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

PMRA Submission Number {.....} EPA MRID Number 45405211

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

SMILES string:

Primary Reviewer: Mary Thomas
Dynamac Corporation

Signature:
Date:

QC Reviewer: Joan Harlin
Dynamac Corporation

Signature:
Date:

Secondary Reviewer: Cheryl Sutton, Ph.D.
EPA

Signature: Cheryl Sutton
Date: 11/21/02

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

CITATION: Staudenmaier, H. 2000. Anaerobic metabolism of BAS 510 F in soil (^{14}C-pyridine label). Unpublished study performed BASF Aktiengellsellschaft, Ecology and Environmental Analytics, Limburgerhof, Germany. Sponsored by BASF Corporation, Research Triangle Park,

NC. Study Code 41858; BASF Registration Document No. 2000/1014990. Study initiated July 27, 1998 and completed December 18, 2000 (p. 12).

ABSTRACT

Metabolism - Anaerobic Soil

The biotransformation of [pyridine-3-^{14}C]-labeled 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide (BAS 510 F) was studied in sandy loam soil (pH 7.5, organic carbon 1.7%) 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 -185 to -233 mV. [^{14}C]BAS 510F was applied at a nominal rate of 1.0 mg/kg (reported to be equivalent to 750 g a.i./ha based on a 5-cm depth and a bulk density of 1.5 g/cm^3). 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 CO_2 and volatile organics. Duplicate soil samples were collected after 0, 3, 7, 14, 30, 62, 90, and 120 days of anaerobic incubation; one sample was designated for immediate analysis and the remaining sample was frozen as a backup. The soil and floodwater water 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. [^{14}C]BAS 510 F and its transformation product M510F47 were separated by HPLC and identified by comparison to reference standards. The identification of the transformation product M510F47 (2-chloronicotinic acid) was confirmed by HPLC/MS.

Overall material balances were 96.7-102.8% of the applied during the study period, with no pattern of decline.

[^{14}C]BAS 510 F was 96.4% of the applied in the sample immediately posttreatment, 86.2% at 30 days, and 77.0-78.0% at 90 and 120 days posttreatment (final sampling interval). M51047F was a maximum 6.7% of the applied at 120 days posttreatment. A minor unidentified transformation product, designated "unknown" (retention time 34 min), was ≤0.8% of the applied throughout the study. Volatilized $^{14}CO_2$ totaled 0.4% of the applied at 120 days; organic volatiles were not detected during the study.

Total extractable [^{14}C]residues decreased from 97.3% of the applied at day 0 to 84.2% at 120 days posttreatment. Total nonextractable [^{14}C]residues slowly increased from 1.5% of the applied at day 0 to 14.4% at 120 days posttreatment. At 120 days, 4.3% of the applied was associated with the fulvic acid fraction, 3.9% with humic acid, and 7.5% with humin.

It was proposed that BAS 510F degrades to M510F47, and both parent and transformation product are degraded to CO_2 and are converted to bound soil residues.

The calculated half-life (linear regression assuming first-order kinetics) was 365 days (r^2 = 0.9086). 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 diphenyl-labeled parent compound yielded a half-life of 267 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 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 (68% sand, 17% silt, 15% clay, pH 7.5 [$CaCl_2$], organic carbon 1.7%, CEC 16 mVal/100 g dry soil; p. 39) collected in 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. 40). Each tube was equipped with inlet/outlet ports. The tubes were housed within an incubation chamber. The samples were incubated for 32 days (temperature not specified). At the end of the pre-incubation period, the redox potential of the soil averaged -204 mV (n = 16; -233 to -185 mV; Table 1, p. 22), indicating that anaerobic conditions were established.

The flooded soils were then treated with [pyridine-3-^{14}C]-labeled BAS 510 F (2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide; radiochemical purity >99%; specific activity 5.16 MBq/mg; Batch No: 640-2037; p. 12), dissolved in acetonitrile (~0.08% by volume; reviewer-calculated; 84.8 µL/100 g dry soil), at a nominal application rate of 1 mg/kg (reported to be equivalent to 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. 14). Duplicate soil samples were collected after 0, 3, 7, 14, 30, 62, 90, and 120 days of anaerobic incubation; one sample was designated for immediate analysis and the remaining sample was frozen as a backup. 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 collected organic volatiles and 0.5 M sodium hydroxide to trap CO_2

(pp. 14, 40). Volatile trapping solutions were collected and replaced with fresh trapping solution at each sampling interval. Control samples were not employed in the study.

The soil and water phases were not separated prior to analysis. On the day of sampling, 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: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 B)

BAS 510 F was identified by comparison to a radiolabeled reference standard. The procedure used to identify the metabolite M510F47 was not clear, but appears to have involved comparison to a reference standard. The identities of parent and metabolite M510F47 were confirmed using HPLC-MS/MS in the ESI mode (pp. 17, 45). The peak recovered from the non-adsorbed material from the SPE-C18 column was cleaned up on a Luna reverse-phase HPLC column, and the resulting material was methylated with diazomethane (p. 19). Both non-methylated and methylated metabolite M510F467 peaks were analyzed by HPLC-MS/MS.

Due to the highly variable material balances obtained for the original samples, the registrant analyzed the set of "spare" samples that was in frozen storage. The "spare" samples were analyzed as described for the original samples.

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 five 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 analyzed by LSC. The precipitate (humic acid) was redissolved in 0.5 M sodium hydroxide and analyzed by LSC. [^{14}C]Residues remaining in the extracted soil (humin) were calculated by subtracting radioactivity in the sodium hydroxide extract (humic acids) from the unextracted residues. For samples collected at 120 days, the fulvic acid fraction was subjected to solid phase partitioning. The samples were adjusted to pH 3.5-4 and passed through a C18/ENV+/SOLUTE SPE column; radioactivity adsorbed to the column was desorbed with methanol.

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

RESULTS/DISCUSSION

[Pyridine-3-^{14}C]-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 calculated half-life of 365 days (first-order kinetics, $r^2 = 0.91$) in anaerobic (flooding plus nitrogen atmosphere) sandy loam soil that was incubated in the dark at 20 ± 2°C, following 32 days of anaerobic (flooding) incubation. However, the half-life is of questionable value since it was determined beyond the scope of the data. Additionally, the soil and floodwater water were not separated prior to analysis and replicate data were not obtained. Based on HPLC analysis, BAS 510 F was 96.4% of the applied in the soil immediately posttreatment, 86.2% at 30 days, and 77.0-78.0% at 90 and 120 days posttreatment (Table 3, p. 24). The transformation product,

2-chloronicotinic acid (M510F47; p. 13),

was a maximum of 6.7% of the applied at 120 days posttreatment. A minor unidentified transformation product, designated "unknown," was ≤0.8% of the applied throughout the study.

Total extractable [^{14}C]residues decreased from 97.3% of the applied at day 0 to 84.2% at 120 days posttreatment (Table 2, p. 23). Total nonextractable [^{14}C]residues slowly increased from 1.5% of the applied at day 0 to 14.4% at 120 days posttreatment. At 120 days, 4.3% of the

applied was associated with the fulvic acid fraction, 3.9% with humic acid, and 7.5% with humin (Table 4, p. 24). Volatilized $^{14}CO_2$ totaled 0.4% of the applied at 120 days; organic volatiles were not detected during the study. Overall material balances were 96.7-102.8% of the applied during the study period, with no pattern of decline.

REVIEWER'S COMMENTS

1. The registrant submitted this study under USEPA Subdivision N Guideline 162-2, anaerobic soil metabolism. However, the experimental design of the study was inappropriate to meet this data requirement. In this study, the soil was flooded and incubated under a nitrogen atmosphere for 32 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. The study design indicated that only one sample was intended for analysis at each sampling interval. Two samples were prepared and collected at each sampling interval. However, only one sample was intended for analysis and the second sample was collected and placed in frozen storage in the event that there was a problem with the analysis of the first sample. Due to the high variability in material balances obtained for the original set of samples (82.7-107.2% of the total radioactive residues), the "spare" samples that were held in frozen storage were analyzed. Data obtained for the "spare" samples were used instead of the data for the original samples. It is preferred that at least two replicate samples be analyzed at each sampling interval to allow for statistical analysis and to quantify sample variability.

3. The treated soils were reported to have been incubated at 20 ± 2°C during the study. Supporting records were not included in the study report. In addition, supporting records confirming that anaerobic conditions were maintaining following treatment were not provided. Redox potentials were provided only for the day of treatment.

4. A DT50 value of 345 days ($r^2 = 0.91$) 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 13, p. 37). 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.

5. 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. 39). The physical, chemical, and microbiological properties 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.

6. The identity of metabolite M510F47 appears to have been confirmed by comparison to a reference standard of the metabolite. However, only radiolabeled parent compound was identified as a reference compound in the study report (p. 13).

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/cm^3 (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. 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.

10. The "spare" samples were stored frozen (temperature not specified) at intervals up to 115 days (ca. 3.8 months) prior to extraction. Storage stability data are required for samples stored for longer than 30 days.

11. Representative HPLC-MS/MS chromatograms presented in Figures 10-11 (pp. 34-35) indicated good separation of peaks.

12. An anaerobic metabolism study conducted using a German soil treated with diphenyl [U-^{14}C]BAS 510 F is currently under review by the Agency (MRID 45405212). The rate of degradation of [^{14}C]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 [^{14}C]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 $^{14}CO_2$ (≤0.4% of the applied), and no detected organic volatiles. The transformation product M51047F was detected in the [pyridine-3-^{14}C]-labeled sample extracts (≤6.7% of the applied), but not in [diphenyl-U-^{14}C]-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

RIN-0333-06

MRID No. 45405211

Page ____ is not included in this copy.

Pages 12 through 17 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.

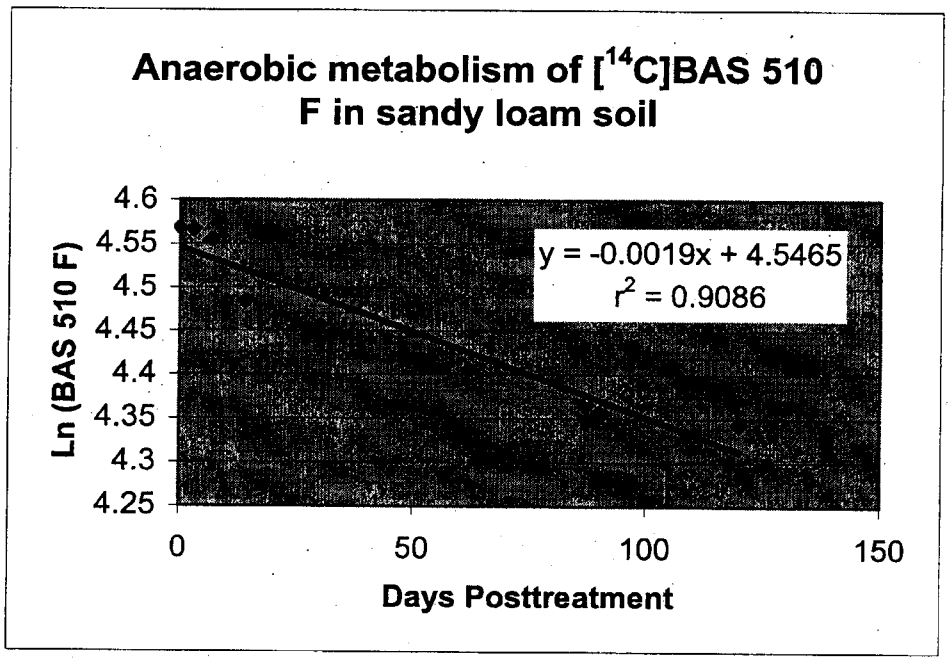
The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

ATTACHMENT 2
Excel Workbook

Chemical Name	BAS 510F
PC Code	128008
MRID	45405211
Guideline No.	162-2
Label	Pyridine

Half-life (days) = 365

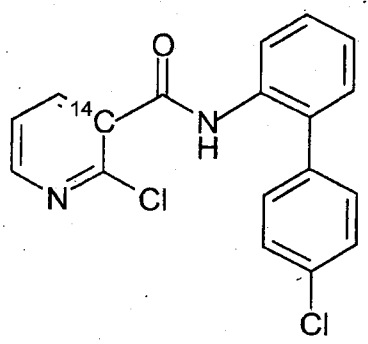
Days Posttreatment	[^{14}C]BAS 510 F (Percent of Applied)	Ln ([^{14}C]BAS 510 F)
0	96.4	4.568506202
3	96.2	4.566429358
7	95.1	4.55492897
14	88.6	4.484131858
30	86.2	4.456670178
62	81.9	4.405498991
90	78.0	4.356708827
120	77.0	4.343805422



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.



[Pyridine-3-^{14}C]-labeled BAS 510 F