

Data Evaluation Report on the anaerobic biotransformation of BAS 510 F in soil

PMRA Submission Number {.....}

EPA MRID Number 45405212

Data Requirement: PMRA Data Code:
EPA DP Barcode: D278387
OECD Data Point:
EPA Guideline: 162-2

Note — This study was submitted by the registrant to satisfy USEPA Subdivision N Guideline 162-2, anaerobic soil metabolism. The study was not conducted according to the guidelines for 162-2, but it was also not conducted in a manner that would fulfill any other Subdivision N Guidelines. Therefore, the study was evaluated under the guideline requirement identified by the registrant.

Test material:

Common name: BAS 510 F

Chemical name

IUPAC: 2-Chloro-N-(4-chlorobiphenyl-2-yl)-nicotinamide
CAS name: 2-Chloro-N-(4-chloro[1,1-biphenyl]-2-yl)-3-pyridinecarboxamide
CAS No: 188425-85-6
Synonyms: 2-Chloro-N-(4'-chlorobiphenyl-2-yl)-nicotinamide
Nicobifen
~~BAS 516 02 F~~

SMILES string:

Primary Reviewer: Mary Thomas
Dynamac Corporation

Signature: *Mary Thomas*
Date: 1/14/02

QC Reviewer: Joan Harlin
Dynamac Corporation

Signature: *Joan Harlin*
Date: 1/15/02

Secondary Reviewer: Cheryl Sutton
EPA

Signature: *Cheryl Sutton*
Date: 1/1/02

Company Code: [for PMRA]
Active Code: [for PMRA]
Use Site Category: [for PMRA]
EPA PC Code: 128008

CITATION: Staudenmaier, H and C. Schäfer. 2000. Anaerobic metabolism of BAS 510 F in soil (¹⁴C-diphenyl label). Unpublished study performed BASF Aktiengesellschaft, Ecology and



2017216

1

Data Evaluation Report on the anaerobic biotransformation of BAS 510 F in soil

PMRA Submission Number {.....}

EPA MRID Number 45405212

Triangle Park, NC. Study Code 42382; BASF Registration Document No. 2000/1014986. Study initiated April 6, 1999 and completed September 27, 2000 (p. 11).

Data Evaluation Report on the anaerobic biotransformation of BAS 510 F in soil

PMRA Submission Number {.....}

EPA MRID Number 45405212

ABSTRACT

Metabolism - Anaerobic Soil

The biotransformation of [diphenyl-U-¹⁴C]-labeled 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide (BAS 510 F) was studied in German sandy loam soil (pH 7.2, organic carbon 1.63%) incubated for 120 days under anaerobic conditions (flooding plus nitrogen atmosphere) in darkness at 20 ± 2°C. Anaerobic conditions were established in the soil prior to treatment; redox potentials at the time of treatment were -236 to -160 mV. [¹⁴C]BAS 510F was applied at a nominal rate of 1.0 mg/kg (equivalent to 750 g a.i./ha). The test system consisted of glass vessels containing the treated flooded soil samples maintained in a flow-through apparatus with traps for the collection of CO₂ and volatile organics. Single soil samples were collected for analysis after 0, 3, 7, 14, 30, 58, 90, and 120 days of anaerobic incubation. An additional sample was treated at an exaggerated nominal rate of 30 mg/kg and incubated as described for 127 days. The soil and floodwater were not separated prior to analysis. The soil plus water were sequentially extracted three times with methanol and three times with methanol:water (1:1, v:v). The extracts and extracted soil were analyzed for total radioactivity using LSC and LSC following combustion, respectively. [¹⁴C]BAS 510 F and its transformation products were separated by HPLC and identified by comparison to the retention time of reference standards. To aid in the identification of BAS 510 F and its transformation products, a single sample treated at an exaggerated nominal rate of 30 mg/kg was incubated as described for 127 days and analyzed by HPLC/MS.

Overall material balances decreased from 101.4% of the applied at 0 day to 94.9% at 30 days, and 89.2-89.8% at 90 and 120 days posttreatment.

[¹⁴C]BAS 510 F was 100.9% of the applied in the sample immediately posttreatment, 85.9% at 30 days, and 73.6% at 120 days posttreatment (final sampling interval). Two minor unidentified transformation products were each ≤0.6% of the applied at 58-120 days. Volatilized ¹⁴CO₂ totaled 0.1% of the applied at 120 days; organic volatiles were not detected during the study.

HPLC/MS analysis of the high-dose sample isolated the minor transformation products M510F08, M510F49, M510F50, and various isomers, each at <1% of the applied.

Total extractable [¹⁴C]residues decreased from 100.9% of the applied at day 0 to 73.9% at 120 days posttreatment. Total nonextractable [¹⁴C]residues slowly increased from 0.5% of the applied at day 0 to 15.8% at 120 days posttreatment. At 90 and 120 days, 3.4-4.2% of the applied was associated with the fulvic acid fraction, 1.1-1.4% with humic acid, and 8.5-9.5% with humin.

Data Evaluation Report on the anaerobic biotransformation of BAS 510 F in soil

PMRA Submission Number {.....}

EPA MRID Number 45405212

It was proposed that BAS 510F initially degrades to M510F08, M510F49, M510F50, and various isomers, all of which then degrade to CO₂ or bind to the soil (Figure 19, p. 46).

The calculated half-life (linear regression assuming first-order kinetics) was 266.5 days ($r^2 = 0.9399$). However, the calculated half-life is beyond the scope of the data and is, therefore, of questionable value. It is noted that the corresponding study on the pyridinyl-labeled parent compound yielded a half-life of 365 days, also calculated beyond the scope of the data.

Study Acceptability: This study is classified as not acceptable. The data are not adequate for a valid half-life determination. The parent did not decrease to 50% of the applied by the end of the study, and replicate data were not obtained. The study does not fulfill the guideline requirement for an anaerobic biotransformation study in soil (§162-2) because the pesticide was applied to anaerobic soil, rather than being applied to aerobic soil and incubated for 30 days prior to the introduction of anaerobic conditions. Additionally, a foreign soil was used rather than a domestic (U.S.) soil, and the samples were not analyzed by phase (i.e., water and soil), but were analyzed only as a whole system. An anaerobic soil metabolism study is not required because an acceptable anaerobic aquatic metabolism study (MRID 45405213) has been submitted.

MATERIALS AND METHODS

Samples (100 g dry weight) of sieved (2 mm) German sandy loam soil (65.14% sand, 25.05% silt, 9.80% clay, pH 7.2 [CaCl₂], organic carbon 1.63%, CEC 12.7 mVal/100 g dry soil; p. 49) collected from Limburgerhof, Germany, were weighed into glass vessels (not further described) and flooded (1-2 mm depth) with 50 mL of distilled water; the final sediment:water ratio was 2:1 (w:v, reviewer-calculated, p. 13). The dishes were placed on metal trays, and the trays were placed in incubation tubes (p. 50). Each tube was equipped with inlet/outlet ports. The tubes were housed within an incubation chamber. The samples were incubated for 29 days (temperature not specified). At the end of the preincubation, the redox potential of the soil averaged -204 mV (n = 8; -236 to -160 mV; Table 1, p. 25), indicating that anaerobic conditions were established.

The flooded soils were then treated with [diphenyl-U-¹⁴C]-labeled BAS 510 F (2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide; radiochemical purity >99%; specific activity 4.85 MBq/mg; Batch No: 641-1101; p. 12) dissolved in acetonitrile (~0.03% by volume; reviewer-calculated, 29.3 µL/100 g dry soil) at a nominal application rate of 1 mg/kg (750 g a.i./ha, p. 13). The treated samples were returned to the metabolism apparatus and incubated in the dark at 20 ± 2°C for up to 120 days posttreatment (p. 13). Single soil samples were collected after 0, 3, 7, 14, 30, 58, 90, and 120 days of anaerobic incubation. To aid in metabolite identification, a single soil sample was treated with the test substance dissolved in acetonitrile (~0.88% by volume; reviewer-calculated, 878.2 µL/100 g dry soil) at an

Data Evaluation Report on the anaerobic biotransformation of BAS 510 F in soil

PMRA Submission Number {.....}

EPA MRID Number 45405212

exaggerated nominal application rate of 30 mg/kg, incubated as described, and collected at 127 days posttreatment (p. 13). During the study, the incubation tubes were purged with moistened nitrogen gas; exiting gases were passed sequentially through ethylene glycol and 0.5 M sulfuric acid to collect organic volatiles and 0.5 M sodium hydroxide to trap CO₂ (pp. 13, 50). Volatile trapping solutions were collected and replaced with fresh trapping solution at each sampling interval. Control samples were not employed in the study. The redox potential was measured at each sampling interval (p. 13).

The soil and water phases were not separated prior to analysis. The samples were stored refrigerated or frozen for up to 36 days prior to extraction (p. 56). The flooded soils were extracted three times with 60 mL of methanol and three times with 100 mL of methanol:water (1:1, v:v) by shaking on a laboratory shaker at 40°C (p. 14). Following each extraction, the samples were centrifuged then decanted through a filter. Aliquots were analyzed for total radioactivity using LSC. Like extracts were combined and concentrated on a rotary evaporator at 40°C. The resulting residues were redissolved in acetonitrile or acetonitrile:water for HPLC analysis. Samples were analyzed by reverse-phase HPLC using the following conditions (p. 16):

HPLC		
Column:	Phenomenex Luna 5 µm, C18, 250 × 4.6 mm	
Solvent A:	Water:acetonitrile:formic acid (900:100:0; v:v:v)	
Solvent B:	Water:acetonitrile:formic acid (50:950:2; v:v:v)	
Gradient:	Time (min)	B (%)
	0-5	0
	17.5-37.5	35
	60	90
	65	100
	70	0
Flow Rate:	1.5 mL/min	
Detection	UV (Kontron 430) Radiodetection (Berthold, LB 507 A)	

The method used to identify BAS 510 F and its transformation products analyzed by HPLC was not stated but appears to be comparison to the retention time of unlabeled reference standards.

The soil sample treated at the exaggerated rate was extracted five times with methanol and twice with methanol:water (1:1, v:v; p. 14). The methanol extracts were combined, reduced in volume, and analyzed by HPLC/MS in the ESI mode. The methanol:water extracts were

Data Evaluation Report on the anaerobic biotransformation of BAS 510 F in soil

PMRA Submission Number {.....}

EPA MRID Number 45405212

not analyzed. The identities of parent and transformation products were confirmed using HPLC/MS in the ESI mode (pp. 17, 53).

The extracted soil was oven-dried at 60°C, homogenized in a small mill, and aliquots were analyzed for total radioactivity by LSC following combustion (p. 14). The combustion efficiency was >92%; radioactive residues in the test samples were corrected for oxidizer efficiency (p. 16). Unextracted residues from the methanol and methanol:water extractions were further characterized by sodium hydroxide extraction (p. 14). The unextracted residues were extracted four times with 100 mL of 0.5 M sodium hydroxide. Following each extraction, the samples were centrifuged and the supernatant was filtered and brought up to volume. The extracts were analyzed by LSC and then like extracts were combined. The alkaline extracts were acidified and the resulting precipitate was removed by centrifugation. The supernatant (fulvic acid) was extracted three times with ethyl acetate, and the acidic water-soluble and ethyl acetate-soluble residues were analyzed by LSC. The precipitate (humic acid) was redissolved in 0.5 M sodium hydroxide and analyzed by LSC.

[¹⁴C]Residues remaining in the extracted soil following sodium hydroxide extraction were designated as humin (p. 23).

Aliquots of each trapping solution were analyzed for total radioactivity by LSC.

RESULTS/DISCUSSION

[Diphenyl-U-¹⁴C]-labeled 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide (BAS 510 F; radiochemical purity >99%), at a nominal concentration of 1 mg/kg, degraded with a reviewer-calculated half-life of 266.5 days (first-order kinetics, $r^2 = 0.94$) in anaerobic (flooding plus nitrogen atmosphere) sandy loam soil that was incubated in the dark at 20 ± 2°C, following 29 days of anaerobic (flooding) incubation. The soil and floodwater water were not separated prior to analysis. Based on HPLC analysis, BAS 510 F was 100.9% of the applied in the soil immediately posttreatment, 85.9% at 30 days, and 73.6% at 120 days posttreatment (Table 3, p. 27). Two minor unidentified transformation products were each ≤0.6% of the applied at 58-120 days.

HPLC/MS analysis of the high-dose sample isolated minor transformation products M510F08, M510F49, M510F50, and various isomers, each <1% of the applied (p. 24, Figure 19, p. 46).

Total extractable [¹⁴C]residues decreased from 100.9% of the applied at day 0 to 73.9% at 120 days posttreatment (Table 2, p. 26). Total nonextractable [¹⁴C]residues slowly increased from 0.5% of the applied at day 0 to 15.8% at 120 days posttreatment. At 90 and 120 days, 3.4-4.2% of the applied was associated with the fulvic acid fraction, 1.1-1.4% with humic acid, and 8.5-9.5% with humin (Table 4, p. 27). Volatilized ¹⁴CO₂ totaled 0.1% of the

Data Evaluation Report on the anaerobic biotransformation of BAS 510 F in soil

PMRA Submission Number {.....}

EPA MRID Number 45405212

applied at 120 days; organic volatiles were not detected during the study. Overall material balances decreased from 101.4% of the applied at 0 day to 94.9% at 30 days, and 89.2-89.8% at 90 and 120 days posttreatment.

Redox potentials ranged from -195 to -159 mV at 0-120 days posttreatment (Table 1, p. 25).

Data Evaluation Report on the anaerobic biotransformation of BAS 510 F in soil

PMRA Submission Number {.....}

EPA MRID Number 45405212

REVIEWER'S COMMENTS

1. The registrant submitted this study under USEPA Subdivision N Guideline 162-2, anaerobic soil metabolism. However, the study design is incorrect to meet this data requirement. In this study, the soil was flooded and incubated under a nitrogen atmosphere for 29 days, then treated with BAS 510 F and incubated (with frequent sampling) for an additional 120 days. In an anaerobic soil metabolism study, soil should be treated with the pesticide and aged aerobically for 30 days, then converted to anaerobic conditions. This study is similar to an anaerobic aquatic metabolism study in that the flooded soil was anaerobic at the time of treatment. However, the study cannot fulfill anaerobic aquatic data requirements because it was conducted for only 120 days, rather than 1 year, and because the soil and water fractions were not analyzed separately. Therefore, the study was reviewed as intended by the registrant. An anaerobic soil metabolism study is not required because an acceptable anaerobic aquatic metabolism study (MRID 45405213) has been submitted.

In the anaerobic aquatic metabolism study (MRID 45405213), the reviewer-calculated half-life value of BAS 510 F in anaerobic clay loam soil was 385 days ($r^2 = 0.8145$; linear regression assuming first-order kinetics). The concentration of BAS 510F at 88 days posttreatment (which was the closest interval to 120 days) was 61-62% of the applied.

2. Only one sample was analyzed for each sampling interval. Replicate sampling is preferred, so that normal variability can be quantified and outliers can be identified.
3. For HPLC analyses, it was not stated how BAS 510 F and its transformation products were identified in the soil extracts. Based on the HPLC chromatograms provided, it appears that BAS 510 F and its transformation products were identified by comparison to the retention times of unlabeled reference standards (Figures 3-9, pp. 30-36). However, it was stated that no reference compounds were used in the study (p. 12). Clarification should be provided by the registrant.
4. The treated soils were reported to have been incubated at $20 \pm 2^\circ\text{C}$ during the study. Supporting records were not included in the study report.
5. A DT50 value of 261 days ($r^2 = 0.94$) was determined by the study author using an analysis based on multi-compartment models as calculated by ModelMaker v. 3.0.4 (p. 17; Figure 20, p. 47). The study author stated that the DT90 value was not reported since it exceeded the study duration and was beyond the period of reliable extrapolation. It is noted, however, that the DT50 also exceeded the duration of the study.
6. The German test soil was classified as a loamy sand soil (DIN 4220) according to the German soil textural classification system and as a sandy loam soil according to the USDA Soil Textural Classification system (p. 49). The physical, chemical, and microbiological properties

Data Evaluation Report on the anaerobic biotransformation of BAS 510 F in soil

PMRA Submission Number {.....}

EPA MRID Number 45405212

of this soil are similar to soils from the U.S. In this study, the test soil is referred to as a sandy loam soil.

7. The nominal application rate was reported to be equivalent to a field application rate of 750 g a.i./ha, based on equal distribution in the upper 5 cm soil layer and a soil density of 1.5 g/cm³ (p. 13). Generally, field application rates are expressed based on a surface depth of six inches.
8. Control samples were not employed in the study.
9. The temperatures at which the stored soil samples, extracts, and fractions were kept refrigerated or frozen prior to analysis were not reported.
10. Method detection limits for LSC and HPLC analysis were not reported. Limits of detection and quantitation should be reported to allow the reviewer to evaluate the adequacy of the test.
11. Representative HPLC/MS chromatograms presented in Figures 10-17 (pp. 37-45) indicated good separation of peaks.
12. An anaerobic metabolism study conducted using a German soil treated with pyridine [3-¹⁴C]-labeled BAS 510 F is currently under review by the Agency (MRID 45405211). The rate of dissipation of [¹⁴C]BAS 510 F was similar with the two treatments, decreasing from 96.4-100.9% of the applied at day 0 to 73-78% at 120 days posttreatment. Extractable [¹⁴C]residues for both treatments decreased from 97.3-100.9% of the applied at day 0 to 73.9-84.2% at 120 days. Other similarities between the treatments were the formation of soil bound residues (14.4-15.8% of the applied at 120 days), minimal amounts of volatilized ¹⁴CO₂ (≤0.4% of the applied), and no detected organic volatiles. The transformation product M51047F was detected in the [pyridine-3-¹⁴C]-labeled sample extracts (≤6.7% of the applied) but not in the [diphenyl-U-¹⁴C]-labeled sample extracts.
13. BAS 510 F chemical name 2-chloro-*N*-(4'-chlorobiphenyl-2-yl)-nicotinamide, as presented in the study report, was identified as the IUPAC name by the Compendium of Pesticide Common Names (<http://www.hclrss.demon.co.uk/nicobifen.html>). The CAS name 2-chloro-*N*-(4-chloro[1,1-biphenyl]-2-yl)-3-pyridinecarboxamide was also obtained from the Compendium of Pesticide Common Names. The following BAS 510 F synonyms were obtained from USEPA/OPP Chemical Databases (<http://www.cdpr.ca.gov/cgi-bin/epa/chemidetriris.pl?pccode=128008> and (http://www.cdpr.ca.gov/cgi-bin/mon/bycode.pl?p_chemcode=5790): 2-chloro-*N*-(4'-chlorobiphenyl-2-yl)-nicotinamide, nicobifen, and BAS 516 02 F.
14. Signed and dated Good Laboratory Practice Compliance, Quality Assurance, and Certificate of Authenticity statements were provided with the study.

ATTACHMENT 1
Data Critical to the Study Interpretation

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

MRID NO. 45405212

Page is not included in this copy.

Pages 11 through 16 are not included in this copy.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
- The product confidential statement of formula.
- Information about a pending registration action.
- X FIFRA registration data.
- The document is a duplicate of page(s) .
- The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

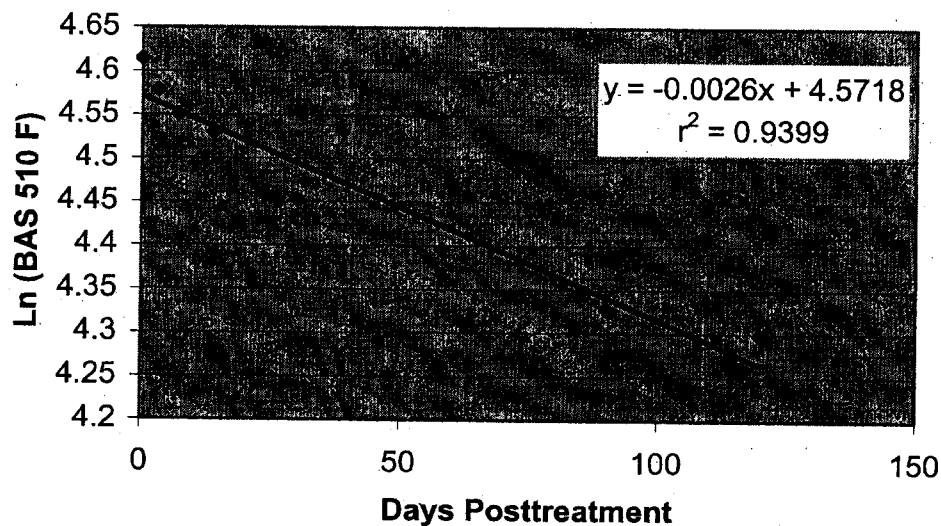
ATTACHMENT 2
Excel Workbook

Chemical Name BAS 510F
 PC Code 128008
 MRID 45405212
 Guideline No. 162-2
 Label Diphenyl

Half-life (days) = 266.5

Days Posttreatment	[¹⁴ C]BAS 510 F (Percent of Applied)	Ln ([¹⁴ C]BAS 510 F)
0	100.9	4.614129927
3	97.4	4.578826211
7	94.7	4.550714
14	92.8	4.53044664
30	85.9	4.453183829
58	81.1	4.395682961
90	75.8	4.328098293
120	73.6	4.298645026

Anaerobic metabolism of [¹⁴C]BAS 510 F (diphenyl label) in sandy loam soil



ATTACHMENT 3
Structure of Parent

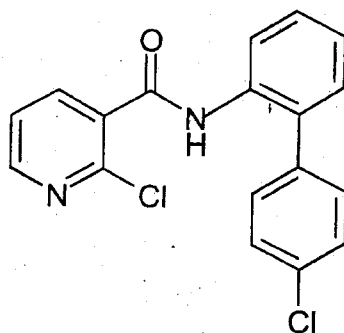
BAS 510 F

IUPAC name: 2-Chloro-*N*-(4-chlorobiphenyl-2-yl)-nicotinamide.

CAS name: 2-Chloro-*N*-(4-chloro[1,1'-biphenyl]-2-yl)-3-pyridinecarboxamide.

CAS No: 188425-85-6.

Synonyms: 2-Chloro-*N*-(4'-chlorobiphenyl-2-yl)-nicotinamide, Nicobifen. BAS 516 02 F.



[Diphenyl- $U-^{14}C$]-labeled BAS 510 F

