / 10.08
Cation Exchange Capacity [meq/100 g][#]	7.6	6.6
Total Nitrogen [%][*,2]	0.26 / 0.25 / 0.31	0.09 / 0.12 / 0.12
Total Phosphorus (Olsen) [mg/kg] [*,2]	600 / 590 / 730	330 / 330 / 340
Redox Potential E_h [mV][1,4]	227.7	500.1
1 Measurement at day of sampling	2 start of acclimation / DAT-0 / DAT-120	
3%organic matter = %organic carbon x 1.724	n.a. = not analyzed	
4 Eh = Eobs + Uref [Uref = reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at given Temperature; 210 mV at 20 °C, extrapolated from manufacturer information]		

B. Materials

1. Experimental conditions: The test systems consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. The individual static test systems were kept at aerobic conditions at 20 ± 2 °C for a maximum period of 120 experimental days. Each vessel was filled with 175 mL of wet sediment (i.e. 118.3 and 247.9 g dry weight sediment for HW and AW) and about 525 mL of water, equivalent to approx. 6 cm in height, resulting in a volume ratio of water to sediment of 3:1. After pre-equilibration, aliquots of the application solution were directly applied onto the water surface of each system. Thereafter, the systems were closed with the trap attachment for absorbing volatile compounds from DAT 1 onwards. During incubation the supernatant water was agitated gently.

The amount of radiolabelled BYI02960 for the treatment of the individual test systems was based on a single field use rate of 400 g/ha, calculated to a water depth of 100 cm, which resulted in a nominal application rate was 20.8 µg/batch. The actual application rate set as 100 % of applied radioactivity (100 % AR) corresponding to 1887.68 Bq/500 µL, 1885.15 Bq/500 µL and 1871.07 Bq/500 µL in the regular water/sediment systems. The material balance for the regular test systems was based on the average amount of radioactivity (RA) recovered from these measurements: 94065 Bq or 20.9 µg of test item per vessel.

2. Sampling: Duplicate samples were taken on DAT-0, 3, 7, 14, 28, 63, 91, and 119 days after application for both test systems. The water was decanted and centrifuged. The sediment was extracted with 3 x 80 mL acetonitrile/water (70/30, v/v), followed by one extraction with 80 mL acetonitrile (combination: ambient extracts). Finally, the sediment was extracted once with 80 mL acetonitrile/water (70/30, v/v) by microwave-accelerated solvent extraction (aggressive extract). Volatile organics and $^{14}CO_2$ were trapped with solid trapping attachments containing soda lime for absorption of $^{14}CO_2$ and polyurethane foam for volatile organic compounds.

3. Description of analytical procedures: The radioactivity of the supernatant and sediment samples was radio-assayed by LSC and HPLC. Volatiles were analysed by LSC.

For HPLC analysis of the water phase, 10 mL of the water phase were concentrated to about 1 mL. For representative sampling dates (DAT 0, 14, 28, 63, 91 and 119), aliquots of 200 µL of the water phases were investigated by TLC without a concentration step (confirmation method).
Sediment samples were exhaustively extracted once with 80 mL acetonitrile/water (70/30, v/v) by microwave-accelerated solvent extraction for 10 minutes (temperature approx. 70°C) with magnetic stirring. After extraction and centrifugation (approx. 15 min, 2500 x g) the supernatant was decanted (aggressive organic extracts) and analysed with LSC. Prior to HPLC-analysis, aliquots of 10.0 mL from the ambient organic extracts and aliquots of 4 mL from the aggressive organic extracts were concentrated to volumes of about 1 mL. 200 µL aliquots of representative ambient and aggressive organic extracts (taken on DAT-0, 14, 28, 63, 91 and 119) were investigated by TLC as a confirmatory method.

C. Determination of Degradation Kinetics

Dissipation rates from the water phase and rates of degradation for the total system were calculated by use of the software KinGui, version 1.1. The kinetic evaluation included the fitting of data with kinetic models SFO, FOMC and DFOP to the experimental data and their assessment according to FOCUS guidance to result in values for comparison with trigger endpoints.

II. RESULTS

A. Findings

The test conditions outlined in the study protocol were maintained throughout the study.

Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period

Table 7.8.3- 2: Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (HW)

		Water Phase				Sediment Layer		
DAT	Replicate	pH	Oxygen Conc.	Redox Potential		pH	Redox Potential	
				SenTix ORP Electrode	E_h*		SenTix ORP Electrode	E_h*
	JR53-		[mg O_2/L]	[mV]	[mV]		[mV]	[mV]
Pre-incubation								
	-19	7.1	8.3	250	460	6.7	-22	188
	-15	6.7	8.3	252	462	6.8	37	247
	-11	6.6	8.4	178	388	6.6	53	263
	-8	7.1	8.6	181	391	6.9	91	301
	-5	7.1	8.4	201	411	6.6	55	265
	-1	7.1	8.8	183	393	7.1	42	252
	HW1-D0	7.1	8.7	174	384	6.8	58	268
0	HW2-D0	7.0	8.9	168	378	6.8	36	246
	Mean	**7.1**	**8.8**	**171**	**381**	**6.7**	**47**	**257**
	HW1-D3	6.9	8.6	198	408	6.9	69	279
3	HW2-D3	7.1	8.6	210	420	6.8	59	269
	Mean	**7.0**	**8.6**	**204**	**414**	**6.9**	**64**	**274**
	HW1-D7	6.9	8.2	226	436	6.8	54	264
7	HW2-D7	6.9	8.4	236	446	6.8	64	274
	Mean	**6.9**	**8.3**	**231**	**441**	**6.8**	**59**	**269**
	HW1-D14	7.1	8.8	198	408	6.5	51	261
14	HW2-D14	6.8	8.6	208	418	6.4	66	276
	Mean	**7.0**	**8.7**	**203**	**413**	**6.5**	**59**	**269**
	HW1-D28	6.7	8.3	228	438	6.9	72	282
28	HW2-D28	7.1	8.5	236	446	6.8	61	271
	Mean	**6.9**	**8.4**	**232**	**442**	**6.9**	**67**	**277**
	HW1-D63	7.3	8.8	234	444	6.9	97	307
63	HW2-D63	7.3	8.9	238	448	6.8	86	296
	Mean	**7.3**	**8.8**	**236**	**446**	**6.9**	**92**	**302**
	HW1-D91	6.7	8.6	246	456	6.6	67	277
91	HW2-D91	6.8	8.5	238	448	6.5	78	288
	Mean	**6.8**	**8.5**	**242**	**452**	**6.5**	**73**	**283**
	HW1-D119	7.0	8.7	213	423	6.5	60	270
119	HW2-D119	6.9	8.7	211	421	6.5	64	274
	Mean	**7.0**	**8.7**	**212**	**422**	**6.5**	**62**	**272**
overall Mean#		7.0	8.6	216	426	6.7	65	275
overall Minimum#		6.7	8.2	168	378	6.4	36	246
overall Maximum#		7.3	8.9	246	456	6.9	97	307

* $E_h = E_{obs}$ + 210 mV [reference potential of SenTix ORP-electrode (WTW) vs. standard hydrogen electrode at 20°C; manufacturer information]

without pre-incubation

Table 7.8.3- 3: Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (AW)

		Water Phase				Sediment Layer		
DAT	Replicate JR53-	pH	Oxygen Conc. [mg O_2/L]	Redox Potential SenTix ORP Electrode [mV]	E_h* [mV]	pH	Redox Potential SenTix ORP Electrode [mV]	E_h* [mV]
Pre-incubation								
-19		8.1	9.3	180	390	7.3	135	345
-15		8.2	8.9	233	443	7.3	230	440
-11		8.2	8.6	187	397	7.4	237	447
-8		8.2	8.8	186	396	7.4	156	366
-5		8.3	8.8	221	431	7.4	195	405
-1		8.4	9.0	170	380	7.5	181	391
0	AW1-D0	8.4	9.0	201	411	7.6	186	396
	AW2-D0	8.4	9.0	196	406	7.6	178	388
	Mean	**8.4**	**9.0**	**199**	**409**	**7.6**	**182**	**392**
3	AW1-D3	8.3	9.0	196	406	7.5	180	390
	AW2-D3	8.3	9.0	203	413	7.4	184	394
	Mean	**8.3**	**9.0**	**200**	**410**	**7.4**	**182**	**392**
7	AW1-D7	8.5	8.8	238	448	7.5	207	417
	AW2-D7	8.5	8.8	225	435	7.5	196	406
	Mean	**8.5**	**8.8**	**232**	**442**	**7.5**	**202**	**412**
14	AW1-D14	8.3	9.0	192	402	7.5	184	394
	AW2-D14	8.4	9.0	189	399	7.3	181	391
	Mean	**8.4**	**9.0**	**191**	**401**	**7.4**	**183**	**393**
28	AW1-D28	8.5	8.8	205	415	7.7	171	381
	AW2-D28	8.4	8.8	220	430	7.7	196	406
	Mean	**8.4**	**8.8**	**213**	**423**	**7.7**	**184**	**394**
63	AW1-D63	8.5	8.9	224	434	7.9	181	391
	AW2-D63	8.5	8.9	212	422	7.8	174	384
	Mean	**8.5**	**8.9**	**218**	**428**	**7.8**	**178**	**388**
91	AW1-D91	8.5	8.8	204	414	7.5	171	381
	AW2-D91	8.5	8.8	215	425	7.5	166	376
	Mean	**8.5**	**8.8**	**210**	**420**	**7.5**	**169**	**379**
119	AW1-D119	8.5	8.8	203	413	7.5	183	393
	AW2-D119	8.5	8.8	210	420	7.6	176	386
	Mean	**8.50**	**8.81**	**207**	**417**	**7.5**	**180**	**390**
overall Mean#		8.4	8.9	208	418	7.5	182	392
overall Minimum#		8.3	8.8	189	399	7.3	166	376
overall Maximum#		8.5	9.0	238	448	7.9	207	417

* $E_h = E_{obs}$ + 210 mV [reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at 20°C; manufacturer information]
# without pre-incubation

A. Data

A summary of key data on total recovery and the distribution of radioactivity into the various components formed in water and sediment is given for system [redacted] in Table 7.8.3- 4 and in Table 7.8.3- 5 for system [redacted].

B. Mass Balance

During the study, the total recovery of radioactivity in individual test vessels ranged from 96.5 to 100.6% (mean 98.9%, RSD 1.2 %) for [redacted] and from 95.7 to 100.8% (mean 98.0%, RSD 1.6%) for [redacted]. Complete material balances found for all sampling dates

demonstrating that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

Table 7.8.3- 4: Biotransformation of [PYR-^{14}C]BYI 02960 in [redacted] under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT) 0	3	7	14	28	63	91	119
Parent BYI 02960	Water Layer	96.7	68.2	52.1	40.2	26.3	17.7	14.7	11.4
		± 0.3	± 0.1	± 1.7	± 5.3	± 4.7	± 3.4	± 1.4	± 0.4
	Sediment	1.8	27.0	42.5	53.3	59.2	59.4	55.8	52.6
		± 0.1	± 0.2	± 1.5	± 4.3	± 3.5	± 1.4	± 2.3	± 0.3
	Entire System	98.5	95.2	94.6	93.6	85.4	77.2	70.5	64.0
		± 0.4	± 0.3	± 0.2	± 1.1	± 1.1	± 4.8	± 3.7	± 0.1
Reg 1	Water Layer	n.d.	n.d.	n.d.	n.d.	0.8	0.6	n.d.	n.d.
						± 0.0	± 0.0		
	Sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	n.d.	n.d.	n.d.	n.d.	0.8	0.6	n.d.	n.d.
						± 0.0	± 0.0		
Reg 2	Water Layer	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	0.4	1.4
						± 0.0		± 0.0	± 0.2
	Sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	n.d.	n.d.	n.d.	n.d.	0.4	n.d.	0.4	1.4
						± 0.0		± 0.0	± 0.2
Non-characterized Radioactivity	Water Layer	0.4	< LOD	0.3	< LOD	< LOD	< LOD	< LOD	< LOD
		± 0.1		± 0.0					
	Sediment	< LOD	< LOD	< LOD	< LOD	< LOD	0.3	< LOD	0.4
							± 0.2		± 0.0
	Entire System	0.4	0.5	0.6	0.4	0.4	0.4	0.3	0.5
		± 0.1	± 0.1	± 0.1	± 0.0	± 0.1	± 0.2	± 0.0	± 0.0
Total Extractable Residues	Water Layer	97.0	68.5	52.4	40.4	27.6	18.5	15.1	12.9
		± 0.2	± 0.0	± 1.7	± 5.4	± 5.1	± 2.8	± 1.8	± 0.2
	Sediment	1.9	27.2	42.8	53.6	59.4	59.8	56.1	53.0
		± 0.1	± 0.2	± 1.5	± 4.3	± 3.5	± 1.5	± 2.3	± 0.3
	Entire System	98.9	95.7	95.1	94.0	87.0	78.3	71.2	65.9
		± 0.3	± 0.2	± 0.2	± 1.1	± 1.6	± 4.3	± 4.0	± 0.2
$^{14}CO_2$	Entire System	n.a.	< 0.1	0.1	0.3	1.2	2.8	5.3	6.8
			± 0.0	± 0.0	± 0.1	± 0.3	± 0.0	± 0.2	± 0.1
Organic Volatiles	Entire System	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
			± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Non-Extractable Residues (NER)	Sediment	0.4	2.6	4.5	6.3	11.6	18.3	20.1	25.0
		± 0.0	± 0.1	± 0.1	± 0.7	± 1.4	± 3.0	± 3.6	± 0.1
Total Recovery	Water Layer	97.0	68.5	52.4	40.4	27.6	18.5	15.1	12.9
		± 0.2	± 0.0	± 1.7	± 5.4	± 5.1	± 2.8	± 1.8	± 0.2
	Sediment	2.2	29.8	47.2	59.9	71.0	78.0	76.1	78.0
		± 0.1	± 0.1	± 1.5	± 5.0	± 4.9	± 1.4	± 1.3	± 0.3
	Entire System	99.3	98.3	99.7	100.6	99.8	99.3	96.5	97.7
		± 0.4	± 0.1	± 0.2	± 0.4	± 0.1	± 1.4	± 0.6	± 0.0

± = SD, standard deviation, < LOD = < 0.3% of AR (Minimum LOD)

Table 7.8.3- 5: Biotransformation of [PYR-^{14}C]BYI 02960 in [redacted] under aerobic conditions, expressed as percent of AR, mean ± SD (MEF-11/907)

Compound	Source	Days After Treatment (DAT) 0	3	7	14	28	63	91	119
Parent BYI 02960	Water Layer	96.5 ± 0.0	75.7 ± 0.3	69.5 ± 0.8	63.3 ± 0.1	47.6 ± 0.0	32.1 ± 1.4	26.8 ± 0.0	22.3 ± 0.0
	Sediment	1.0 ± 0.2	20.4 ± 1.0	26.5 ± 0.5	33.4 ± 0.1	42.4 ± 0.1	47.5 ± 0.8	48.7 ± 0.3	49.5 ± 0.3
	Entire System	97.5 ± 0.2	96.1 ± 0.6	96.0 ± 0.4	96.7 ± 0.0	90.0 ± 0.1	79.6 ± 2.3	75.5 ± 0.3	71.7 ± 0.2
Reg 2	Water Layer	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.0	0.6 ± 0.1	0.8 ± 0.0
	Sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.0	0.6 ± 0.1	0.8 ± 0.0
Reg 3	Water Layer	n.d.	n.d.	n.d.	n.d.	n.d.	1.3 ± 0.0	1.1 ± 0.1	1.3 ± 0.2
	Sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Entire System	n.d.	n.d.	n.d.	n.d.	n.d.	1.3 ± 0.0	1.1 ± 0.1	1.3 ± 0.2
Non-characterized Radioactivity	Water Layer	0.5 ± 0.0	0.3 ± 0.0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Sediment	< LOD	< LOD	< LOD	< LOD	< LOD	0.3 ± 0.0	< LOD	< LOD
	Entire System	0.6 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.0
Total Extractable Residues	Water Layer	97.1 ± 0.0	76.0 ± 0.3	69.8 ± 0.8	63.5 ± 0.1	47.8 ± 0.0	34.0 ± 2.0	28.8 ± 0.0	24.6 ± 0.2
	Sediment	1.1 ± 0.2	20.4 ± 0.9	26.7 ± 0.4	33.6 ± 0.1	42.5 ± 0.1	47.8 ± 0.8	48.9 ± 0.4	49.7 ± 0.2
	Entire System	98.1 ± 0.3	96.5 ± 0.6	96.4 ± 0.3	97.1 ± 0.0	90.3 ± 0.1	81.8 ± 2.8	77.7 ± 0.3	74.3 ± 0.0
$^{14}CO_2$	Entire System	n.a.	0.1 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	3.0 ± 0.0	6.0 ± 1.5	7.4 ± 0.4	8.5 ± 0.5
Organic Volatiles	Entire System	n.a.	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0
Non-Extractable Residues (NER)	Sediment	< 0.1 ± 0.0	1.1 ± 0.0	1.8 ± 0.0	3.0 ± 0.0	6.5 ± 0.0	9.3 ± 0.5	10.7 ± 0.3	13.6 ± 0.3
Total Recovery	Water Layer	97.1 ± 0.0	76.0 ± 0.3	69.8 ± 0.8	63.5 ± 0.1	47.8 ± 0.0	34.0 ± 2.0	28.8 ± 0.0	24.6 ± 0.2
	Sediment	1.1 ± 0.2	21.5 ± 1.0	28.4 ± 0.5	36.6 ± 0.1	49.1 ± 0.1	57.1 ± 0.4	59.6 ± 0.7	63.3 ± 0.5
	Entire System	98.2 ± 0.3	97.6 ± 0.6	98.4 ± 0.3	100.8 ± 0.0	99.9 ± 0.1	97.2 ± 0.8	95.7 ± 0.2	96.4 ± 0.2

± = SD, standard deviation, < LOD = < 0.3% of AR (Minimum LOD)

C. Volatilization

Formation of $^{14}CO_2$, mineralization of [PYR-^{14}C]BYI 02960, was observed in both water/sediment test systems. At termination of the study, the $^{14}CO_2$ recovery (mean values of duplicates) in test system from HW was 6.8% of AR. In AW test system, $^{14}CO_2$ accounted for 8.5% of AR at study termination.

Most of the $^{14}CO_2$ was collected in the soda lime fraction of the trap attachments, ≤ 0.3% of AR in [redacted] and ≤ 1.5% of AR in [redacted] was dissolved CO_2 in water.
No significant amounts of organic volatiles were found (< 0.1% of AR).

D. Residues in water, bound and extractable residues in sediment

The radioactivity in the water phase of HW test system decreased steadily from 97.0% at day 0 to 12.9% of AR at study termination. The extractable ^{14}C residues in sediment increased from 1.9% at DAT 0 to 59.8% of AR at DAT 63 and declined to 53.0% of AR towards the end of the study. NER was 0.4% of AR at day 0 which increased to 25.0% of AR at study termination.
The radioactivity in the water phase of AW test system decreased from 97.1% at day 0 to 24.6% of AR towards the end of the study. Extractable ^{14}C residues in the sediment increased from 1.1% at day 0 to 49.7% of AR at study termination. The amount of bound residues was <0.1% of the applied radioactivity at day 0 and increased to 13.6% towards the end of the incubation period.
The NER of extracted sediments of DAT 119 from HW (both replicates) was found to be of heterogeneous nature. Portions of 4.5 and 5.4% of AR were fractionated together with the humic acids, whereas similar amounts of radioactivity were associated with the fulvic acids (9.1 and 9.3% of AR) or strongly integrated into the insoluble humin of the sediment matrix (10.7 and 9.8% of AR).

E. Transformation of Parent Compound

The percentages of BYI 02960 and its residues determined in water and sediment extracts are presented in Table 7.8.3-4 for the system HW, and in Table 7.8.3-5 for the system AW. The elimination of BYI 02960 from the water body occurred mainly via partition into the sediment phase and partly via degradation. In the sediment phase of HW, the amounts of BYI 02960 increased from 1.8% at day 0 to a maximum of 59.4% of AR at DAT-63, followed by a decrease to 52.6% of AR towards the end of the study. In the AW sediment the amounts of BYI 02960 increased from 1.0% at day 0 to 49.5% of AR at study termination.
Three very minor transformation products were observed in the water phase. One of those was detected in the water phase of both test systems, and accounted for up to 1.4 and 0.8% of AR, respectively. The others were either detected in the water phase from HW (≤ 0.8% of AR) or [redacted] (≤ 1.3% of AR). The maximum amount of the non-characterized radioactivity in the water phase was 0.5% of AR. No transformation products were detected in the sediment extracts from HW and AW. The maximum amount of the non-characterized radioactivity was 0.4% of AR.

F. Dissipation and Degradation Kinetics

After fitting of data to the three kinetic models SFO, FOMC (Gustafson-Holden) and DFOP the quality of fits was assessed according to FOCUS kinetic guidance. For the dissipation from water the best-fit kinetic models for the determination of trigger values were the FOMC kinetic model for HW and the DFOP kinetic model for AW with DT_{50} values of 8.5 and 34.5 days, respectively (Table 7.8.3-6). The corresponding modeling endpoints were calculated using the DFOP kinetic model. The DT_{50} values for the slow degradation phase were 63.0 and 63.6 days for the water phases of [redacted] and [redacted], respectively.

In the entire water/sediment systems, [PYR]BYI 02960 was degraded more slowly the best fit kinetics was single first-order kinetics (SFO). The DT_{50} values were 193.1 and 246.9 days for systems from

[redacted] and [redacted], respectively. These values were used as trigger values and modeling endpoints (Table 7.8.3- 7).

Table 7.8.3- 6: Summary of dissipation kinetics of BYI 02960 from the supernatant water

Water Phase of Test System	Kinetic Model	DT$_{50}$ [days]	DT$_{90}$ [days]	Chi2 Error [%]
[redacted]	SFO	14.9	49.6	19
	FOMC	**8.5**	**174.6**	**1.6**
	DFOP	8.2	125.4	3.8
[redacted]	SFO	48.5	161	10.2
	FOMC	27.3	705.5	4.6
	DFOP	**34.5**	**181.8**	**4.4**

Table 7.8.3- 7: Summary of degradation kinetics of BYI 02960 from the entire water/sediment test system

Entire System	Kinetic Model	DT$_{50}$ [days]	DT$_{90}$ [days]	Chi^2 Error [%]
[redacted]	**SFO**	**193.1**	**641.3**	**1.2**
	FOMC	251.9	> 1000	1.1
	DFOP	204.2	716.3	1.1
[redacted]	**SFO**	**246.9**	**820.1**	**1.3**
	FOMC	360.3	> 1000	1.2
	DFOP	> 1000	> 1000	1.3

Bold: best fit, SFO = Single First Order Model, FOMC = First Order Multi Compartment Model, DFOP = Double First Order in Parallel Model

In the supplemental test, i.e. the test systems sterilized by gamma radiation or steam pressure and then incubated for 0, 60 or 120 days, no CO_2 was formed (≤ 0.1% AR). The amount of radioactivity in the water phase, predominantly represented by parent compound, was about two times higher than in that from the microbial active samples. Further, there was a clear trend that NER formation in sediment was lower than in the microbial active test flasks. This shows that the NER were at least partly formed by microbial processes.

III. CONCLUSIONS

[PYR] BYI 02960 is microbially degraded in aerobic water/sediment systems. The DT$_{50}$ values for BYI 02960 in the entire water/sediment systems were 193 and 247 days for [redacted] and [redacted], respectively. The only major transformation products were to carbon dioxide and formation of NER.

Table 7.8.3- 8: Results Synopsis

Parameter	[redacted]	[redacted]
Material Balance [% AR]	96.5 – 100.6	95.7 – 100.8
Water Phase [% AR]	12.9 – 97.0	24.6 – 97.1
Sediment Extract [% AR]	1.9 – 59.8	1.1 – 49.7
Major transformation products *	CO_2 (max. 6.8%) NER (max. 25.0%)	CO_2 (max. 8.5%) NER (max. 13.6%)

* Criteria for "major": ->10% of AR at any DAT; >5% of AR at two successive DATs, increasing towards study end

Kinetics Evaluation, Trigger Values		DT_{50} [days]	Chi² Err [%]	Best Fit Kinetic Model	t-probability
Supernatant water	[redacted]	8.5	1.6	FOMC	-
	[redacted]	34.5	4.4	DFOP	-
Entire system	[redacted]	193.1	1.2	SFO	-
	[redacted]	246.9	1.3	SFO	-
Kinetics Evaluation, Modeling Endpoints					
Supernatant water	[redacted]	63.0*	3.8	DFOP	< 0.001
	[redacted]	63.6*	4.4	DFOP	< 0.001
Entire system	[redacted]	193.1	1.2	SFO	< 0.001
	[redacted]	246.9	1.3	SFO	< 0.001

SFO = Single First-Order Model, FOMC = First Order Multi Compartment Model, DFOP = Double First Order in Parallel Model
* DT_{50} value of the slow degradation phase

Report:	**KIIA 7.8.3/02, [redacted], [redacted], 2012**
Title:	[Furanone-4-[14]C] and [Ethyl-1-[14]C]BYI 02960: Aerobic Aquatic Metabolism
Report No & Document No:	MEF-10/730 M-426504-01-1
Guidelines:	**OECD TG No. 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, adopted April 24, 2002** **US EPA Fate, Transport and Transformation Test Guidelines, OPPTS 835.4300 and OPPTS 835.4400, Aerobic and Anaerobic Aquatic Metabolism, 2008**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The aerobic transformation of [FUR-[14]C] and [ETH-[14]C]BYI 02960 was studied in two water/sediment systems, [redacted] (HW) and [redacted] (AW) for a maximum of 120 days in the dark at 20 ± 2°C. The test systems consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. Individual flasks filled a volume ratio of water to sediment of 3:1 were treated with [[14]C]BYI 02960. The actual application rates were 20.86 and 20.80 µg/batch for label FUR and label ETH test systems from [redacted] and 20.63 and 20.53 µg/batch for label FUR and label ETH test systems from [redacted], corresponding to approx. 40 µg/L water assuming a field application rate of 400 g BYI 02960/ha. During incubation the supernatant water was agitated gently.

Duplicate test systems were processed and analyzed after 0, 1, 3, 7, 14, 30, 45/44, 60/58, 87/86 and 120 days of incubation for test systems from HW and AW (both labels), respectively. The water samples were decanted and analyzed for radioactivity after centrifugation and filtration. Prior to HPLC analysis, a concentration step was performed. The sediment samples were extracted three times with

acetonitrile/water (70/30, v/v), followed by one extraction step with pure acetonitrile, all at ambient temperature (ambient organic extracts). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v). The combined extracts from the ambient extractions and the aggressive extract were analyzed by LSC and via HPLC after a concentration step. For selected samples, the HPLC analysis was confirmed by TLC. Identification of the two test items was achieved by HPLC-MS, HPLC-MS/MS, NMR and/or Co-chromatography. The major metabolite detected in label ETH test systems was characterized by HPLC-MS. The non-extractable residues (NER) in sediment samples were determined, and those from the last sampling date were separated into humin, humic acid and fulvic acid fractions.

The test conditions outlined in the study protocol were maintained throughout the study, and the mean material balances in all four test series ranged from 98.2 - 100.4% of AR. The complete material balance demonstrates that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

The radioactivity in FUR and ETH test systems from HW water decreased steadily from 96.0 and 97.2% of AR at day 0 to 14.3 and 14.2% at study termination. The radioactivity in FUR and ETH test systems from AW water decreased from 97.9 and 98.3% of AR at day 0 to 37.3 and 44.1% towards the end of the study. Extractable ^{14}C sediment residues in FUR and ETH test systems from HW increased from 4.2 and 3.8% of AR at day 0 to 54.7 and 54.1% at study termination, respectively. Extractable ^{14}C residues in FUR and ETH sediments from AW increased from 2.2 and 2.4% of AR at day 0 to maxima of 37.7 and 40.1% of AR at study termination. The maxima of non-extractable ^{14}C residues (mean values of duplicates) in the sediments were 22.6 and 26.6% of AR for FUR and ETH test systems from HW, and 17.9 and 15.2% of AR for FUR and ETH test systems from AW, respectively.

At the end of the study period, 3.9 and 1.5% of AR were present as $^{14}CO_2$ in systems from HW, and 5.5 and 0.9% of AR were present as $^{14}CO_2$ in systems from AW, each for FUR and ETH label, respectively. The total amount mainly accounted for of $^{14}CO_2$ trapped in soda lime, but as well for the amount of $^{14}CO_2$ present in the water phase (all sampling intervals) and sediments (only DAT-120). Organic volatile compounds amounted to ≤ 0.2% of the applied radioactivity in both systems and for both labels.

In the water phase from HW, BYI 02960 decreased from 95.5 and 96.8% of AR at day 0 to 14.3 and 14.1% at study termination for FUR and ETH test systems, respectively. In AW water of FUR and ETH test systems, BYI 02960 decreased from 97.4 and 97.9% of AR at day 0 to 35.6 and 36.8% at study termination. In the sediment phase of FUR and ETH test systems from HW, BYI 02960 increased from 4.1 and 3.7% of AR at day 0 to maximum amounts of 58.7 and 58.3% at DAT-60 or DAT-45, and then slightly declined to 54.3 and 52.6% towards study termination, respectively. In AW sediment (FUR and ETH, respectively) BYI 02960 increased from 2.2 and 2.3% of AR at day 0 to 37.6 and 38.9% at study termination.

DFA (difluoroacetic acid) was observed as a degradation product of [ETH]BYI 02960 in the water phases and in the sediment extracts of both water/sediment systems. In the water phases it accounted for up to 1.1% (HW) and 6.0% (AW) of AR, in the sediment extracts, DFA accounted for only 0.8% and 0.9% of AR, respectively. Two very minor metabolites were detected in the water phases of ETH test systems (≤ 1.1% of AR), and three very minor metabolites were detected in the water phases of FUR test systems (≤ 1.0% of AR). In the sediments, one very minor metabolite was detected in the

ETH test systems (≤ 0.5% of AR) and the FUR test systems (≤ 0.1% of AR). The maximum amount of the non-characterized radioactivity was 0.5% of AR for all test systems and compartments.

The dissipation of BYI 02960 from the water phase was mainly characterized by a fast translocation into the sediment. The best-fit kinetic model for the determination of trigger values was the DFOP kinetic model with DT_{50} values of 9.8 and 9.4 days for FUR and ETH test systems from HW, and DT_{50} values of 59.2 and 66.2 days for FUR and ETH test systems from AW. The corresponding modeling endpoints were also calculated using the DFOP kinetic model. The DT50 values for the slow degradation phase were 48.5 and 50.2 days for the water phase of HW, but 123.8 and 117.5 days for the water phase of AW.
In the entire water/sediment systems, BYI 02960 was degraded slowly, the degradation was best described using single first-order kinetics (SFO). The estimated DT_{50} values were 208.2 and 202.4 days for FUR and ETH systems from HW, and 246.1 and 285.0 for FUR and ETH test systems from AW. These values are to be used as trigger values and modelling endpoints.

I. MATERIALS AND METHODS

A. Materials

1. Test Items: BYI02960, CAS No: 951659-40-8
[Furanone-4-^{14}C]BYI02960, sample ID: KATH 6352
Specific activity = 3.94 MBq/mg (106.46 µCi/mg)
Radiochemical purity: >99% (acc. radio-HPLC and -TLC)
Chemical purity: >99% (HPLC, UV detection at 210 nm)

[Ethyl-1-^{14}C]BYI02960, sample ID: KATH 6358
Specific activity = 3.93 MBq/mg (106.28 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, near [redacted], Germany;) and [redacted] (AW, [redacted]).
[redacted] is an artificially dammed pond in the course of [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 chosen systems are well characterized.
Physical-chemical characteristics of the water/sediment systems are summarized in Table 7.8.3- 9.
Water and sediment samples were taken separately and poured into plastic containers. The collected sediments were sieved down to 2 mm mesh-size to remove parts of e.g. plants and stones. The collected water phases were filtered through a 0.06 mm sieve.

Table 7.8.3- 9: Physico-chemical characteristics of water and sediment

Parameter	[redacted] (HW)	[redacted] (AW)
Geographic Location	close to [redacted] Germany	[redacted] Germany
Properties of Water		
Temperature [°C][1]	13.1	16.5
pH[1]	6.5	6.9
Hardness [°dH]*	2.3	9.7
Oxygen Concentration (saturation) [mg/L][1]	8.59	9.02
Total Organic Carbon (TOC) [mg/L][2]	5 / 3 /11	< 2 / < 2 / 2
Dissolved Organic Carbon (DOC) [mg/L]	4	< 2
Total Nitrogen [mg/L]	< 10	4.6
Total Phosphorus [mg/L]	< 0.3	< 0.03
Redox Potential E_h [mV][1,4]	+ 445	+ 418
Properties of Sediments		
Soil Taxonomic Classification (USDA)	Loam	Sand
Sand (2000 – 50 µm) [%]	44	88
Silt (< 50 – 2 µm) [%]	45	8
Clay (< µm) [%]	11	4
pH[#]	4.8 ($CaCl_2$); 5.0 (H_2O)	6.8 ($CaCl_2$); 7.2 (H_2O)
Organic Matter [%][2,3]	8.8	0.7
Organic Carbon [%][2]	5.1 / n.a. / 4.8	0.4 / n.a. / 0.6
% $CaCO_3$ [#]	0.2	0.4
Sediment Microbial Activity [mg CO_2 /hr/kg sediment (dry wt)][2]	22.50 / 20.83 / 12.42	7.92 / 6.67 / 4.5
Cation Exchange Capacity [meq/100 g]	8.5	3.5
Total Nitrogen [%]	0.39	0.02
Total Phosphorus (Olsen) [ppm]	784	188
Redox Potential E_h [mV][1,4]	+ 142	+ 331

[1] Measurement at day of sampling
[2] start of acclimation / DAT-0 / DAT-120
[3] %organic matter =%organic carbon x 1.724
n.a. = not analyzed
[4] $E_h = E_{obs} + U_{ref}$ [U_{ref} = reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at given Temperature; 210 mV at 20 °C, extrapolated from manufacturer information]

B. Materials

1. Experimental conditions: The test systems consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. The individual static test systems were kept at aerobic conditions at 20 ± 1 °C for a maximum period of 120 days. Entire vessels filled with either 82.2 g (HW) or 279.7 g (AW) dry weight sediment and 525 mL of supernatant water (volume ratio of water to sediment: 3:1) were applied with [FUR-^{14}C] or [ETH-^{14}C]BYI 02960, resulting in four parallel test series. After pre-equilibration, aliquots of the application solution were directly applied onto the water surface of each microcosm system. Thereafter, the systems were closed with the trap attachment for absorbing volatile compounds from DAT 1 onwards. During incubation the supernatant water was agitated gently.

The nominal application rate of 20.8 µg/batch corresponds to about 40 µg/L water and was selected based on a field application rate of 400 g/ha. The actual application rates were 20.86 and 20.80 µg/flask for FUR and ETH test systems from [redacted] and 20.63 and 20.53 µg/flask for FUR and ETH test systems from [redacted].

2. Sampling: Duplicate flasks were processed and analyzed after 0, 1, 3, 7, 14, 30, 45/44, 60/58, 87/86 and 120 days of incubation for test systems from HW and AW, respectively. The water phase was decanted and analyzed for radioactivity after centrifugation and filtration. Prior to HPLC analysis, a concentration step was performed. The sediment was extracted three times with acetonitrile/water (70/30, v/v), followed by one extraction step with pure acetonitrile, all at ambient temperature (ambient organic extracts). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v). The extracted sediment phase was freeze-dried, homogenized and combusted in an oxidizer. The evolved CO_2 was trapped in a scintillation cocktail and measured by LSC to determine the non-extractable residues.

3. Description of analytical procedures: The radioactivity of the supernatant and sediment samples was radio-assayed by LSC and HPLC. The combined extracts from the ambient extractions and the aggressive extract were analyzed by LSC and via HPLC after a concentration step. For selected samples, the HPLC analysis was confirmed by TLC. Identification of the two test items was achieved by HPLC-MS, HPLC-MS/MS, NMR and/or Co-chromatography. The major metabolite detected in the ETH test systems was characterized by HPLC-MS. Volatile organics and $^{14}CO_2$ were trapped with solid trapping attachments containing soda lime for absorption of $^{14}CO_2$ and polyurethane foam for volatile organic compounds. The non-extractable residues were separated into humin, humic acid and fulvic acid fractions for the last sampling interval.

C. Determination of Degradation Kinetics

Dissipation rates from the water phase and rates of degradation for the total system were calculated by use of the software KinGui, version 1.1. The kinetics evaluation included the fitting of data with kinetic models SFO, FOMC and DFOP to the experimental data and their assessment according to FOCUS guidance to result in values for comparison with trigger endpoints.

II. RESULTS

The test conditions outlined in the study protocol were maintained throughout the study

Table 7.8.3- 10: Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (HW)

		Water Phase								Sediment Layer					
DAT	Replicate JR48-H-	pH		Oxygen Conc. [mg O_2/L]		Redox Electrode [mV]	Redox E_h* [mV]	Redox Electrode [mV]	Redox E_h* [mV]	pH		Redox Electrode [mV]	Redox E_h* [mV]	Redox Electrode [mV]	Redox E_h* [mV]
Pre-incubation/Label		F	E	F	E	F	F	E	E	F	E	F	F	E	E
-11		6.1	6.2	8.1	8.1	130	340	130	340	6.2	6.2	-41	169	-41	169
-7		6.1	6.1	7.8	7.8	141	351	141	351	6.1	6.1	42	252	42	252
-5		6.1	6.0	8.5	8.5	218	428	218	428	6.0	6.0	70	280	70	280
-3		5.9	6.0	8.3	8.3	212	422	212	422	6.0	6.0	100	310	100	310
0	D0-F/E1	5.8	5.8	7.7	7.6	220	430	205	415	5.9	5.9	70	280	98	308
	D0-F/E2	5.8	5.8	7.6	7.7	210	420	202	412	5.9	6.0	65	275	110	320
	Mean	**5.8**	**5.8**	**7.6**	**7.6**	**215**	**425**	**204**	**414**	**5.9**	**6.0**	**68**	**278**	**104**	**314**
1	D1-F/E1	6.3	6.2	7.7	7.7	248	458	238	448	6.2	6.1	98	308	100	310
	D1-F/E2	6.2	6.2	7.7	7.7	246	456	236	446	6.2	6.1	95	305	108	318
	Mean	**6.2**	**6.2**	**7.7**	**7.7**	**247**	**457**	**237**	**447**	**6.2**	**6.1**	**97**	**307**	**104**	**314**
3	D3-F/E1	6.5	6.5	7.6	7.6	237	447	234	444	6.3	6.2	98	308	104	314
	D3-F/E2	6.5	6.5	7.6	7.5	239	449	231	441	6.2	6.3	113	323	109	319
	Mean	**6.5**	**6.5**	**7.6**	**7.5**	**238**	**448**	**233**	**443**	**6.2**	**6.3**	**106**	**316**	**107**	**317**
7	D7-F/E1	6.2	6.2	7.7	7.9	245	455	242	452	6.0	6.1	106	316	110	320
	D7-F/E2	6.2	6.2	7.5	7.8	240	450	236	446	6.0	6.0	111	321	104	314
	Mean	**6.2**	**6.2**	**7.6**	**7.8**	**243**	**453**	**239**	**449**	**6.0**	**6.0**	**109**	**319**	**107**	**317**
14	D14-F/E1	6.4	6.5	7.4	7.7	251	461	243	453	6.2	6.1	114	324	101	311
	D14-F/E2	6.5	6.5	7.4	7.8	249	459	238	448	6.1	6.2	108	318	108	318
	Mean	**6.4**	**6.5**	**7.4**	**7.7**	**250**	**460**	**241**	**451**	**6.2**	**6.1**	**111**	**321**	**105**	**315**
30	D30-F/E1	6.7	6.7	7.6	7.6	241	451	228	438	6.2	6.2	128	338	116	326
	D30-F/E2	6.7	6.7	7.5	7.7	237	447	233	443	6.2	6.2	116	326	110	320
	Mean	**6.7**	**6.7**	**7.5**	**7.6**	**239**	**449**	**231**	**441**	**6.2**	**6.2**	**122**	**332**	**113**	**323**
45	D45-F/E1	6.8	7.0	7.3	7.5	238	448	228	438	6.2	6.2	120	330	113	323
	D45-F/E2	6.8	7.0	7.3	7.4	234	444	238	448	6.2	6.2	111	321	106	316
	Mean	**6.8**	**7.0**	**7.3**	**7.4**	**236**	**446**	**233**	**443**	**6.2**	**6.2**	**116**	**326**	**110**	**320**
60	D60-F/E1	6.9	6.8	7.4	7.6	252	462	242	452	6.2	6.2	131	341	128	338
	D60-F/E2	6.7	6.9	7.5	7.6	246	456	246	456	6.3	5.9	122	332	115	325
	Mean	**6.8**	**6.9**	**7.4**	**7.6**	**249**	**459**	**244**	**454**	**6.2**	**6.0**	**127**	**337**	**122**	**332**
87	D87-F/E1	6.8	6.8	7.3	7.3	238	448	244	454	6.2	6.1	138	348	148	358
	D87-F/E2	6.8	6.8	7.3	7.3	240	450	248	458	6.2	6.1	145	355	148	358
	Mean	**6.8**	**6.8**	**7.3**	**7.3**	**239**	**449**	**246**	**456**	**6.2**	**6.1**	**142**	**352**	**148**	**358**
120	D120-F/E1	6.6	6.6	7.2	7.3	232	442	238	448	6.0	5.9	141	351	136	346
	D120-F/E2	6.6	6.6	7.2	7.2	238	448	243	453	5.9	5.9	133	343	134	344
	Mean	**6.6**	**6.6**	**7.2**	**7.3**	**235**	**445**	**241**	**451**	**6.0**	**5.9**	**137**	**347**	**135**	**345**
overall Mean#		6.5	6.5	7.5	7.6	239	449	235	445	6.1	6.1	113	323	115	325
overall Minimum#		5.8	5.8	7.2	7.2	210	420	202	412	5.9	5.9	65	275	98	308
overall Maximum#		6.9	7.0	7.7	7.9	252	462	248	458	6.3	6.3	145	355	148	358

* $E_h = E_{obs}$ + 210 mV [reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at 20°C; manufacturer information]

without pre-incubation

Table 7.8.3- 11: Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (AW)

		Water Phase								Sediment Layer					
DAT	Replicate JR48-A-	pH		Oxygen Conc. [mg O_2/L]		Redox Potential				pH		Redox Potential			
						Electrode [mV]	E_h* [mV]	Electrode [mV]	E_h* [mV]			Electrode [mV]	E_h* [mV]	Electrode [mV]	E_h* [mV]
Pre-incubation/Label		F	E	F	E	F	F	E	E	F	E	F	F	E	E
-7		7.5	7.5	7.7	7.7	234	444	234	444	6.9	6.9	199	409	199	409
-5		7.7	7.7	8.3	8.3	236	446	236	446	7.0	7.0	203	413	203	413
-3		7.8	7.8	8.3	8.3	234	444	234	444	7.1	7.1	211	421	211	421
0	D0-F/E1	7.9	7.8	8.3	8.4	236	446	233	443	7.1	7.1	204	414	211	421
	D0-F/E2	7.9	7.4	8.4	8.4	233	443	230	440	7.1	7.0	207	417	208	418
	Mean	**7.9**	**7.6**	**8.3**	**8.4**	**235**	**445**	**232**	**442**	**7.1**	**7.1**	**206**	**416**	**210**	**420**
1	D1-F/E1	8.1	8.1	8.0	8.1	205	415	210	420	7.1	7.1	215	425	208	418
	D1-F/E2	8.1	8.1	8.1	7.9	203	413	208	418	7.1	7.1	208	418	204	414
	Mean	**8.1**	**8.1**	**8.0**	**8.0**	**204**	**414**	**209**	**419**	**7.1**	**7.1**	**212**	**422**	**206**	**416**
3	D3-F/E1	7.9	8.0	8.1	8.0	215	425	210	420	7.1	7.1	222	432	222	432
	D3-F/E2	8.0	8.0	8.0	8.1	208	418	211	421	7.1	7.2	225	435	218	428
	Mean	**8.0**	**8.0**	**8.0**	**8.0**	**212**	**422**	**211**	**421**	**7.1**	**7.1**	**224**	**434**	**220**	**430**
7	D7-F/E1	8.0	8.0	8.4	8.4	203	413	219	429	7.2	7.1	228	438	221	431
	D7-F/E2	8.1	8.0	8.3	8.5	199	409	217	427	7.2	7.0	230	440	216	426
	Mean	**8.0**	**8.0**	**8.3**	**8.4**	**201**	**411**	**218**	**428**	**7.2**	**7.0**	**229**	**439**	**219**	**429**
14	D14-F/E1	8.3	8.2	8.1	8.2	228	438	213	423	7.0	7.1	185	395	193	403
	D14-F/E2	8.3	8.2	8.2	8.2	218	428	214	424	7.0	7.1	190	400	195	405
	Mean	**8.3**	**8.2**	**8.2**	**8.2**	**223**	**433**	**214**	**424**	**7.0**	**7.1**	**188**	**398**	**194**	**404**
30	D30-F/E1	8.4	8.3	8.3	8.3	230	440	229	439	7.2	7.2	183	393	198	408
	D30-F/E2	8.4	8.3	8.4	8.3	226	436	230	440	7.2	7.2	196	406	201	411
	Mean	**8.4**	**8.3**	**8.3**	**8.3**	**228**	**438**	**230**	**440**	**7.2**	**7.2**	**190**	**400**	**200**	**410**
44	D44-F/E1	8.3	8.2	8.3	8.2	236	446	238	448	7.1	7.1	193	403	209	419
	D44-F/E2	8.3	8.3	8.2	8.2	237	447	234	444	7.1	7.0	205	415	196	406
	Mean	**8.3**	**8.2**	**8.2**	**8.2**	**237**	**447**	**236**	**446**	**7.1**	**7.0**	**199**	**409**	**203**	**413**
58	D58-F/E1	8.4	8.4	8.1	8.2	198	408	201	411	7.0	7.0	186	396	190	400
	D58-F/E2	8.4	8.4	8.2	8.2	205	415	206	416	7.0	7.0	194	404	188	398
	Mean	**8.4**	**8.4**	**8.2**	**8.2**	**202**	**412**	**204**	**414**	**7.0**	**7.0**	**190**	**400**	**189**	**399**
86	D86-F/E1	8.5	8.5	8.5	8.5	221	431	217	427	7.0	6.9	186	396	186	396
	D86-F/E2	8.5	8.5	8.5	8.5	223	433	218	428	7.0	7.0	180	390	188	398
	Mean	**8.5**	**8.5**	**8.5**	**8.5**	**222**	**432**	**218**	**428**	**7.0**	**7.0**	**183**	**393**	**187**	**397**
120	D120-F/E1	8.6	8.6	8.2	8.2	228	438	225	435	7.2	7.2	194	404	198	408
	D120-F/E2	8.6	8.6	8.2	8.2	225	435	226	436	7.1	7.1	198	408	196	406
	Mean	**8.6**	**8.6**	**8.2**	**8.2**	**227**	**437**	**226**	**436**	**7.2**	**7.1**	**196**	**406**	**197**	**407**
overall Mean#		8.3	8.2	8.2	8.2	219	429	219	429	7.1	7.1	201	411	202	412
overall Minimum#		7.9	7.4	8.0	7.9	198	408	201	411	7.0	6.9	180	390	186	396
overall Maximum#		8.6	8.6	8.5	8.5	237	447	238	448	7.2	7.2	230	440	222	432

* $E_h = E_{obs}$ + 210 mV [reference potential of SenTix ORP electrode (WTW) vs. standard hydrogen electrode at 20°C; manufacturer information]
# without pre-incubation

A. Data

. A summary of key data on total recovery and the distribution of radioactivity into the various components formed in water and sediment is given in Table 7.8.3- 12 and Table 7.8.3- 13 for test system [redacted] and in Table 7.8.3- 14 and Table 7.8.3- 15 for test system [redacted].

B. Mass Balance

During the study, the total recovery of radioactivity in individual test vessels of the HW test systems ranged from 94.9 to 100.7% (mean 98.2%, RSD 2.0%) for FUR and from 96.5 to 101.4% (mean 98.7%, RSD 1.4%) for ETH. The radioactivity in individual test vessels of the AW test systems ranged from 98.4 to 101.8% (mean 100.4%, RSD 1.1%) for FUR and from 99.4 to 101.2% (mean 100.3%, RSD 0.5%) for ETH. The complete material balance found for all sampling intervals demonstrates that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

Table 7.8.3- 12: Biotransformation of [FUR-^{14}C]BYI 02960 in [redacted] under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT)									
		0	1	3	7	14	30	45	60	87	120
Parent BYI 02960	Water Layer	95.5	86.4	69.3	57.5	47.0	32.7	27.0	21.1	15.6	14.3
		±0.6	±1.1	±1.1	±0.7	±1.5	±1.0	±0.8	±1.2	±1.8	±1.2
	Sediment	4.1	10.9	27.1	37.2	47.4	55.1	57.2	58.7	54.9	54.3
		±0.7	±0.3	±0.6	±0.3	±0.9	±0.4	±0.5	±1.5	±0.7	±2.9
	Entire System	99.6	97.3	96.4	94.7	94.4	87.9	84.2	79.8	70.5	68.6
Reg 2	Water Layer	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0
		±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.1	±0.0
	Sediment	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
		±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.1
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.1
Non-characterized radioactivity	Water Layer	0.5	0.3	0.4	0.3	0.2	0.2	0.1	0.1	0.1	0.0
		±0.0	±0.1	±0.0	±0.0	±0.1	±0.1	±0.0	±0.0	±0.0	±0.0
	Sediment	0.1	0.4	0.1	0.1	0.2	0.2	0.3	0.2	0.3	0.2
		±0.0	±0.0	±0.0	±0.0	±0.1	±0.1	±0.0	±0.0	±0.1	±0.1
Total extractable residues	Water Layer	96.0	86.7	69.7	57.7	47.3	32.9	27.1	21.3	16.3	14.3
		±0.6	±1.0	±1.1	±0.7	±1.5	±1.1	±0.8	±1.2	±1.9	±1.2
	Sediment	4.2	11.3	27.3	37.3	47.6	55.3	57.5	58.9	55.3	54.7
		±0.8	±0.3	±0.6	±0.3	±0.0	±0.5	±0.5	±1.5	±0.6	±3.1
Total $^{14}CO_2$	Entire System	0.0	0.0	0.1	0.2	0.4	1.0	1.2	1.8	3.3	3.9
		±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.1	±0.0	±0.7	±0.3
Total volatile organics	Entire System	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0
		±0.0	±0.0	±0.0	±0.1	±0.1	±0.0	±0.0	±0.0	±0.0	±0.0
NER	Sediment	0.4	1.0	2.5	4.1	5.2	8.9	11.9	14.9	19.9	22.6
		±0.0	±0.0	±0.3	±0.1	±0.3	±0.6	±0.5	±0.6	±1.0	±3.7
Total recovery	Water Layer	96.0	86.7	69.7	57.7	47.3	32.9	27.1	21.3	16.3	14.3
		±0.6	±1.0	±1.1	±0.7	±0.2	±1.1	±0.8	±1.2	±1.9	±1.2
	Sediment	4.6	12.3	29.7	41.4	52.8	64.3	69.4	73.8	75.2	77.3
		±0.8	±0.3	±0.6	±0.3	±0.9	±0.5	±0.5	±1.5	±0.6	±3.1
	Entire System	100.7	99.0	99.6	99.4	100.5	98.2	97.8	97.0	94.9	95.5
		±0.1	±0.7	±0.1	±0.5	±0.2	±0.1	±0.3	±0.9	±0.8	±0.3

n.d.: not detected; n.a. : not analysed; DAT : day after treatment, ± = standard deviation.

Table 7.8.3- 13: Biotransformation of [ETH-^{14}C]BYI 02960 in [redacted] under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT) 0	1	3	7	14	30	45	60	87	120
Parent BYI 02960	Water Layer	96.8 ±0.3	82.1 ±0.1	70.1 ±0.4	57.4 ±0.6	44.6 ±0.8	31.9 ±1.0	28.2 ±0.4	21.5 ±0.1	14.8 ±0.2	14.1 ±0.7
	Sediment	3.7 ±0.1	14.2 ±0.2	26.8 ±0.0	37.2 ±0.1	48.6 ±0.6	56.0 ±0.6	58.3 ±0.2	57.2 ±0.6	55.9 ±0.6	52.6 ±1.8
	Entire System	100.6	96.2	96.8	94.6	93.2	87.9	86.5	78.8	70.7	66.7
DFA	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.6 ±0.0	0.0 ±0.0	1.1 ±0.0	0.7 ±0.1	0.0 ±0.0
	Sediment	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	0.0 ±0.0	0.8 ±0.1	0.7 ±0.5
	Entire System	0.0	0.0	0.0	0.0	0.0	0.6	0.0	1.1	1.4	0.7
Reg 2	Water Layer	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	1.0 ±0.0	0.8 ±0.0	0.0 ±0.0
	Sediment	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	0.0 ±0.0	0.0 ±0.0	0.5 ±0.5
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.8	0.5
Non-characterized radioactivity	Water Layer	0.4 ±0.0	0.3 ±0.0	0.4 ±0.0	0.3 ±0.0	0.2 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
	Sediment	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0	0.3 ±0.0	0.2 ±0.1	0.3 ±0.0	0.2 ±0.1
Total extractable residues	Water Layer	97.2 ±0.3	82.4 ±0.1	70.4 ±0.4	57.6 ±0.5	44.8 ±0.8	32.6 ±1.0	28.3 ±0.4	23.8 ±0.1	16.4 ±0.2	14.2 ±0.7
	Sediment	3.8 ±0.1	14.2 ±0.2	26.9 ±0.0	37.5 ±0.1	48.8 ±0.6	56.2 ±0.6	58.6 ±0.2	57.5 ±0.6	57.0 ±0.8	54.1 ±0.7
Total $^{14}CO_2$	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.2 ±0.0	0.6 ±0.0	1.1 ±0.0	1.5 ±0.3
Total volatile organics	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.1 ±0.1	0.0 ±0.0	0.1 ±0.0	0.1 ±0.0
NER	Sediment	0.3 ±0.0	1.5 ±0.3	2.1 ±0.1	4.2 ±0.0	6.0 ±0.6	9.4 ±0.1	12.0 ±0.3	16.3 ±0.5	22.5 ±1.0	26.6 ±0.8
Total recovery	Water Layer	97.2 ±0.3	82.4 ±0.1	70.4 ±0.4	57.6 ±0.5	44.8 ±0.8	32.6 ±1.0	28.3 ±0.4	23.8 ±0.1	16.4 ±0.2	14.2 ±0.7
	Sediment	4.1 ±0.1	15.8 ±0.2	29.0 ±0.0	41.6 ±0.1	54.7 ±0.6	65.6 ±0.6	70.6 ±0.2	73.8 ±0.6	79.5 ±0.8	54.1 ±0.7
	Entire System	101.4 ±0.4	98.2 ±0.5	99.4 ±0.3	99.3 ±0.4	99.6 ±0.8	98.4 ±0.3	99.2 ±0.3	98.2 ±0.0	97.0 ±0.2	96.5 ±0.3

n.d.: not detected; n.a. : not analysed; DAT : day after treatment, ± = standard deviation.

Table 7.8.3- 14: Biotransformation of [FUR-^{14}C]BYI 02960 in [redacted] under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT) 0	1	3	7	14	30	45	60	87	120
Parent BYI 02960	Water Layer	97.4 ±0.8	89.4 ±1.6	85.1 ±0.3	78.8 ±0.2	69.6 ±0.1	59.3 ±2.8	52.3 ±0.8	46.0 ±0.9	41.5 ±0.8	35.6 ±1.6
	Sediment	2.2 ±0.1	10.4 ±1.9	14.2 ±0.3	19.4 ±0.0	26.4 ±0.2	31.9 ±1.8	34.8 ±0.3	34.9 ±0.8	37.6 ±0.4	37.6 ±0.3
	Entire System	99.6	99.8	99.4	98.2	96.0	91.2	87.1	80.9	79.1	73.1
Reg 4	Water Layer	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	1.0 ±0.1	0.8 ±0.0	0.6 ±0.2
	Sediment	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	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.8	0.6
Reg 2	Water Layer	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	0.8 ±0.0	0.7 ±0.1	0.9 ±0.1
	Sediment	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	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.7	0.9
Reg 3	Water Layer	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	0.5 ±0.0	0.0 ±0.0	0.0 ±0.0
	Sediment	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	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Non-characterized radioactivity	Water Layer	0.5 ±0.1	0.2 ±0.0	0.3 ±0.1	0.4 ±0.0	0.4 ±0.0	0.2 ±0.1	0.3 ±0.1	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0
	Sediment	0.1 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.1 ±0.1	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0	0.2 ±0.0	0.1 ±0.1
Total extractable residues	Water Layer	97.9 ±0.7	89.6 ±1.6	85.5 ±0.2	79.1 ±0.2	70.0 ±0.1	59.5 ±2.7	52.6 ±0.9	48.6 ±1.0	43.2 ±0.8	37.3 ±1.4
	Sediment	2.2 ±0.1	10.4 ±1.9	14.2 ±0.3	19.5 ±0.0	26.5 ±0.0	32.1 ±1.8	35.0 ±0.3	35.1 ±0.8	37.8 ±0.5	37.7 ±0.2
Total $^{14}CO_2$	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.3 ±0.0	0.5 ±0.0	0.7 ±0.1	1.6 ±0.1	2.4 ±0.1	3.3 ±0.3	5.5 ±0.7
Total volatile organics	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.1	0.0 ±0.0	0.0 ±0.0	0.1 ±0.1	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
NER	Sediment	0.5 ±0.0	0.9 ±0.0	1.6 ±0.0	2.6 ±0.1	4.7 ±0.0	8.4 ±0.1	10.3 ±0.4	13.0 ±0.0	15.1 ±0.1	17.9 ±0.2
Total recovery	Water Layer	97.9 ±0.7	89.6 ±1.6	85.5 ±0.2	79.1 ±0.2	70.0 ±0.0	59.5 ±2.7	52.6 ±0.9	48.6 ±1.0	43.2 ±0.8	37.3 ±1.4
	Sediment	2.7 ±0.1	11.3 ±1.9	15.9 ±0.3	22.1 ±0.0	31.3 ±0.2	40.5 ±1.8	45.3 ±0.3	48.1 ±0.8	52.9 ±0.5	37.7 ±0.2
	Entire System	100.6 ±0.6	101.0 ±0.4	101.4 ±0.5	101.6 ±0.3	101.8 ±0.0	100.7 ±1.1	99.5 ±0.3	99.1 ±0.0	99.4 ±0.0	98.4 ±0.2

n.d.: not detected, n.a. : not analysed, DAT : day after treatment, ± = standard deviation.

Table 7.8.3- 15: Biotransformation of [ETH-[14]C]BYI 02960 in [redacted] under aerobic conditions, expressed as percent of AR, mean ± SD

Compound	Source	Days After Treatment (DAT) 0	1	3	7	14	30	45	60	87	120
Parent BYI 02960	Water Layer	97.9 ±1.1	91.6 ±0.1	87.4 ±1.5	80.6 ±0.7	73.9 ±0.1	62.1 ±0.7	54.7 ±0.0	49.7 ±0.0	43.2 ±0.9	36.8 ±0.3
	Sediment	2.3 ±0.2	7.5 ±0.1	11.5 ±0.6	17.3 ±0.5	22.3 ±0.5	29.4 ±0.2	33.6 ±0.6	34.4 ±0.0	38.0 ±0.2	38.9 ±0.0
	Entire System	100.2	99.1	98.8	97.8	96.2	91.5	88.3	84.1	81.2	75.7
DFA	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	1.5 ±0.0	2.2 ±0.1	2.6 ±0.1	4.2 ±0.7	6.0 ±0.2
	Sediment	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	0.5 ±0.0	0.6 ±0.0	0.9 ±0.1
	Entire System	0.0	0.0	0.0	0.0	0.0	1.5	2.2	3.1	4.8	6.9
Reg 2	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.8 ±0.2	0.9 ±0.1	1.0 ±0.0	1.1 ±0.2
	Sediment	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	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.9	1.0	1.1
Reg 3	Water Layer	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.6 ±0.1	0.4 ±0.0	0.0 ±0.0	0.0 ±0.0
	Sediment	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	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	Entire System	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.4	0.0	0.0
Non-characterized radioactivity	Water Layer	0.4 ±0.0	0.3 ±0.1	0.4 ±0.1	0.4 ±0.0	0.5 ±0.2	0.1 ±0.0	0.3 ±0.0	0.3 ±0.1	0.3 ±0.1	0.2 ±0.0
	Sediment	0.1 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0	0.2 ±0.0	0.2 ±0.0	0.1 ±0.0	0.2 ±0.0
Total extractable residues	Water Layer	98.3 ±1.1	91.9 ±0.0	87.7 ±1.4	81.0 ±0.7	74.3 ±0.1	63.8 ±0.6	58.6 ±0.2	53.9 ±0.1	48.6 ±0.3	44.1 ±0.1
	Sediment	2.4 ±0.3	7.5 ±0.1	11.5 ±0.6	17.3 ±0.5	22.3 ±0.0	29.5 ±0.2	33.8 ±0.6	35.1 ±0.0	38.7 ±0.1	40.1 ±0.0
Total $^{14}CO_2$	Entire System	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.1 ±0.0	0.2 ±0.0	0.3 ±0.0	0.5 ±0.0	0.9 ±0.0
Total volatile organics	Entire System	0.0 ±0.0	0.0 ±0.0	0.1 ±0.1	0.1 ±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	Sediment	0.5 ±0.0	0.6 ±0.0	1.1 ±0.0	2.0 ±0.1	3.4 ±0.0	6.0 ±0.1	7.4 ±0.1	10.5 ±0.1	13.1 ±0.3	15.2 ±0.2
Total recovery	Water Layer	98.3 ±1.1	91.9 ±0.0	87.7 ±1.4	81.0 ±0.7	74.3 ±0.6	63.8 ±0.6	58.6 ±0.2	53.9 ±0.1	48.6 ±0.3	44.1 ±0.1
	Sediment	2.9 ±0.3	8.2 ±0.1	12.6 ±0.6	19.3 ±0.5	25.7 ±0.5	35.5 ±0.2	41.3 ±0.6	45.6 ±0.0	51.8 ±0.1	40.1 ±0.0
	Entire System	101.2 ±0.8	100.1 ±0.1	100.4 ±0.9	100.3 ±0.2	100.1 ±0.6	99.4 ±0.7	100.1 ±0.7	99.9 ±0.1	100.9 ±0.6	100.3 ±0.0

n.d.: not detected; n.a. : not analysed; DAT : day after treatment, ± = standard deviation.

C. Volatilization

Formation of $^{14}CO_2$, i.e. mineralization of BYI 02960, was observed in all water/sediment systems. At termination of the study, the $^{14}CO_2$ recovery (mean values of duplicates) in systems from HW was 3.9

and 1.5% of AR for FUR and ETH, respectively. In AW water/sediment systems, $^{14}CO_2$ accounted for 5.5 and 0.9% of AR at study termination for FUR and ETH, respectively.
Organic volatiles were < 0.2% of the applied radioactivity for both systems and labels.

D. Residues in water, bound and extractable residues in sediment

The radioactivity in FUR and ETH test systems from [redacted] water decreased steadily from 96.0 and 97.2% of AR at day 0 to 14.3 and 14.2% of AR at study termination. The radioactivity in FUR and ETH test systems from [redacted] water decreased from 97.9 and 98.3% of AR at day 0 to 37.3 and 44.1% of AR towards the end of the study. The elimination of BYI 02960 from the water body occurred mainly via translocation into the sediment phase and partly via degradation.

The extractable radioactivity in FUR and ETH test systems from HW increased from 4.2 and 3.8% of AR at day 0 to 54.7 and 54.1% of AR at study termination, respectively. Extractable ^{14}C residues in FUR and ETH sediment from [redacted] increased from 2.2 and 2.4% of AR at day 0 to maxima of 37.7 and 40.1% of AR at study termination. This indicates a rapid partitioning of BYI 02960 residues from the water phases into the sediments.

The NER for FUR and ETH test systems from HW were 0.4 and 0.3% of AR at day 0 and increased to 22.6 and 26.6% of AR at study termination, respectively. For AW water/sediment systems (FUR and ETH, respectively), the amount of NER was 0.5% of AR at day 0 and increased to 17.9 and 15.2% of AR towards the end of the incubation period. By further characterization it was found that the NER was of a heterogeneous nature in case of both types of sediments and for both radiolabels. In the sediments from HW, portions of only 1.4 and 1.8% of the NER were fractionated together with humic acids, whereas the major portion of radioactivity (53.9 and 59.0% of NER for FUR and ETH, respectively) was associated with fulvic acids. Amounts of 45.2% (FUR) and 42.9% (ETH) of the NER were found strongly integrated into the insoluble humin of the sediment matrix. In the sediment from AW, only 4.8 and 2.4% of the NER were fractionated together with humic acids for FUR and ETH, respectively, whereas similar amounts of radioactivity were associated with fulvic acids or strongly integrated into the insoluble humin of the sediment matrix (45.4 and 47.2% of the NER for FUR; 47.9 and 49.1 for ETH).

E. Transformation of Parent Compound

In the water phase from HW, the amount of BYI 02960 decreased from 95.5 and 96.8% of AR at day 0 to 14.3 and 14.1% of AR at study termination for FUR and ETH test systems, respectively. In the AW water of FUR and ETH test systems, the amount of BYI 02960 decreased from 97.4 and 97.9% of AR at day 0 to 35.6 and 36.8% of AR at study termination.
One major peak was observed in the water phase of ETH test systems, accounting for up to 1.1% of AR in the water phase from HW, and 6.0% of AR in water phases from AW. The metabolite was identified as DFA using HPLC-MS. Further, two very minor metabolites were detected in the water phases of ETH test systems (≤ 1.1% of AR) and three very minor metabolites were detected in the water phases of FUR test systems (≤ 1.0% of AR). The maximum amount of the non-characterized radioactivity was 0.5% of AR for all water phases.

In the sediment phase of FUR and ETH test systems from HW, the amount of BYI 02960 increased from 4.1 and 3.7% of AR at day 0 to maximum amounts of 58.7 and 58.3% of AR at DAT-60 or DAT-45, and then slightly declined to 54.3 and 52.6% of AR towards study termination. In the AW

sediment (FUR and ETH, respectively) the amount of BYI 02960 increased from 2.2 and 2.3% of AR at day 0 to 37.6 and 38.9% of AR at study termination. -DFA was also observed in the ETH sediments with maximum amounts of 0.8% of AR (DAT-87) for HW and 0.9% of AR (DAT 120) for sediment from AW. Additionally one very minor metabolite was detected in the ETH sediments (≤ 0.5% of AR) and FUR sediments (≤ 0.1% of AR), respectively. The maximum amount of the non-characterized radioactivity was 0.4% of AR for all sediments.

The BYI 02960 residues in the <u>entire test systems</u> from HW declined to 68.6 and 66.7% of AR at study termination, FUR and ETH, respectively. In the entire FUR and ETH test systems from AW, 73.1 and 75.7% of AR were found as unchanged test item at the end of the incubation period.
The metabolite DFA was found at maximum amounts of 1.4% ([redacted], DAT-87) and 6.9% of AR ([redacted], DAT-120). The maximum amount of a single minor radioactivity zone in both systems and with both labels was 1.1% of the AR.

F. Dissipation Kinetics

The dissipation of BYI 02960 from the water phase is mainly characterized by a fast translocation into the sediment which is best described using bi-phasic kinetic models. The best-fit kinetic model for the determination of trigger values was the DFOP kinetic model with DT_{50} values of 9.8 and 9.4 days for FUR and ETH test systems from [redacted] and DT_{50} values of 59.2 and 66.2 days for FUR and ETH test systems from [redacted]. The corresponding modeling endpoints were also calculated using the DFOP kinetic model. The DT_{50} values for the slow degradation phase were 48.5 and 50.2 days for the water phase of [redacted], but 123.8 and 117.5 days for the water phase of [redacted] which is much more of sandy type.
In the entire water/sediment systems, BYI 02960 the degradation was best described using single first-order kinetics (SFO). The estimated DT_{50} values were 208.2 and 202.4 days for FUR and ETH systems from [redacted] and 246.1 and 285.0 for FUR and ETH test systems from [redacted]. These values were used as trigger values and modelling endpoints.

Table 7.8.3- 16: Summary of dissipation kinetics of BYI 02960 from the supernatant water; evaluation for trigger values according to FOCUS

Water Phase of Test System	Kinetic Model	DT_{50} [days]	DT_{90} [days]	Chi^2 Error [%]
[redacted] (FUR)	SFO	24.6	81.6	15.6
	FOMC	10.2	292.3	3.7
	DFOP	9.8	118.4	4.1
[redacted] (ETH)	SFO	24.7	82.0	16.2
	FOMC	9.8	308.8	4.4
	DFOP	9.4	120.9	4.9
[redacted] (FUR)	SFO	69.3	230.1	7.6
	FOMC	53.2	> 1000	2.7
	DFOP	59.2	344.9	2.9
[redacted] (ETH)	SFO	73.1	242.8	6.5
	FOMC	61.4	> 1000	2.4
	DFOP	66.2	341.2	2.4

Bold: best fit; SFO = Single First Order Model; FOMC = First Order Multi Compartment Model; DFOP = Double First Order in Parallel Model

Table 7.8.3- 17: Summary of degradation kinetics of BYI 02960 from the entire water/sediment test system; evaluation for trigger values according to FOCUS

Entire Test System	Kinetic Model	DT50 [days]	DT90 [days]	Chi² Error [%]
[redacted] (FUR)	SFO	208.2	691.6	1.5
	FOMC	283.6	> 1000	1.3
	DFOP	*205.4*	706.8	1.4
[redacted] (ETH)	SFO	202.4	672.2	1.6
	FOMC	249.3	> 1000	1.6
	DFOP	197.6	685.2	1.3
[redacted] (FUR)	SFO	246.1	817.4	1.3
	FOMC	475.1	> 1000	0.9
	DFOP	626.4	> 1000	0.9
[redacted] (ETH)	SFO	285.0	946.9	1.0
	FOMC	633.6	> 1000	0.6
	DFOP	420.4	> 1000	0.6

Bold: best fit; SFO = Single First Order Model; FOMC = First Order Multi Compartment Model; DFOP = Double First Order in Parallel Model

Table 7.8.3- 18: Modelling endpoints according to FOCUS obtained for dissipation or degradation of BYI 02960 from the supernatant water

Water Phase of Test System	Kinetic Model	k_2	DT_{50} for k2 [days]	Visual Acceptability	Chi² Err. [%]	t-probability
HW (FUR)	DFOP	0.0143	48.5	Yes	4.1	< 0.001
HW (ETH)	DFOP	0.0138	50.2	Yes	4.9	< 0.001
AW (FUR)	DFOP	0.0056	123.8	Yes	2.9	< 0.001
AW (ETH)	DFOP	0.0059	117.5	Yes	2.4	< 0.001

DFOP = Double First Order in Parallel Model, k_2 = slow degradation rate constant of the DFOP kinetic model

Table 7.8.3- 19: Modelling endpoints according to FOCUS obtained for dissipation or degradation of BYI 02960 from the entire water/sediment system

Entire System	Kinetic Model	DT_{50} [days]	Visual Acceptability	Chi² Err [%]	t-probability
HW(FUR)	SFO	208.2	Yes	1.5	< 0.001
HW(ETH)	SFO	202.4	Yes	1.6	< 0.001
AW (FUR)	SFO	246.1	Yes	1.3	< 0.001
AW (ETH)	SFO	285.0	Yes	1.0	< 0.001

SFO = Single First Order Model

III. CONCLUSIONS

BYI 02960 is translocated from the water phase into the sediment where it is slowly degraded and mineralized. Major transformation products were mineralization to carbon dioxide, formation of NER and one major metabolite, DFA, (max. 6.9% of AR). An overall result synopsis is shown by Table 7.8.3- 20.

Table 7.8.3- 20: Results Synopsis

	[redacted]		[redacted]	
Parameter	**Label FUR**	**Label ETH**	**Label FUR**	**Label ETH**
Material Balance [% AR]	94.9 – 100.7	96.5 – 101.4	98.4 – 101.8	99.4 – 101.2
Water Phase [% AR]	14.3 – 96.0	14.2 – 97.2	37.3 – 97.9	44.1 – 98.3
Sediment Extract [% AR]	4.2 – 58.9	3.8 – 58.6	2.2 – 37.8	2.4 – 40.1
$^{14}CO_2$ [max. %]	3.9	1.5	5.5	0.9
Major transformation products *	NER, max. 22.6%	NER, max. 26.6%	NER, max. 17.9	NER, max. 15.2% DFA, max. 6.9%
DT_{50} (trigger values)				
Water	9.8	9.4	59.2	66.2
Total system	208.2	202.4	246.1	285.0
DT50 (modelling values)				
Water	48.5*	50.2*	123.8*	117.5*
Total system	208.2	202.4	246.1	285.0

* DT50 value of the slow degradation phase of DFOP model

Report:	**KIIA 7.8.3/03, [redacted], E., [redacted], 2011**
Title:	[1-^{14}C]BYI 02960-DFA (BCS-AB60481): Aerobic Aquatic Degradation
Report No & Document No	MEF-11/996 M-422371-01-1
Guidelines:	**OECD TG 308, Aerobic and Anaerobic Transformation in aquatic systems, 2002** **US EPA Fate, Transport and Transformation Test Guidelines, OPPTS 835.4300 and OPPTS 835.4400, Aerobic and Anaerobic Aquatic Metabolism, 2008**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The aerobic transformation of [1-^{14}C] DFA was studied in two water/sediment systems [redacted] (HW) and [redacted] (AW) for a maximum of 99 days in the dark at 19.2 ± 0.1 °C. The application rate of 4.7 µg DFA per test system was the ten-fold overdose of the application rate calculated based on the use rate of the parent compound BYI 02960 (400 g/ha) and the maximum amount of BYI 02960-DFA formed in the parent aerobic aquatic metabolism study (6.9%). The test systems consisted of laboratory microcosm flasks attached to traps for the collection of CO_2 and volatile organic compounds. Entire vessels filled with either 88.4 g (HW) or 210.3 g (AW) dry weight sediment and 520 mL of supernatant water (volume ratio of water to sediment: 3:1) were treated with [1-^{14}C]BYI 02960-DFA. During incubation the supernatant water was in smooth motion.

Samples were taken after 0, 7, 19, 33, 61, 79 and 99 days of incubation. The water layers were decanted and centrifuged. The sediment samples were extracted stepwise with acetonitrile/water mixtures at ambient temperature (ambient organic extracts). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract). The extracted sediment phase was air-dried or freeze-dried, homogenized and combusted in an oxidizer in order to determine the non-extractable residues (NER). The water phases and the combined extracts of the ambient extraction steps were analyzed by LSC and TLC in order to determine the amounts of the test item and its transformation products. The aggressive extracts were only analyzed by LSC since they contained only low amounts of radioactivity. Identification of the test item in application solution was achieved by HPLC-MS, HPLC-MS/MS and NMR.

The test conditions outlined in the study protocol were maintained throughout the study. The mean material balances in the two test series ranged from 97.7 to 105.8% for test systems from [redacted] and from 97.7 to 107.6% for test systems from [redacted].

The radioactivity in the water phase of HW test systems decreased steadily from 97.6% at day 0 to 34.6% of AR at study termination. The radioactivity in the water phase of AW test systems decreased from 100.0% of AR at day 0 to 80.1% at DAT-7 and varied between 75.0% (DAT-79) and 83.8% (DAT-33) afterwards. Extractable sediment ^{14}C residues in test systems from HW increased from 2.8% of at day 0 to 27.7% of AR at DAT-61 and declined to 24.1% towards the end of the study. Extractable ^{14}C residues in the sediment from AW accounted for 1.5% of at day 0 and varied between 16.2% (DAT-7) and 17.2% of AR (DAT-79) afterwards. The maximum of non-extractable ^{14}C residues (NER) in the sediment were 15.8% and 6.5% of AR for test systems HW and AW at DAT-61, respectively. The maximum of $^{14}CO_2$ in the test systems from HW and AW were 25.1% and 7.5% of AR, respectively. The majority of total $^{14}CO_2$ accounted for volatile $^{14}CO_2$ trapped by the soda lime. A very minor portion included in those values was $^{14}CO_2$ present dissolved in the water phases (all sampling dates) and sediments (only DAT-99). Organic volatile compounds were not detected in significant amounts (< 0.1% of the applied radioactivity in all test systems).

In the water phase of HW, BYI 02960-DFA decreased from 93.8% of AR at day 0 to 32.3% at study termination. In the water of AW, BYI 02960-DFA decreased from 95.4% of AR at day 0 to 78.3% at DAT-7 and varied between 72.3% (DAT-79) and 81.1% (DAT-33) afterwards. In the sediment phase of HW, BYI 02960-DFA increased from 2.8% of AR at day 0 to a maximum of 25.2% at DAT-79 and then declined 22.7% towards the end of the study. In the sediment phase of AW, BYI 02960-DFA accounted for 1.5% of AR at day 0 and varied between 13.3% (DAT-33) and 16.5% (DAT-79) afterwards. Just minor transformation products were observed in the water and sediment phase of both test systems.

The dissipation of DFA from the supernatant water phase was characterized by translocation into the sediment and by degradation. This was best described using the DFOP kinetic model with DT_{50} values of 54.2 and 583.9 days for [redacted] and [redacted], respectively. The corresponding modeling endpoints were either determined using the DFOP kinetic model ([redacted]), resulting in a DT_{50} value of 75.3 days for the slow degradation compartment, or the SFO kinetic model ([redacted]) with a DT_{50} value of 371.5 days. In the entire water/sediment systems, -DFA was degraded slowly which was best described using the DFOP kinetic model. The estimated DT_{50} values were 106.5 and 967.1 days for test systems from [redacted] and [redacted]. The corresponding modeling endpoints were determined using the SFO kinetic model with estimated DT_{50} values of 109.0 and 567.2 days for test systems from [redacted] and [redacted],.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: DFA: CAS No: 2218-52-2 (sodium salt), 381-73-7 (free acid),
[1-^{14}C]BYI 02960-DFA, sample ID: KATH 6450
Specific activity = 2.84 MBq/mg (76.68 µCi/mg)
Radiochemical purity: >98% (acc. radio-HPLC -RA)
Chemical purity: >99.5% (HPLC)

[1-^{14}C-]BYI 02960-DFA was identified via NMR, HPLC-MS and HPLC-MS/MS in stock solution, and via NMR and HPLC-MS with accurate mass detection in application solution

2. Test System: The water/ sediment systems were taken from [redacted] (HW), near [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 chosen systems are well characterized. Physical-chemical characteristics of the water/sediment systems are summarized in Table 7.8.3- 21. Water and sediment samples were taken separately and poured into plastic containers. The collected sediments were sieved down to 2 mm mesh-size to remove parts of e.g. plants and stones. The collected water phases were filtered through a 0.06 mm sieve.

Table 7.8.3- 21: Physico-chemical Characteristics of Water and Sediment

Parameter	[redacted] (HW)	[redacted] (AW)
Geographic Location	close to [redacted] Germany [redacted]	[redacted] Germany [redacted]
Properties of Water		
Temperature [°C]1	13.1	17.0
pH1	6.9	7.5
Oxygen Concentration (saturation) [mg/L][1]	9.9	10.3
Total Organic Carbon (TOC) [mg/L][2]	2 / 20 / 4	2 / 5 / 15
Redox Potential Eh [mV][1,4]	462.2	458.8
Properties of Sediment		
Soil Taxonomic Classification (USDA)	Loam	Loamy Sand
Sand (2000 – 50 µm) [%]	49	85
Silt (< 50 – 2 µm) [%]	39	9
Clay (< µm) [%]	12	6
Organic Carbon [%]	3.9	1.4
Organic Matter [%][3]	6.7	2.4
pH[1]	6.6	7.0
pH	5.2 (CaCl2); 5.4 (H2O)	7.0 (CaCl2); 7.2 (H2O)
Temperature [°C][1]	11.3	15.7
Organic Carbon [%][2]	4.4 / 4.5 / 4.3	1.4 / 1.4 / 1.7
Organic Matter [%][3]	7.6 / 7.8 / 7.4	2.4 / 2.4 / 2.9
Sediment Microbial Activity [mg CO2 /hr/kg sediment (dry wt)][2]	14.17 / 9.58 / 6.67	20.00 / 22.08 / 9.00
Cation Exchange Capacity [meq/100 g]	7.9	7.1
Total Nitrogen [%]	0.3	0.11
Total Phosphorus [ppm]	692	266
Redox Potential Eh [mV][1,4]	244.3	387.2
1 Measurement at day of sampling	2 start of acclimatization / DAT-0 / DAT-99	
3 %organic matter =%organic carbon x 1.724	n.a. = not analyzed	
4 Potential difference between used electrode and H2-electrode at 20°C: 210 [mV] Theoretical potential of used buffer solution for Pt-Ag/AgCl electrode at 25°C: 220 [mV]		

B. Methods

1. Experimental conditions: The tests were performed using individual static test systems held at aerobic conditions at 19.2 ± 0.1 °C for a maximum period of 99 experimental days. Each vessel was filled with 175 mL of wet sediment and about 520 mL of water, equivalent to approx. 6 cm in height, resulting in a volume ratio of water to sediment of 3:1. After pre-equilibration, aliquots of the application solution were directly applied onto the water surface of each microcosm system. Thereafter, the systems were closed with a solid trap attachment for absorbing volatile compounds from DAT 1 onwards.

The amount of radiolabelled DFA for the treatment of the test systems was based an application rate of the parent compound (400 g/ha, calculated to a water depth of 100 cm). The actual application rate set as 100 % of applied radioactivity (100 % AR) corresponding to 266.78 Bq/500 µL (prior to application), 265.82 Bq/500 µL (during application) and 270.17 Bq/500 µL (after application) in the water/sediment systems. The material balance was based on the average amount of radioactivity (RA) recovered from these measurements: 13380 Bq or 4.7 µg BYI02960-DFA per test system.

2. Sampling: Duplicate samples were taken on DAT 0, 7, 19, 33, 61, 79, and 99 days after application for test systems from [redacted] and [redacted]. The water was decanted and centrifuged. The sediment samples were exhaustively extracted with 3 x 80 mL acetonitrile/water (50/50, v/v), followed by one extraction 80 mL acetonitrile/water (70/30, v/v) all at ambient temperature (combination: ambient extract). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v) for 10 minutes (temperature approx. 70°C) with magnetic stirring.

Volatile organics and $^{14}CO_2$ were trapped with solid trapping attachments containing soda lime for absorption of $^{14}CO_2$ and polyurethane foam for volatile organic compounds. The extracted sediment phase was air-dried or freeze-dried, homogenized and combusted in an oxidizer in order to determine the non-extractable residues (NER).

3. Description of analytical procedures: The water phase and combined extract of the ambient extraction steps were analyzed by LSC and TLC in order to determine the amounts of the test item and its transformation products. The aggressive extract was only analyzed by LSC since they contained only low amounts of radioactivity. The very minor transformation products detected in the water layers and sediment extracts were characterized according to their R_f-values in TLC-analysis. No identification procedures were needed or performed. Quantification of the microbial activity in the sediments was based on the method of substrate-induced initial respiratory response (SIR).

C. Determination of Degradation Kinetics

Dissipation rates from the water phase and rates of degradation for the total system were calculated by use of the software KinGui, version 1.1. The kinetic evaluation included the fitting of data with kinetic models SFO, FOMC and DFOP to the experimental data and their assessment according to FOCUS guidance to result in values for comparison with trigger endpoints.

II. RESULTS

The test conditions outlined in the study protocol were maintained throughout the study.

Table 7.8.3- 22: Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (HW)

DAT	Sample	Water Layer				Sediment			Buffer solution
		O_2	Redox E_{obs}	Redox E_H	pH	Redox E_{obs}	Redox E_H	pH	Redox E_{obs}
	WU53 H-	[mg/L]	[mV]	[mV]		[mV]	[mV]		[mV]
0*	D0-A	9.0	173	372	7.3	-13	186	6.3	231
	D0-B	9.0	180	379	7.3	49	248	6.9	
7	D7-A	8.9	211.8	414	8.5	31	233	6.7	228**
	D7-B	8.8	212.2	414	8.0	68	270	6.5	
19	D19-A	8.5	214	416	6.8	47	249	6.7	228
	D19-B	8.5	211	413	6.8	43	245	6.7	
33	D33-A	8.5	276	482	5.5	-96	111	6.0	224
	D33-B	8.6	239	445	5.5	-89	117	6.1	
61	D61-A	8.4	209	407	7.2	219	417	6.2	232
	D61-B	8.4	205	403	7.2	227	425	6.2	
79	D79-A	8.5	245	453	4.9	119	327	5.2	222
	D79-B	8.6	239	447	4.9	121	329	5.2	
99	D99-A	8.4	211	412	7.2	196	397	6.5	229
	D99-B	8.4	209	410	7.3	212	413	6.5	
	min	8.4	173	372	4.9	-96	111	5.2	222
	max	9.0	276	482	8.5	227	425	6.9	232
	mean	8.6	217	419	6.7	81	283	6.3	228

Table 7.8.3- 23: Oxygen Saturation, pH and Redox Potential Measurements of the Aerobic Test Systems throughout the Study Period (AW)

DAT	Sample ID	Water Layer				Sediment			Buffer solution
		O_2	Redox E_{obs}	Redox E_H	pH	Redox E_{obs}	Redox E_H	pH	Redox E_{obs}
	WU53 A-	[mg/L]	[mV]	[mV]		[mV]	[mV]		[mV]
0*	D0-A	8.9	248	447	8.2	82	281	7.7	231
	D0-B	8.8	244	443	8.2	89	288	7.7	
7	D7-A	8.7	230.6	433	8.5	153.2	355.2	7.7	228**
	D7-B	9.2	218.6	421	8.5	19.1	221.1	7.6	
19	D19-A	8.3	196	398	8.7	203	405	7.6	228
	D19-B	8.3	197	399	8.7	202	404	7.6	
33	D33-A	8.9	182	388	8.3	-43	163	7.3	224
	D33-B	8.8	191	397	8.4	-42	164	7.4	
61	D61-A	8.7	207	405	8.2	153	351	7.7	232
	D61-B	8.7	202	400	8.3	155	353	7.7	
79	D79-A	8.5	154	362	8.1	226	434	7.9	222
	D79-B	8.5	160	368	8.1	230	438	7.9	
99	D99-A	8.6	202	403	8.4	151	352	7.7	229
	D99-B	8.7	199	400	8.5	159	360	7.7	
	min	8.3	153.6	362	8.1	-43	163	7.3	222
	max	9.2	248.0	447	8.7	230	438	7.9	232
	mean	8.7	202.2	404	8.4	124	326	7.7	228

A. Data

A summary of key data on total recovery and the distribution of radioactivity into the various components formed in water and sediment is given for system [redacted] in Table 7.8.3- 24 and in Table 7.8.3- 25 for system [redacted].

B. Mass Balance

During the study, the total recovery of radioactivity in individual test vessels ranged from 97.7 to 105.8% of AR (mean 100.7%, RSD 2.7 %) for HW and from 97.7 to 107.6% of AR (mean 103.0%, RSD 2.9%) for AW. The complete material balance found for all sampling dates demonstrated that no significant portion of radioactivity dissipated from the vessels or was lost during processing (for detailed results see resp. tables).

C. Volatilization

Formation of $^{14}CO_2$ was observed in all water/sediment systems. At termination of the study, the $^{14}CO_2$ recovery (mean values of duplicates) in systems from HW was 25.1% of the applied radioactivity. In AW test system, $^{14}CO_2$ accounted for max. 7.5% of the applied radioactivity at DAT-79. From these data it can be concluded that DFA is mineralized in water/sediment systems. No significant amounts of organic volatiles were found (< 0.1% of AR).

D. Residues in Water, Bound and Extractable Residues in Sediment

The radioactivity in the water phase of HW test system decreased steadily from 97.6% of AR at day 0 to 34.6% at study termination. The extractable radioactivity increased from 2.8% of AR at DAT 0 to 27.7% at DAT 61 and declined to 24.1% towards the end of the study. The bound residues were 5.4% of the applied radioactivity at day 0 and slightly increased during the course of the study. The maximum amount of bound residues (15.8% of AR) was detected on DAT 61.

The radioactivity in the water phase of AW test systems decreased to 80.1% of AR at day 7 and varied between 75.0% (DAT 79) and 83.8% (DAT 33) afterwards. Extractable ^{14}C residues in the sediments accounted for 1.5% of AR at day 0 and varied between 16.2% (DAT-7) and 17.2% (DAT-79) afterwards. The amount of bound residues was 4.6% of AR at day 0. During the entire study period the amounts of bound residues varied between 1.0% (DAT 19) and 6.5% (DAT 61). Due to their low amounts (< 20%), the non-extractable residues were not further characterized.

Along with the overall metabolism of BYI 02960-DFA bound residues were formed. Their maximum amounts were comparatively low and accounted for 15.8 and 6.5% of AR for water/sediment systems from [redacted] and [redacted], respectively

E. Transformation of Test Item

The BYI 02960-DFA residues in the entire test systems from HW declined from 96.6% of AR at DAT 0 to 55.0% of AR at study termination. In the entire test systems from AW, the decrease of the test item was less pronounced. There, unchanged test item accounted for 89.7% of AR at the end of the incubation period. The maximum amount of a single minor radioactivity zone in the water phase of both test systems was 2.7% of AR; in the sediment it was max. 3.3% of AR.

Table 7.8.3- 24: Biotransformation of DFA in [redacted] under aerobic conditions, expressed as percent of AR

Compound	Sample	DAT 0	7	19	33	61	79	99
DFA	Water Layer	93.8	72.1	64.3	58.3	43.9	35.7	32.3
		± 0.1	± 1.5	± 1.6	± 0.6	± 1.0	± 1.5	± 0.1
	Sediment	2.8	21.4	20.8	21.8	24.8	25.2	22.7
		± 0.2	± 0.6	± 1.0	± 1.4	± 0.1	± 0.1	± 0.6
	Entire System	96.6	93.5	85.0	80.0	68.6	60.9	55.0
		± 0.1	± 0.1	± 0.6	± 0.8	± 1.2	± 1.4	± 0.5
Origin	Water Layer	0.4	0.2	n.d.	< LOD	< LOD	n.d.	n.d.
		± 0.0	± 0.0					
	Sediment	n.d.	n.d.	< LOD	0.3	0.4	n.d.	0.4
					± 0.0	± 0.1		± 0.1
	Entire System	0.4	0.2	< LOD	0.5	0.5	n.d.	0.4
		± 0.0	± 0.0		± 0.0	± 0.2		± 0.1
ROI 3	Water Layer	0.5	0.3	0.4	0.2	0.7	1.1	1.5
		± 0.1	± 0.1	± 0.0	± 0.0	± 0.2	± 0.1	± 0.1
	Sediment	n.d.	n.d.	0.3	0.9	0.8	n.d.	n.d.
				± 0.0	± 0.0	± 0.2		
	Entire System	0.5	0.3	0.8	1.1	1.6	1.1	1.5
		± 0.1	± 0.0	± 0.0	± 0.2	± 0.0	± 0.1	± 0.1
ROI 4	Water Layer	n.d.	n.d.	0.4	n.d.	< LOD	n.d.	n.d.
				± 0.0				
	Sediment	n.d.	n.d.	0.2	0.6	0.4	n.d.	n.d.
				± 0.0	± 0.3	± 0.2		
	Entire System	n.d.	n.d.	0.7	0.6	0.6	n.d.	n.d.
				± 0.1	± 0.3	± 0.2		
Non-characterized Radioactivity	Water Layer	2.9	1.8	1.7	1.8	1.3	0.9	0.7
		± 0.2	± 0.2	± 0.0	± 0.3	± 0.2	± 0.2	± 0.2
	Sediment	n.d.	1.7	2.1	3.3	1.3	1.8	1.0
			± 0.1	± 0.0	± 0.1	± 0.1	± 0.2	± 0.0
	Entire System	2.9	3.6	3.7	5.1	2.6	2.7	1.8
		± 0.2	± 0.1	± 0.0	± 0.2	± 0.1	± 0.0	± 0.1
Total Extractable Residues	Water Layer	97.6	74.4	66.8	60.5	46.1	37.7	34.6
		± 0.1	± 1.1	± 1.7	± 0.7	± 1.3	± 1.2	± 0.2
	Sediment	2.8	23.1	23.5	26.9	27.7	27.1	24.1
		± 0.2	± 0.5	± 1.0	± 1.8	± 0.1	± 0.1	± 0.5
	Entire System	100.4	97.6	90.3	87.4	73.9	64.7	58.7
		± 0.3	± 0.6	± 0.6	± 1.1	± 1.3	± 1.4	± 0.7
$^{14}CO_2$	Entire System	n.a.	1.2	3.7	6.3	14.5	21.1	25.1
			± 0.0	± 0.3	± 0.2	± 0.2	± 1.0	± 0.1
Organic Volatiles	Entire System	n.a.	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
			± 0.0	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
Non-Extractable Residues	Sediment	5.4	1.8	3.7	6.8	15.8	13.2	14.0
		± 0.1	± 0.1	± 0.2	± 0.8	± 11.7	± 0.5	± 0.1
Total Recovery *	Water Layer	97.6	74.4	66.8	60.5	46.1	37.7	34.6
		± 0.1	± 1.1	± 1.7	± 0.7	± 1.3	± 1.2	± 0.2
	Sediment	8.2	24.9	27.3	33.7	55.3	40.3	38.2
		± 0.3	± 0.5	± 1.2	± 2.6	± 11.6	± 0.3	± 0.4
	Entire System	105.8	100.5	97.7	100.5	103.1	99.0	97.9
		± 0.5	± 0.5	± 0.2	± 2.1	± 12.8	± 0.1	± 0.5

DAT: day after treatment, SD: standard deviation, < LOD = Peak contained less than 0.22 Bq
* Values taken from Material Balance, ± = SD = standard deviation

Table 7.8.3- 25: Biotransformation of DFA in [redacted] under aerobic conditions, expressed as percent of AR

		DAT						
Compound	**Source**	**0**	**7**	**19**	**33**	**61**	**79**	**99**
DFA	Water Layer	95.4 ± 0.3	78.3 ± 1.2	79.0 ± 0.7	81.1 ± 2.1	75.9 ± 1.2	72.3 ± 0.6	74.8 ± 1.7
	Sediment	1.5 ± 0.0	15.1 ± 0.3	14.3 ± 1.7	13.3 ± 1.1	15.3 ± 0.4	16.5 ± 0.2	14.9 ± 1.9
	Entire System	97.0 ± 0.3	93.3 ± 1.5	93.3 ± 1.1	94.5 ± 1.1	91.2 ± 0.8	88.8 ± 0.8	89.7 ± 0.2
Origin	Water Layer	0.5 ± 0.0	n.d.	n.d.	0.3 ± 0.1	< LOD	n.d.	1.2 ± 0.0
	Sediment	n.d.	< LOD	n.d.	< LOD	< LOD	n.d.	0.2 ± 0.0
	Entire System	0.5 ± 0.0	< LOD	n.d.	0.5 ± 0.1	< LOD	n.d.	1.4 ± 1.2
ROI 3	Water Layer	1.3 ± 0.4	0.2 ± 0.0	1.0 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	1.2 ± 0.3	1.4 ± 0.1
	Sediment	n.d.	< LOD	0.5 ± 0.2	0.6 ± 0.1	0.7 ± 0.2	< LOD	< LOD
	Entire System	1.3 ± 0.4	0.4 ± 0.2	1.4 ± 0.2	1.3 ± 0.0	1.7 ± 0.1	1.4 ± 0.3	1.5 ± 0.1
ROI 4	Water Layer	n.d.	n.d.	0.4 ± 0.0	n.d.	0.7 ± 0.0	n.d.	n.d.
	Sediment	n.d.	< LOD	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	n.d.	< LOD
	Entire System	n.d.	< LOD	0.7 ± 0.3	0.2 ± 0.0	0.9 ± 0.1	n.d.	< LOD
Non-characterized Radioactivity	Water Layer	2.7 ± 0.4	1.6 ± 0.3	2.1 ± 0.2	1.6 ± 0.3	1.2 ± 0.1	1.5 ± 0.7	1.3 ± 0.1
	Sediment	n.d.	0.8 ± 0.0	1.8 ± 0.5	1.9 ± 0.2	0.8 ± 0.3	0.6 ± 0.1	0.5 ± 0.0
	Entire System	2.7 ± 0.4	2.4 ± 0.3	3.9 ± 0.4	3.5 ± 0.1	2.0 ± 0.2	2.1 ± 0.8	1.8 ± 0.1
$^{14}CO_2$	Entire System	n.a.	0.3 ± 0.0	1.4 ± 0.1	1.3 ± 0.1	4.1 ± 0.0	7.5 ± 0.4	5.6 ± 1.0
Organic Volatiles	Entire System	n.a.	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0	< 0.1 ± 0.0
Non-Extractable Residues	Sediment	4.6 ± 0.0	1.1 ± 0.1	1.0 ± 0.1	1.7 ± 0.0	6.5 ± 24.5	2.7 ± 0.2	2.1 ± 0.2
Total Recovery	Water Layer	100.0 ± 0.3	80.1 ± 1.3	82.4 ± 0.2	83.8 ± 2.6	78.8 ± 1.4	75.0 ± 0.4	78.7 ± 2.8
	Sediment	6.1 ± 0.0	17.3 ± 0.3	17.8 ± 1.0	17.9 ± 1.5	48.1 ± 24.7	19.9 ± 0.1	18.0 ± 1.9
	Entire System	106.1 ± 0.3	97.7 ± 1.5	101.6 ± 0.7	103.1 ± 1.0	107.6 ± 23.4	102.4 ± 0.0	102.3 ± 0.2

DAT: day after treatment, SD: standard deviation, < LOD = Peak contained less than 0.22 Bq

Remark: Due to the low amounts of radioactivity present in the aggressive organic extracts, only the ambient organic extracts were subjected to TLC-analysis. The amounts of DFA and its metabolites in the sediment were calculated for the sum of ambient and aggressive organic extracts, assuming that the distribution of radioactivity in the aggressive extracts is equal to the distribution in the ambient extracts.

F. Dissipation Kinetics

The dissipation of BYI 02960-DFA from the supernatant water phase was characterized by translocation into the sediment and by degradation. This was best described using the DFOP kinetic model with DT_{50} values of 54.2 and 583.9 days for [redacted] and [redacted], respectively (see Table 7.8.3- 26). The corresponding modeling endpoints were either determined using the DFOP kinetic model ([redacted]), resulting in a DT_{50} value of 75.3 days for the slow degradation compartment, or the SFO kinetic model ([redacted]) with a DT_{50} value of 371.5 days (see Table 7.8.3- 27).

In the entire water/sediment systems, BYI 02960-DFA was degraded slowly which was best described using the DFOP kinetic model. The estimated DT_{50} values were 106.5 and 967.1 days for test systems from [redacted] and [redacted], respectively (see Table 7.8.3- 26). The corresponding modeling endpoints were determined using the SFO kinetic model with estimated DT_{50} values of 109.0 and 567.2 days for test systems from [redacted] and [redacted], respectively (see Table 7.8.3- 27).

Table 7.8.3- 26: Kinetics evaluation (trigger values according to FOCUS) of the dissipation of DFA

Supernatant Water of Test System	Kinetic Model	DT_{50} [days]	DT_{90} [days]	Chi^2 Error [%]
[redacted]	SFO	63.2	210.1	6.6
	FOMC	50.7	> 1000	4.7
	DFOP	**54.2**	**229.9**	**1.6**
[redacted]	SFO	371.5	> 1000	4.8
	FOMC	> 1000	> 1000	2.2
	DFOP	**583.9**	**> 1000**	**2**
Entire Test System				
[redacted]	SFO	109.0	362.2	2.9
	FOMC	125.0	> 1000	2.1
	DFOP	**106.5**	**397.8**	**0.6**
[redacted]	SFO	567.2	> 1000	3.0
	FOMC	> 1000	> 1000	1.2
	DFOP	**967.1**	**> 1000**	**0.9**

Bold: best fit

III. CONCLUSION

The dissipation of DFA from the supernatant water phase was characterized by translocation into the sediment and by slowly degradation. With the exception of mineralization to carbon dioxide and formation of NER no major metabolite was found. An overall result synopsis is shown by Table 7.8.3-27.

Table 7.8.3- 27: Results Synopsis

Parameter	[redacted]	[redacted]
Material Balance [% AR]	97.7 – 105.8	97.7 – 107.6
Water Phase [% AR]	34.6 – 97.6	75.0 -100.0
Sediment Extract [% AR]	2.8 – 27.7	1.5 – 17.2
$^{14}CO_2$ [max. %]	25.1	7.5
Major transformation products * [max. %]	NER: 15.8	NER: 6.5
Kinetics Evaluation, Trigger Values		
Supernatant water	54.2	583.9
Entire system	106.5	967.1
Kinetics Evaluation, Modeling Endpoints		
Supernatant water	75.3#	371.5
Entire system	109.0	567.2

* Criteria for "major": ->10% of AR; >5% of AR at two successive DAT, increasing towards study end
# DT50 value of the slow degradation phase as a worst case scenario for risk assessment

Supportive Study: Degradation in Outdoor Microcosm Pond

Report:	**KIIA 7.8.3/04, [redacted], 2012**
Title:	Fate of BYI 02960 (tech.) in Outdoor Microcosm Ponds Simulating Actual Exposure Conditions in Agricultural Use
Report No & Document No	EBRVP109 M-427167-01-1
Guidelines:	**OECD Guidance Document "Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms)", April 2006 Guidance Document on Testing Procedures for Pesticides in Freshwater Microcosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991) Community-Level Aquatic System Studies – Interpretation Criteria (Guidance Document from the CLASSIC Workshop, SETAC 2002) OECD Guideline 308, but only in part (where applicable)**
GLP:	**Yes (fully GLP compliant and certified laboratory)**

EXECUTIVE SUMMARY

The fate of BYI 02960 (tech.) was determined in pond water and sediment in outdoor microcosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels.
For this purpose, 4 microcosms were treated with two different concentrations of the test item. During the consecutive months samples of water and sediment were taken and the content of the test item in these compartments was analyzed. Additionally several parameters of water and sediment were monitored to characterise the system.
In November 2009 a pond with the shape of a stub pyramid (bottom 53 m^2, surface 80 m^2) was filled with a layer of natural sediment (0.15 m height) and water (0.7 m height). The walls and bottom of the pond is made of plastic foil. The sediment originated from a drinking water reservoir system, the water was composed of local ground water and water from a natural pond. Twenty-five enclosures (= microcosms) made of polycarbonate (diameter 0.97 m. height 0.9 m) were inserted into the pond on May 4, 2010 (= about 4 weeks before application). Overall, the microcosms are representative for small stagnant water bodies.

The test substance BYI 02960 (tech.) (Batch ID: 2009-000239) was applied once on June 02, 2010 onto the water surface of four microcosms. Two treatment levels, 10 and 100 µg a.i./L, were tested with two replicates each:. Two microcosms were kept untreated as controls. The microcosms were

investigated for a period of 148 days after the treatment. Several times during the study period water and sediment samples were taken and analyzed to demonstrate the initial test concentrations and the fate of the test substance in these compartments. The physic-chemical water parameters were also evaluated. Furthermore the abundance of filamentous algae und the turbidity of the water was assessed.

The results of initial concentrations demonstrated that the nominal concentrations had been applied. A steady decline of BYI 02960 in the water phase was shown. At the end of the study (≈ 5 months after application) the a.i. concentration was 22% of applied amount at the test concentration of 10 µg a.s./L, and 40% of applied amount at 100 µg a.s./L concentration level. The analyzed concentrations of BYI 02960 in the sediment did not show a clear trend during the entire study period, although a slight increase of BYI 02960 concentrations was observed towards the end of the study. For the 10 µg a.s./L-level, 2.3 to 7.5% of applied amount was found in the sediment; for the 100 µg a.s./L-level, 1.9 to 11.4 % of the applied amount were detected in the sediment.

The half-life calculations for BYI 02960 in the microcosm water using SFO kinetics model resulted on average for both concentration level in 80.6 days, and in the entire system (water + sediment) in 95.1 days. A calculation for the fate in sediment was not given, since the fate of BYI 02960 in this matrix did not show a clear pattern of decline.

I. MATERIALS AND METHODS

A. Materials

1. Test Item: BYI 02960 (tech.)
Origin Batch No. 2009-000239
Batch code BYI 02960-01-03
Specification No. 102000022313)
Analyzed a.i. content: 96.2% w/w
Application solution: 6.34 g of test item was dissolved in 50 mL DMF, which was suspended in pure water to get nominal 10 and 100 µg a.s./L concentration level after treatment

2. Test System: In Nov. 2009 a pond with the shape of a stub pyramid (bottom 56 m^2, surface 80 m^2) was filled with a layer of natural sediment (0.15 m height) and water (0.7 m height). The walls and bottom of the pond consist of a plastic foil. The sediment originated from "[redacted]", part of the water reservoir system "[redacted] ([redacted])", 50 km distant from the testing facility. The sediment was taken in November 2009 by means of an excavator, transported in containers to [redacted] and uniformly distributed among the pond bottom thereafter. The water was composed of local ground water and water from a natural pond.

Twenty-five cylindrical enclosures (= microcosms) made of polycarbonate (diameter 0.97 m. height 0.9 m, previous to light) were inserted into the pond on May 4, 2010 (= about 4 weeks before application). Six of these microcosms) were used for the present fate-o-cosm. To ensure conditions as close to nature as possible, the tanks are not roofed and are exposed to outdoor weather conditions. Overall, the microcosms are representative for small stagnant water bodies.

B. Methods

1. Experimental conditions: On day 0 (= 2010-06-02) an aqueous suspension of the test substance was applied using a pipette and a small glass plate. The glass plate was held onto the water surface. The application suspension was transferred onto the glass plate using a glass pipette. The application suspension did run via the glass plate into the microcosms simulating a run off scenario. The mean water height in the microcosms on day 0 was 0.66 m corresponding to 488 L water / microcosm.
Two treatment levels were tested, with two replications each: 10 and 100 µg a.i./L. Two microcosms were kept untreated as controls. The microcosms were investigated for a period of 148 days after the treatment. Several times during the study period water and sediment samples were taken and analyzed to demonstrate the initial test concentrations and the fate of the test substance in these compartments. The physic-chemical water parameters were also evaluated and reported. Furthermore the abundance of filamentous algae und the turbidity of the water was assessed.

At least once per week the oxygen content, temperature, pH and conductivity were measured in the centre of the water column. The measurements were made directly in the pond water with electronic measuring instruments. Several times during the study ammonium, nitrite, nitrate, total nitrogen, total phosphate, ortho-phosphate, carbonate hardness, sum of alkaline earth's (=total hardness) were determined. Carbonate hardness and sum of alkaline earth were measured by means of commercial tests. Ammonium, nitrite, nitrate, total nitrogen total phosphate and ortho-phosphate were determined by means of commercial photometric tests. Redox potential in water was determined monthly during the study. Total organic carbon (TOC) in water and dissolved organic carbon (DOC) in water were determined at study start and on days 84 and 148.

Parameters at day 0 were determined in non-treated sediment outside of the microcosms. At day 84 and 148 (=study end), the parameters were determined in the sediment of the control microcosms (mixed sample of both microcosms). The Redox potential was determined directly in the sediment of both control microcosms. At the start of the study, on day 84 and at the end of the study the following parameters of the sediment were determined: Total organic carbon and microbial biomass. The redox potential was measured monthly parallel to the water. This measurement was performed according to common guidelines.
The characteristics of test system are summarized in Table 7.8.3- 28.

Table 7.8.3- 29: Characteristics of test system

Redox potential TOC and DOC measured in the test water

Date 2010	Study day	Redox potential Ct/1	Redox potential Ct/2	TOC (mg/L)	DOC(mg/L)
02.06.	0	233	175	9	9
30.06.	28	121	125		
27.07.	55	178	176		
25.08.	84	202	197	13	12
30.09	120	200	201		
28.10	148	209	212	11	10

Water levels in the microcosms (cm) around the time of sampling dates

Date 2010	Study Day	Control (1)	Control (2)	10 µg a.i./L (1)	10 µg a.i./L (2)	100 µg a.i./L (1)	100 µg a.i./L (2)
03.06	1	65	64	64	66	68.5	66
09.06	7	63	61.3	58.5	63.8	64	63
16.06.	14	61	58.5	53	61.5	59.5	60
01.07.	29*)	56.5	53	48	55	54	57
28.07.	56	61	58.5	53	61.5	58	60
25.08.	84	65	63	59	65.5	65.5	67.5
29.09.	119	69	68	64.5	68.5	69	68.5
28.10.	148	69	67	65	69	68	69

*) water level before refill with water

Characterization of the sediment

Particle size distribution: 56 % sand, 36 % Silt, 8% Clay = sandy loam[USDA]

Date 2010	Study day	Microbial biomass (mg CO2/hr/kg soil DW)	Redox potential Ct/1	Redox potential Ct/2	TOC (gew.-%)
02.06.	0	30.0	- 103	- 96	5.3
30.06.	28		- 148	- 142	
27.07.	55		- 124	- 131	
25.08.	84	29.6	- 100	- 109	5.6
30.09	120		- 118.3	- 85.7	
28.10	148	37.8	-31.3	-24.6	4.8

Climatic data (recorded at the nearby weather station of the Bayer CropScience AG)

Month (2010)	Temperature (mean °C)	Precipitation (mm)	Sunshine duration (hrs)	Insolation (kWh/m2)
June	19.3	19.1	197	181
July	22.9	111	211	175
August	19.4	233	136	114
September	14.6	93.1	131	152
October	10.8	43.1	118	101

2. Sampling: Samples were taken on 0, 1, 7, 14, 28, 56, 84, 120 and 148 days after application.
Mixed water samples out of the entire water column were taken for analysis to investigate the fate of the substance in water during the study period. For this purpose the water samples were obtained with a flask attached to a metal rod. The flask (1.0 L glass bottle) was moved around in the microcosm during filling to obtain water from different sites. 6 x 20 mL of the water samples were filled into 50 mL amber glass bottles and deep frozen at < - 18 °C until analysis.
Sediment samples were taken by means of a corer (inner diameter of 5.0 cm) at two to three sampling points each sampling time. The sample points were noted to avoid multiple sampling at one sampling point. Water covering the sediment was decanted carefully and the sampled sediment-column was filled into a glass-beaker with the same dimensions as the core. As it can be assumed that the test substance primarily adsorbed to the upper layer of the sediment, the upper 2 to 3 cm of the sediment

samples were mixed for analyses (= ca. 170 g wet weight). The resulting sediment samples were frozen and stored at < -18°C until analysis. Sediment samples of the control microcosms were also taken at each time.

3. Description of analytical procedures: The water samples were analysed according to Method 01182: Method 01182 for the determination of BYI 02960 in test water from aquatic toxicity tests by HPLC-MS/MS. In the method 01182 the linearity of the detector was checked for BYI 02960 in the range 0.05 to 11 µg/L with an injection volume of 10 µL. The correlation coefficient was 0.9993.

In the present study the method was validated concurrently with the sample analyses of the study by evaluation of the standard injections. To the samples from day 84 until 148 a volume of 1 mL acetonitrile was in addition. Before measurement the samples were added with 0.1 mL formic acid in. The water samples were directly injected into the HPLC-MS/MS instrument. The injection volume was 10 µL. Each sample was injected in duplicate. The limit of quantification (LOQ) of BYI 02960 in the pond water was 1.141 µg a.i./L.

Sediment samples of 20 g were extracted with 40 mL of acetonitrile/water (1/4; v/v) for 3 minutes in a microwave at 250 W. An aliquot of 1.5 mL was taken and centrifuged for 5 minutes at > 12000 g to remove fine particles of the sediment. The supernatant was taken and injected into the HPLC-MS/MS system. Two replicates (A + B) of 20 g each were analysed from each sample. The mean value of both replicates is reported. The sediment samples were analysed according to the following method: Analytical Method 01074 for the Determination of BYI 02960 in Soil using LC/MS/MS. Additional recovery experiments for sediment at a LOQ level of 1 µg/kg were conducted. The limit of quantification (LOQ) was 5.0 µg BYI 02960/kg dry weight of sediment.

C. Determination of Degradation Kinetics

The dissipation kinetics of BYI 02960 in the microcosm ponds was evaluated based on a simple first order, single compartment model. An exponential decline curve was fitted to the experimental data, and the rate constant k, as well as the characteristic dissipation times DT50 (dissipation time of 50% of the test item) was derived from this calculation. The decline curves and regression analyses for BYI 02960 were calculated with the Single First Order mathematical model (SFO). The used program was KinGUI Version 2. Model input data sets were the residual concentrations of BYI 02960 in water found in each pond at each sampling interval (displayed as % of nominal content). The total residues in each pond were calculated based on the measured residues in water plus sediment (displayed as mg a.i./microcosm). All data points were weighted equally.

II. RESULTS

A. Data

The analyzed concentrations of the application solutions indicated that 118 and 112% of nominal test concentration were applied into the pond water in case of the two treatment levels of 10 and 100 µg a.i./L.

B. Water Layer

The analyzed concentrations of the mixed samples are shown in Table 7.8.3- 30 and in Table 7.8.3- 31 are represented as percentage of nominal application. For the calculation of percentage values the actual water volume in the microcosms at the time of sampling was taken into consideration.

Table 7.8.3- 30: Results of analysis in water [as µg BYI 02960/L], average of two measurements

Date 2010	Study Day	Control (1)	Control (2)	10 µg a.i./L (1)	10 µg a.i./L (2)	100 µg a.i./L (1)	100 µg a.i./L (2)
03.06	1	<LOQ	<LOQ	10.3	10.0	105	100
09.06	7	nd	nd	9.15	8.28	106	104
16.06.	14	nd	nd	9.61	8.38	98.9	87.9
30.06.	28	nd	nd	7.73	7.18	89.5	88.9
28.07.	56	nd	nd	5.85	5.09	69.7	68.2
25.08.	84	nd	nd	4.30	3.77	58.0	53.9
30.09.	119	nd	nd	2.63	2.85	44.1	42.3
28.10.	148	nd	nd	2.13	2.15	40.0	37.2

nd = not determined; (1)= replicate 1, (2) = replicate 2

Table 7.8.3- 31: Results of analysis in water [as % of nominal BYI 02960], average of two measurements

Date 2010	Study Day	Control (1)	Control (2)	10 µg a.i./L (1)	10 µg a.i./L (2)	100 µg a.i./L (1)	100 µg a.i./L (2)
03.06	1	<LOQ	<LOQ	99.8	99.9	108.9	99.9
09.06	7	nd	nd	81.1	79.9	102.7	99.2
16.06.	14	nd	nd	77.1	78.0	89.1	79.9
30.06.	28	nd	nd	56.2	59.8	73.2	76.7
28.07.	56	nd	nd	47.0	47.4	61.2	62.0
25.08.	84	nd	nd	38.4	37.4	57.5	55.1
30.09.	119	nd	nd	25.7	26.5	46.0	43.9
28.10.	148	nd	nd	21.0	22.5	41.2	38.9

nd = not determined; (1)= replicate 1, (2) = replicate 2

C. Sediment

The results of the analysis of BYI 02960 in sediment are summarized in Table 7.8.3- 32. The samples for analysis were taken from the uppermost 2-3 cm layer. The total dry weight of this sediment layer was around 7.13 kg per microcosm. The analyzed concentrations were multiplied by this value calculated as % of the initial applied.

Table 7.8.3- 32: Results of analysis of BYI 02960 in sediment average of two measurements

Date 2010	Study Day	Control (1)	Control (2)	10 µg a.i./L (1) µg BYI 02960 /kg dry weight		10 µg a.i./L (2) % of applied BYI 02960		100 µg a.i./L (1) µg BYI 02960 /kg dry weight		100 µg a.i./L (2) % of applied BYI 02960	
03.06	1	nd	nd	nd		nd		nd		nd	
09.06	7	<LOQ	<LOQ	36.3	5.3	30.3	4.4	128	1.9	127	1.9
16.06.	14	<LOQ	<LOQ	38.8	5.7	30.1	4.4	140	2.0	247	3.6
30.06.	28	<LOQ	<LOQ	34.6	5.1	15.9	2.3	252	3.7	415	6.1
28.07.	56	<LOQ	<LOQ	26.4	3.9	46.3	6.8	258	3.8	152	2.2
25.08.	84	<LOQ	<LOQ	29.6	4.3	23.8	3.5	152	2.2	260	3.8
30.09.	119	<LOQ	<LOQ	47.0	6.9	45.0	6.6	142	2.1	185	2.7
28.10.	148	<LOQ	<LOQ	47.3	6.9	51.4	7.5	781	11.4	701	10.2

nd = not determined; (1)= replicate 1, (2) = replicate 2

F. Dissipation Kinetics

The dissipation of BYI 02960 from the supernatant water phase was characterized by translocation into the sediment and by degradation. The half-life calculations for BYI 02960 in the microcosm water

and the total system (water + sediment) are displayed in Table 7.8.3- 33. A calculation for the decline in sediment was not possible, since the fate of BYI 02960 in this matrix did not show a continuous pattern.

Table 7.8.3- 33: SFO DT_{50}- values calculated for BYI 02960 in water and the entire test system

Nominal test concentration (µg a.i./L)	DT_{50} - Water (days)	DT_{50} – Total System (water+sediment) (days)
10	62.1	74.1
100	99.1	116
mean	80.6	95.1

III. CONCLUSIONS

BYI 02960 dissipated from the supernatant water phase with a mean DT_{50} of 81 days due to translocation into the sediment and by degradation. The overall degradation (mean of 95 days) was faster under the prevailing outdoor conditions in comparison to the laboratory water sediment studies. Considering that BYI 02960 is rapidly degraded by photolysis there may be an enhanced degradation effect of sunlight under the outdoor test conditions.

Degradation of BYI 02960 in Aquatic Systems - Summary

The hydrolysis study of BYI 02960 in sterile buffer solutions of pH 4, 7 and 9 showed that the active substance is hydrolytically stable under environmental conditions.

Photolytically BYI 02960 degraded very rapidly in sterile buffer and natural water studies. Based on these findings and dependent on time of the year and location, BYI 02960 should degrade with a DT_{50} of few days to one week in an aqueous environment, if exposed to sunlight. The major degradates were identified as BYI 02960-succinamide (found at max. 39.6% of applied) and BYI 02960-azabicyclosuccinamide (found at max. 25.9% of applied). The findings were included in the proposal for the pathway of degradation of BYI 02960 in the aqueous environment (see Figure 7.8- 1).

BYI 02960 is regarded stable under anaerobic aquatic condition, and no major metabolites were formed.

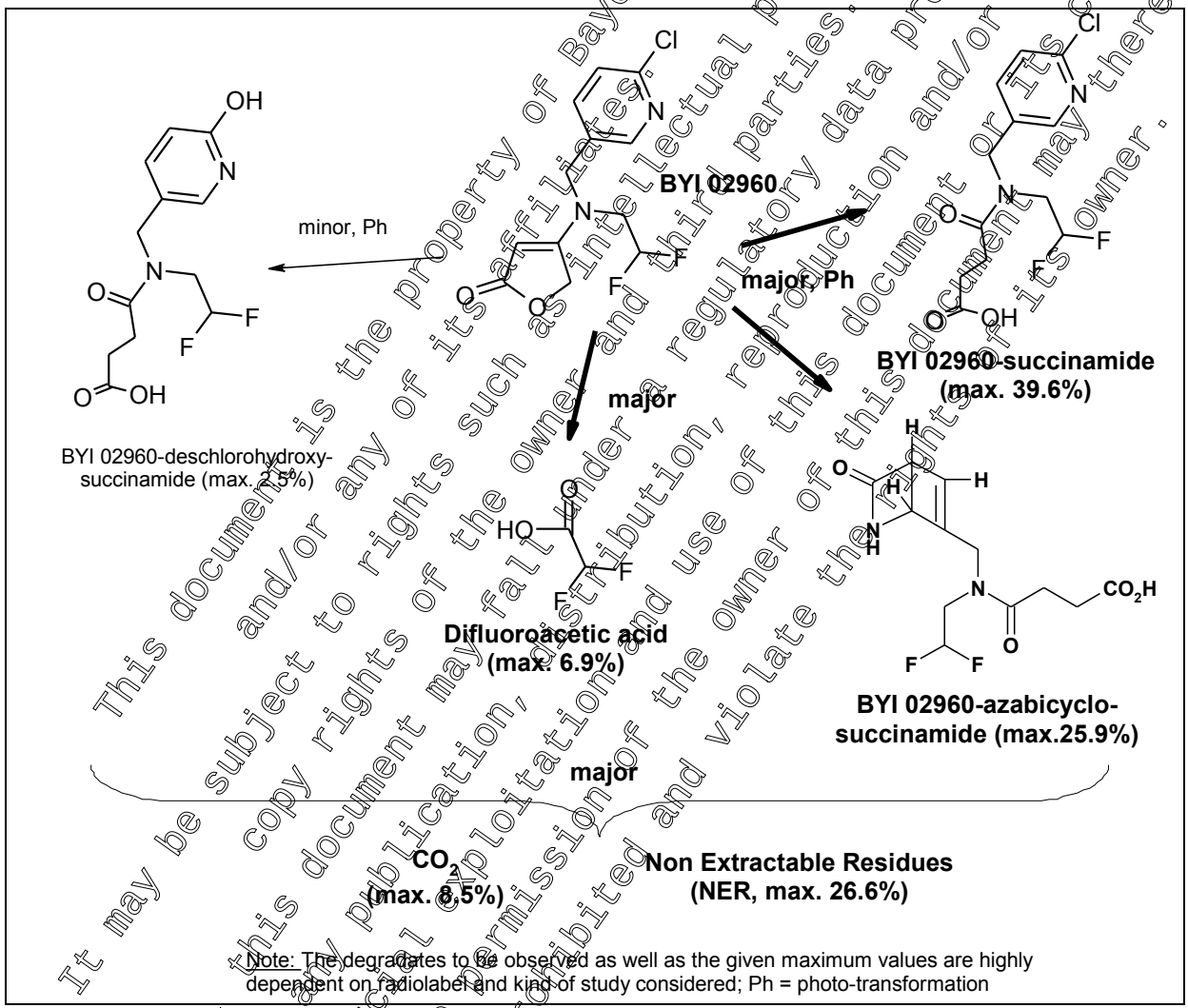
The aerobic biotransformation of BYI 02960 was studied in two water-sediment systems, [redacted] (sand) and [redacted] (loam), for a maximum of 120 days in the darkness at 20°C. The test item was applied with three radiolabels per test system, using [furanone-4-^{14}C]-, [ethyl-1-^{14}C]-, and [pyridine-2,6-^{14}C]-labelled BYI 02960. Dissipation of BYI 02960 from the water phase was mainly characterized by rapid partitioning into the sediment where it is slowly degraded and mineralized. DFA (difluoroacetic acid) was observed as a degradation product of [ethyl-1-^{14}C]BYI 02960 in both water/sediment systems tested. In the water phases DFA accounted for up to 6.0%, in the sediment extracts for max. 0.9% of the applied radioactivity. No further significant degradation products were observed in the studies except mineralization to carbon dioxide (max. 8.5% of applied) and formation of NER (max. 26.6% of applied). The DT_{50} value for BYI 02960 in the entire water/sediment systems was in the range of 193 to 285 days for [redacted] and [redacted], respectively.

In a supportive study the fate of BYI 02960 (tech.) was investigated in pond water and sediment in outdoor microcosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. The dissipation of BYI 02960 from the supernatant water phase with a mean

of 81 days was caused by translocation into the sediment and by degradation. The overall degradation (mean of 95 days) was faster under the prevailing outdoor conditions compared to the standardized laboratory water sediment studies considering the rapid degradation due to photolysis this may due to the enhanced degradation due to sunlight under the outdoor test conditions.

In a further water-sediment study the degradation behavior of [DFA applied as test item was investigated. Mineralization to carbon dioxide (max. 25.1% of applied) and formation of NER (max. 15.8% of applied) was measured during the study period. In conclusion, a total system degradation half-life of 249 days can be used for degradation of DFA in water and for the sediment compartment.

Figure 7.8- 1: Proposed degradation pathway of BYI 02960 in aqueous environment



IIA 7.9 Degradation in the saturated zone

The degradation behavior of BYI 02960 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.

IIA 7.10 Rate and route of degradation in air

Based on an estimation according to structure-activity relationship (SAR) methods developed by [redacted] *et al.*, the half-life time in air of the insecticidal active substance BYI 02960 was assessed with the computer program AOPWIN^{TM}, version 1.92a (U.S. EPA, 2008).

Report:	**KIIA 7.10/01, [redacted], E.; 2010**
Title:	BYI 02960: Calculation of the chemical half-life in the troposphere
Report No & Document No	MEF-10/896 M-398741-01-2
Guidelines:	**Not applicable**
GLP	**Not applicable (calculation)**

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 BYI 02960 in the air was assessed by the program AOPWIN^{TM}, version 1.92a (U.S. EPA, 2008). The half-life time ($t_{1/2}$) was estimated within a range of 4.4 hours (short-term scenario) to 13.1 hours (long-term scenario), depending on the mean concentration of hydroxyl radicals present in the troposphere.
In addition, BYI 02960 is susceptible to reactions with ozone, however, that attack and its resulting chemical half-life is considered to be slower by a factor of 2 to 10.
As a consequence of the short half-life in air, no long-range transport of BYI 02960 in the atmosphere is likely to occur nor an accumulation of BYI02960 in the environmental compartment air. From the low vapor pressure of the substance it is concluded that very low, if any, quantities of BYI02960 are expected to enter the atmosphere from volatilization.

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 ki and the concentrations c(yi) of the potential reaction partners are known.
With an exception to be made for fully halogenated compounds, the abiotic degradability of an organic xenobiotic compounds can be well predicted from estimated reaction rate constants (ki) of the individual processes, and the concentrations c(yi) of the potential reaction partners, i.e. in particular the concentrations of OH radicals and ozone.
An experimental determination of the hydroxyl radical reaction rate (kOH), however, is very laborious and gas phase measurements are difficult for molecules showing a comparatively low vapor pressure. The approach of Atkinson et al. was based on a comprehensive set of experimental data to result in a quantitative structure-activity relationship (QSAR) mathematic model that allows for estimation by calculation, starting from the molecular structure of a test compound. The calculation procedure has been transferred into the personal computer program "Atmospheric Oxidation Program" (AOP) by Meylan & Howard. The current version 1.92a (U.S. EPA, 2008) was used for the calculations.

AOPWINTM requires only the chemical structure and atmospheric concentrations of the potential reaction partners as inputs.

The AOPWINTM estimation method adds up the partial reaction rates ki of the reaction of photochemically generated active species with subgroups of the test molecule (increments), resulting in the overall reaction rate. The following hydroxyl and/or ozone reactions are considered:

- hydrogen abstraction
- addition to double bonds
- addition to triple bonds
- reaction with N, S and hydroxyl groups
- addition to aromatic rings
- addition to fused rings

The listed increments (group rate constants for hydrogen abstraction, addition to double and triple bonds, ring systems, and reaction with heteroatoms) 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).
The reliability of the method used by AOPWINTM was examined by comparison of estimated and experimentally determined hydroxyl radical rate constants for 647 chemicals. More than 90 percent of the estimated rate constants were within a factor of two of the experiment value for these chemicals. More than 95 percent of the estimated values differed within a factor of three from measured values.

Considering the chemical structure of BYI 02960 it can be concluded that reactions with photochemical produced hydroxyl radicals will mainly determine its degradation rate (Ktotal, indirect photoreaction ≈ kOH) in the air. However, also reactions with ozone are expected to have a significant influence on the overall assessment.
Since a chemical in the troposphere is usually at a very low concentration and a steady-state concentration of OH radicals is produced by sunlight, the hydroxyl radical concentration can be treated as a constant so the reaction can be considered a pseudo first-order reaction.
As an estimate the maximum half-life in air from indirect photoreaction-oxidation processes can be derived under consideration of the concentration of hydroxyl radicals by the following formula:

The half-life in the troposphere, $t_{½} = \ln 2 / (k_{OH} \times [OH])$

where k_{OH} is the hydroxyl radical rate constant in units of cm^3/molecule-sec and [OH] is the hydroxyl radical concentration in units of molecules (or radicals) per cm^3. Thus, to calculate the half-life in the troposphere, one needs the estimated or measured rate constant of the chemical as well as some average OH radical concentration. In a U.S. EPA review by [redacted](no date), the author concluded that the diurnally and annually averaged 12-h daylight hydroxyl radical concentration of 1.5×10^6 molecules (radicals)/cm^3 should be used as the default in the AOPWIN program based upon data from [redacted] et al. of 1990. More recent reviews ([redacted] 2003) have suggested 2.0×10^6 molecules (radicals)/cm^3 12-hour daylight [OH].
Twelve hour daylight OH radical concentrations are reasonable for fast reacting chemicals but for chemicals that react more slowly (> a few days) 24 hours averages might be more appropriate. Atkinson (1985) suggested seasonally and diurnally 24 hour averaged hydroxyl radical concentrations at 298 K of 5×10^5 molecules/cm^3 in the northern hemisphere and 6×10^5 molecules/cm^3 in the southern hemisphere

The AOPWIN program allows the user to select 12 or 24 hour time frames and any average hydroxyl radical concentrations. For the current report the default originally set at 1.5×10^6 molecules (radicals)/cm^3 per 12-h of daylight was taken for the short term, and 0.5×10^6 molecules (radicals)/cm^3 per day (24-h) for the long term estimations.
The maximum chemical life-time in air is calculated similarly by use of the formula:

$$\tau = 1 / (k_{OH} \text{ x } [OH])$$

The ozone rate constant estimations produced by AOPWIN are generally important when one or more functional group is attached to any olefinic or acetylenic unit. It is also important for a limited number of chemical classes (e.g., hydrazines, phenols, alkyl lead compounds, and furans). The database of experimental ozone rate constants is not nearly as extensive as the database for hydroxyl radical rate constants. Because of this smaller available database, the number of fragment values that are available for estimations is considerably smaller. Therefore, the number of compounds that can be estimated with reasonable certainty is smaller. If an "assumed value" is used to produce an estimated rate constant, it is a good idea to check the "Show Calculation" screen for ozone to see if a "default value" was used in the calculation. If a default value was used, consider the estimate questionable.
Ozone reaction half-lives for organic chemicals can also be estimated similar to the approach used with hydroxyl radical by using an average ozone concentration and equation

$$\text{The half-life in the troposphere, } t^{1/2} = \ln 2 / (k_{O_{zone}} \text{ x } [\text{ozone}])$$

[redacted] (1984) indicated that [ozone] are around 10-40 ppb (0.25 to 1×10^{-12} molecules/cm^3) at ground level and increases with altitude (30 to 100 ppb at 10 km). These authors used 7×10^{-11} molecules/cm^3 (30 ppb) per 24 hours as representative of an unpolluted lower troposphere and this is the initial default value for AOPWIN, which was used in the current estimations. As with OH radicals, the concentration of ozone varies with amount of pollution, time of the day, climate, and zonal location.

II. Results

The overall reaction rate of BYI 02960 with hydroxyl radicals is estimated to be 29.3590 × 10-12 cm3 × molecule-1 × s-1. This rate is derived mainly from incremental reactions like hydrogen abstraction (10.7929 × 10-12 cm3 × molecule-1 × s-1) and an addition reaction to the C=C bond (18.2490 × 10-12 cm3 × molecule-1 × s-1).
Based on the overall hydroxyl radical reaction rate constant in combination with the "short term" concentration of these radicals in the atmosphere during daylight (i.e. 1.5 x 10+6 OH radicals/cm3) the half-life (t1/2) of BYI 02960 in air is derived to be 4.372 hours.
That estimate should be regarded as worst-case assumption as the approach does not consider the contribution of any other reactive species to the overall atmospheric degradation of BYI 02960 (i.e. by ozone; for its contribution refer to Appendix 3 and Appendix 4 of report). It should be noted that the chemical half-life of the active substance can be expected to be shorter when being applied in the early afternoon since the OH radical concentration in the troposphere may increase to 5 x 10+6 radicals/cm3 during the day while these values are lower in the early morning or late afternoon.

III. Conclusions

BYI 02960 is considered to be susceptible to reactions with hydroxyl radicals to contribute significantly to the overall degradation of the substance in the atmosphere. Various parts of the molecule were identified as potential targets for radical reactions. An attack by hydroxyl radicals and

ozone should result in the formation of multiple primary radicals. Their formation may be followed by secondary oxidation products that can be eliminated from the atmosphere by wet and/or dry deposition.

In addition, BYI 02960 is susceptible to reactions with ozone, however, that attack and its resulting chemical half-life is considered to be slower by a factor of 2 to 10.

As a consequence of the short half-life in air, no long-range transport of BYI 02960 in the atmosphere is likely to occur nor an accumulation of BYI 02960 in the environmental compartment air. From the low vapor pressure of the substance it is concluded that very low, if any, quantities of BYI 02960 are expected to enter the atmosphere from volatilization of soil residues.

IIA 7.11 Definition of the residue

In Europe the definition of the residue for further evaluation is proposed as follows:

Soil: BYI 02960, DFA, 6-CNA
Groundwater: BYI 02960, DFA, 6-CNA
Surface water: BYI 02960, DFA, 6-CNA, BYI 02960-succinamide, BYI 02960-azabicyclosuccinmaide

IIA 7.12 Monitoring data concerning fate and behaviour

Plant protection products containing BYI 02960 are not yet authorized for use. Accordingly, monitoring data concerning fate, behavior and concentration in the environment are not available.

IIA 7.13 Other/special studies

The physico-chemical properties of the metabolites (environmental and plant) are summarized in this section as there is no suitable chapter in the physico-chemical chapter and the results of the studies are used in the environmental and ecotoxicological assessment.

Water Solubility of BYI 02960 Metabolites

Report:	**KIIA 7.13/01, [redacted], [redacted]; 2011**
Title:	BCS-CC98193 (BYI 02960-DFEAF): Water solubility at pH 5, pH 7 and pH 9 (flask method)
Report No & Document No	PA11/018 M-415753-01-1
Guidelines/ Requirements:	**Regulation (EC) No 440/2008, Annex, Part A, method A.6. OECD 105 OPPTS 830.7840**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The water solubility C_S of BCS-CC98193 (BYI 02960-DFEAF) at pH 5, pH 7 and pH 9 was determined according to the "flask method" described in the European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.6., OECD-guideline 105 and EPA Product Properties Test Guideline OPPTS 830.7840.

The concentration of BCS-CC98193 was quantified by HPLC analyses for pH 5, pH 7 and pH 9. The used HPLC method (reversed phase) was found to be valid. The sample purity of 98.5 % w/w was taken into account. The results of the solubility measurements are given in the following table.

Table 7.13- 1: Water solubility of BCS-CC98193 (BYI 02960-DFEAF)

Solubility in	Measured pH [1)]	Solubility C_S at 20°C	RSD
buffer pH 5	5.0	35.5 g/L	5.6 %
buffer pH 7	7.0	36.7 g/L	1.3 %
buffer pH 9	9.0	36.4 g/L	5.9 %

1) resulting from 3 experiments

Report:	**KIIA 7.13/02, [redacted], [redacted]; 2011**
Title:	BCS-CR74729 (BYI 02960-succinamide): Water solubility at pH 5, pH 7 and pH 9 (flask method)
Report No & Document No	PA11/078 M-416651-01-1
Guidelines/ Requirements:	**Regulation (EC) No 440/2008, Annex, Part A, method A.6. OECD 105 OPPTS 830.7840**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The water solubility C_S of BCS-CR74729 (BYI 02960-succinamide) at pH 5, pH 7 and pH 9 was determined according to the "flask method" described in the European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.6., OECD-guideline 105 and EPA Product Properties Test Guideline OPPTS 830.7840.

Due the fact that at all three pH-values the buffer capacity was not sufficient, a modified flask method was applied: Instead of using a buffer solution the pH value was adjusted by adding small amounts of aqueous sodium hydroxide and hydrochloric acid solutions to the solution of the test item.

The concentration of BCS-CR74729 (BYI 02960-succinamide) at pH 5 was quantified by HPLC analyses. The used HPLC method (reversed phase) was found to be valid. The minimum solubility at pH 7 and pH 9 was visually determined. The sample purity (97.8 % w/w) was taken into account. The results of the solubility measurements are given in the following table.

Table 7.13- 2: Water solubility of BCS-CR74729 (BYI 02960-succinamide)

Solubility in	Final pH [1)]	Solubility C_S at 20°C	RSD
buffer pH 5	5.0	5.3 g/L	5.2 %
buffer pH 7	7.0	> 120 g/L	-
buffer pH 9	9.0	> 120 g/L	-

1) measured pH of the saturated solution, resulting from 2 experiments each

Report:	**KIIA 7.13/03, [redacted], [redacted]; 2011**
Title:	BCS-CU93236 (BYI 02960-azabicyclosuccinamide NA-salt): Water solubility at pH 5, pH 7 and pH 9 (flask method)
Report No & Document No	PA11/094 M-417069-01-1
Guidelines/ Requirements:	**Regulation (EC) No 440/2008, Annex, Part A, method A.6. OECD 105 OPPTS 830.7840**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The water solubility C_S of BCS-CU93236 (BYI 02960-azabicyclosuccinamide Na-salt) at pH 5, pH 7 and pH 9 was determined according to the "flask method" described in the European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.6., OECD-guideline 105 and EPA Product Properties Test Guideline OPPTS 830.7840.

The minimum solubility of BCS-CU93236 at pH 5, pH 7 and pH 9 was visually determined. The sample purity of 97.3 % w/w was taken into account. The results of the solubility measurements are given in the following table.

Table 7.13- 3: Water solubility of BCS-CU93236 (BYI 02960-azabicyclosuccinamide Na-salt)

Solubility in	Final pH [1)]	Solubility C_S at 20°C [1)]
buffer pH 5	5.1	> 175 g/L
buffer pH 7	7.0	> 180 g/L
buffer pH 9	9.0	> 180 g/L

[1)] resulting from 2 experiments

Report:	**KIIA 7.13/04, [redacted], [redacted]; 2011**
Title:	Difluoroacetic acid (BCS-AA56716): Miscibility with distilled water and solubility in water in a pH range of 1.6 to 13
Report No & Document No	PA10/042 M-418554-01-1
Guidelines/ Requirements:	**Regulation (EC) No 440/2008, Annex, Part A, method A.6. OECD 105 OPPTS 830.7840**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The water solubility C_S of difluoroacetic acid (BCS-AA56716) in a pH range of 1.6 to 13.0 was determined based on the "flask method" described in the guidelines European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.6., OECD-guideline 105 and EPA Product Properties Test Guideline OPPTS 830.7840.

Due to the extremely high water solubility at all pH values a visual evaluation was sufficient.

In addition the miscibility of difluoroacetic acid with distilled water was evaluated. Difluoroacetic acid (BCS-AA56716) was miscible with distilled water in any ratio at 20 °C. The water solubility of difluoroacetic acid (BCS-AA56716) at approx. 20 °C was above 500 g/L solution in a pH range of 1.6 to 13.0.

The metabolite 6-CNA is a common metabolite with the active substance acetamiprid, the following study has been performed as part of the Acetamiprid regulatory package and access to the study has been granted by the owner of the study.

Report:	**KIIA 7.13/05, [redacted]; 2001**
Title:	Solubility of IC-0 in Water
Report No & Document No	NCAS 01 -129 M-202871-01-1
Guidelines/ Requirements:	**Regulation (EC) No 440/2008, Annex, Part A, method A.6.** **OECD 105** **OPPTS 830.7840**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

Solubility of IC-0, 6-chloronicotinic acid (6-CNA), in distilled water and buffer solutions (pH 4 and 10) was determined using the Shake Flask Method. The solubility of 6-CNA in water was 1.43 g/L at 20°C and 1.30 g/L at 10°C.
No difference was observed for the solubility obtained at two temperatures. The solubility in pH 4 and 10 (buffer solutions) were 4.62 and 18.6 g/L at 20°C, respectively. The pH values for the test solutions of pH 4 and 10 buffer solutions dropped down to 3.2 and 4.4, respectively after incubation.

Partition Coefficient 1-Octanol/Water of BYI 02960 Metabolites

Report:	**KIIA 7.13/06, [redacted], [redacted]; 2011**
Title:	BCS-CR74729 (BYI 02960-succinamide): Partition coefficients 1-octanol / water at pH 5, pH 7 and pH 9 (shake flask method)
Report No & Document No	PAC1/079 M-416883-01-1
Guidelines:	**Regulation (EC) No 440/2008, Annex, Part A, method A.8.** **OECD 107** **OPPTS 830.7550**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The partition coefficients 1-octanol / water of BYI 02960-succinamide (BCS-CR74729) at room temperature (mean 23°C) were determined according to the "shake flask method" described in the European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.8., OECD Guideline 107 and EPA Product Properties Test Guideline OPPTS 830.7550.
The concentration of BYI 02960-succinamide (BCS-CR74729) was quantified by HPLC analysis. The used HPLC method (reversed phase) was found to be valid. The results of the experiments are given in the following table:

Table 7.13- 4: Partition coefficients of BYI 02960-succinamide (BCS-CR74729) at room temperature (mean: 23°C)

Measured in	measured pH	P_{ow}	log P_{ow}
buffer pH 5	pH 4.7	4	0.6
buffer pH 7	pH 6.9	0.05	- 1.3
buffer pH 9	pH 8.9	0.003	- 2.5

Report:	**KIIA 7.13/07, [redacted], [redacted]; 2011**
Title:	BCS-CU93236 (BYI 02960-azabicyclosuccinamide Na-salt): Partition coefficients 1-octanol / water at pH 5, pH 7 and pH 9 (shake flask method)
Report No & Document No	PA11/093 M-416656-01-1
Guidelines:	**Regulation (EC) No 440/2008, Annex, Part A, method A.8. OECD 107 OPPTS 830.7550**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The partition coefficients 1-octanol / water of BCS-CU93236 (BYI 02960-azabicyclosuccin-amide Na-salt) at room temperature (mean 23°C) were determined according to the "shake flask method" described in European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.8., OECD Guideline 107 and EPA Product Properties Test Guideline OPPTS 830.7550.
The concentration of BCS-CU93236 (BYI 02960-azabicyclosuccinamide Na-salt) was quantified by HPLC analysis. The used HPLC method (reversed phase) was found to be valid. The results of the experiments are given in the following table:

Table 7.13- 5: Partition coefficients of BCS-CU93236 (BYI 02960-azabicyclosuccinamide Na-salt) at room temperature (mean: 23°C)

Measured in	measured pH	Pow	log Pow
buffer pH 5	pH 5.3	4.7×10^{-2}	- 1.3
buffer pH 7	pH 7.0	1.9×10^{-3}	- 2.7
buffer pH 9	pH 9.0	2.9×10^{-4}	- 3.5

Report:	**KIIA 7.13/08, [redacted], [redacted]; 2011**
Title:	Difluoroacetic acid (BCS-AA56716): Partition coefficients 1-octanol / water at pH 5, pH 7 and pH 9 (shake flask method)
Report No & Document No	PA10/043 M-416624-01-1
Guidelines:	**Regulation (EC) No 440/2008, Annex, Part A, method A.8. OECD 107 OPPTS 830.7550**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The partition coefficients 1-octanol / water of difluoroacetic acid (BCS-AA56716) at room temperature (mean 23°C) were determined according to the "shake flask method" described in the European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.8., OECD Guideline 107 and EPA Product Properties Test Guideline OPPTS 830.7550.

The concentration of difluoroacetic acid (BCS-AA56716) was quantified by IC analyses. The used IC method was found to be valid. The results of the solubility measurements are given in the following table:

Table 7.13- 6: Partition coefficients of difluoroacetic acid (BCS-AA56716) at RT (mean: 23°C)

Measured in	P_{ow}	log P_{ow}
buffer pH 5	0.00083	- 3.1
buffer pH 7	0.00080	- 3.1
buffer pH 9	0.00030	- 3.5

The metabolite 6-CNA is a common metabolite with the active substance acetamiprid, the following study has been performed as part of the Acetamiprid regulatory package and access to the study has been granted by the owner of the study.

Report:	**KIIA 7.13/09, [redacted], S.; 2001**
Title:	Partition Coefficient (n-octanol/water) of IC-0
Report No & Document No	NCAS-01 -127 M-204285-01-1
Guidelines:	**Regulation (EC) No 440/2008, Annex, Part A, method A.8.** **OECD 107** **OPPTS 830.7550**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The octanol/water partition coefficient (Pow) of IC-0, 6-chloronicotinic acid (6-CNA), was determined by the shake flask method. Prior to the experimental determination, the log P_{OW} of IC-0 was calculated to be 1.55 by CLogP software. The experimental determination was performed in duplicate on each of three different volume ratios of octanol and pH 1.98 buffer solution.

The average P_{OW} value with standard deviation was 33.0 ± 1.5, and the log P_{OW} value was determined to be 1.52 at 25 °C. The value agreed with the calculated value.

Dissociation Constant of BYI 02960 Metabolites in Water

Report:	**KIIA 7.13/10, [redacted], [redacted]; 2011**
Title:	BCS-CC98193 (BYI 02960-DFEAF): Dissociation constant in water
Report No & Document No	PA11/021 M-415757-01-1
Guidelines:	**OECD 112** **OPPTS 830.7370**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The dissociation constant of BCS-CC98193 (BYI 02960-DFEAF) was examined using a spectrophotometric method based on the OECD-Guideline 112 and the EPA Product Properties Test Guideline OPPTS 830.7370.

UV/VIS spectra of the test item were recorded at pH 2.04, pH 6.87 and pH 12.05 at room temperature (approx. 21 °C). The UV/VIS spectra were almost congruent, taking the UV absorption of aqueous sodium hydroxide and hydrochloric acid into account. No relevant difference was found between the UV/VIS absorption at the examined pH values.

No dissociation constant pKa was found in aqueous solution of BCS-CC98193 (BYI 02960-DFEAF) in the pH-range of $2 < pH < 12$.

This finding corresponds to the result that BCS-CC98193 (BYI 02960-DFEAF) shows no acidic or basic properties in the range of approximately $2 < pKa < 12$.

Report:	**KIIA 7.13/11, [redacted]; 2011**
Title:	Difluoro acetic acid (BCS-AA56716): Determination of the dissociation constant in water
Report No & Document No	20100366.02 M-418626-01-1
Guidelines:	**OECD 112** **OPPTS 830.7370**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The purpose of this study was the determination of the dissociation constant of the test item according to the OECD test guideline, OECD 112 (1981), Dissociation Constants in Water and EPA Product Properties Test Guideline OPPTS 830.7370.

The titration method was used. The experiments were performed by potentiometric titration.

The mean value of three determinations at room temperature (approx. 23 °C) was $pK_a = 1.6$

The metabolite 6-CNA is a common metabolite with the active substance acetamiprid, the following study has been performed as part of the Acetamiprid regulatory package and access to the study has been granted by the owner of the study.

Report:	**KIIA 7.13/12, [redacted]; 2001**
Title:	Dissociation Constant of IC-0
Report No & Document No	NCAS 01 -140 M-203097-01-1
Guidelines:	**OECD 112** **OPPTS 830.7370**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The dissociation constant (pKa) of IC-0, 6-chloronicotinic acid (6-CNA), was determined using a titration method. The pKa of IC-0 was 3.28 (20°C).

Vapor pressure of BYI 02960 Metabolites

Report:	**KIIA 7.13/13, [redacted]; 2011**
Title:	BCS-CC98193 (BYI 02960-DFEAF): Vapour pressure
Report No & Document No	20110091.01 M-420457-01-1
Guidelines/ Requirements:	**European Commission Regulation (EC) No. 440/2008, A.4** **OECD 104** **OECD 113** **OPPTS 830.7950**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

Using the vapour pressure balance (effusion method), the vapour pressure values for the test item BCS-CC98193 (BYI 02960-DFEAF) were found to be:

Temperature (°C)	P (hPa)	P (Pa)
20	2.1×10^{-7}	2.1×10^{-5}
25	4.7×10^{-7}	4.7×10^{-5}
50	2.0×10^{-5}	2.0×10^{-3}

Report:	**KIIA 7.13/14, [redacted]; 2011**
Title:	Difluoro acetic acid (BCS-AA56716): Vapour pressure
Report No & Document No	20100366.01 M-418553-01-1
Guidelines:	**European Commission Regulation (EC) No. 440/2008, A.4** **OECD 104** **OECD 113** **OPPTS 830.7950**
GLP	**Yes (fully GLP compliant and certified laboratory)**

SUMMARY

The following vapour pressure values of difluoroacetic acid (BCS-AA56716) were extrapolated based on the measurement results of the dynamic method:

Temperature (°C)	P (hPa)	P (Pa)
20	2.8	2.8×10^2
25	4.0	4.0×10^2
50	20	2.0×10^3

Henry's Law Constant of BYI 02960 Metabolites

Report:	**KIIA 7.13/15, [redacted], [redacted]; 2011**
Title:	BCS-CC98193 (BYI 02960-DFEAF): Calculation of the Henry´s Law constants
Report No & Document No	AF11/029 M-418455-01-1
Guidelines	**Directive 94/37/EEC, Annex 1, Section 2, paragraph 2.3.2**
GLP	**N/A (calculation)**

SUMMARY

This report has been prepared to calculate the Henry's law constants of BCS-CC98193 (BYI 02960-DFEAF) at 20°C at pH 5, pH 7 and pH 9.
The Henry's law constant K at 20 °C was calculated to be approx. 9.5 x 10^{-8} Pa•m^3•mol^{-1} in buffers of pH 5 to 9.

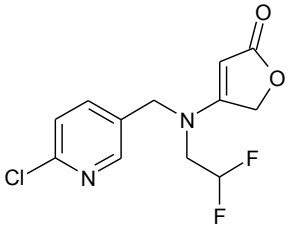
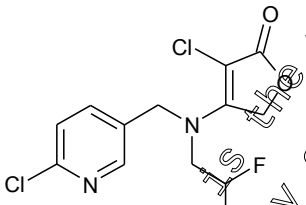
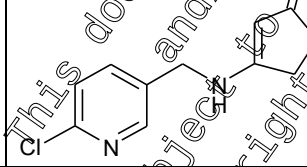
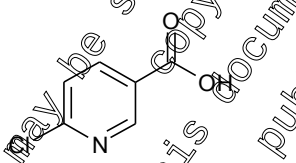
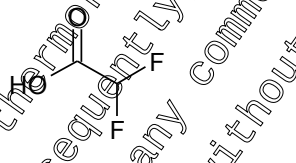
Report:	**KIIA 7.13/16, [redacted], [redacted]; 2011**
Title:	Difluoro acetic acid (BCS-AA56716): Calculation of the Henry's Law constant
Report No & Document No	AF11/017 M-419373-01-1
Guidelines/ Requirements:	**Directive 94/37/EEC, Annex 1, Section 2, paragraph 2.3.2**
GLP	**N/A (calculation)**

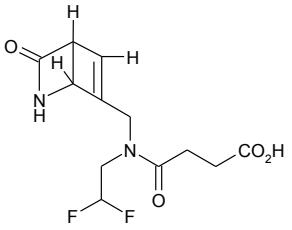
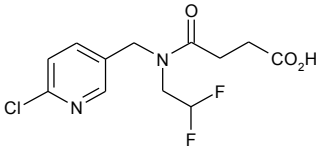
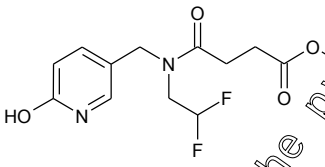
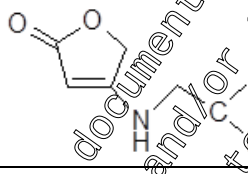
SUMMARY

This report has been prepared to calculate the Henry's law constants of difluoroacetic acid (BCS-AA56716) at 20°C in the pH range of 1.6 to 13.0.
The Henry's law constant K at 20 °C was calculated to be approx. 0.054 Pa•m^3•mol^{-1} in a pH range of 1.3 to 13.

List of BYI 02960 metabolites in plants and in the environment referred to in the current section

In the original study reports on BYI 02960 the metabolites are sometimes named by different synonyms, the metabolites referred to in this section are summarized below. Full details are provided in Document N.

	Name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
a.s.	**BYF 02960 (parent compound)** 	$C_{12}H_{11}ClF_2N_2O_2$ 288.68 g/mol Flupyradifurone	all matrices
M01	**BYI 02960-chloro** 	$C_{12}H_{10}Cl_2F_2N_2O_2$ 323.13 g/mol BCS-CD27046	Plant: Environment Aerobic Soil (minor)
M19	**BYI 02960-des-difluoroethyl** 	$C_{10}H_9ClN_2O_2$ 224.65 g/mol BCS-AB49019	Animal Environment Aerobic soil (minor)
M27	**6-CNA** 	$C_7H_4ClNO_2$ 157.56 g/mol 6-chloronicotinic acid IC-0 (in reports from Nisso) BYI 02960-6-CNA BCS-AA35572	Animal, Plant: Environment Aerobic soil (major)
M44	**DFA** 	$C_2H_2F_2O_2$ 96.03 g/mol difluroacetic acid BYI 02960-DFA BCS-AA56716	Animal, Plant: Environment Aerobic Soil (major) Aerobic water/Sediment (major)
M47	**BYI 02960-azabicyclosuccinamide**		

	Name, Structure IUPAC name CAS name, [CAS number]	Molecular formula molar mass Other names / codes	Occurrence
		$C_{12}H_{14}F_2N_2O_4$ 288.25 g/mol BCS-CS64875	Environment Water – aquatic photolysis (major)
M48	**BYI 02960-succinamide**		
		$C_{12}H_{13}ClF_2N_2O_3$ 306.69 g/mol BCS-CR74729	Environment Water – Aquatic photolysis (major)
M49	**BYI 02960-deschlorohydroxysuccinamide**		
		$C_{12}H_{14}F_2N_2O_4$ 288.25 g/mol DCHS	Environment Water – Aquatic Photolysis (minor)
The following metabolite does not occur in environmental fate studies, however the physico-chemcial properties have been summarised in this chapter aling with the remaining metabolite data.			
M34	BYI 02960-difluoroethyl-amino-furanone 	$C_6H_7F_2NO_2$ 163.12 g/mol DFEAF	Animal, Plant