Data Evaluation Report on the aerobic biotransformation of chloronicotinic acid in soil

PMRA Submission Number {.....} EPA MRID Number 45405209

Data Requirement: PMRA Data Code:
EPA DP Barcode: D278387
OECD Data Point:
EPA Guideline: 162-1

Test material:
Common name: Chloronicotinic acid
Chemical name
IUPAC: 2-Chloronicotinic acid
CAS name: 2-Chloronicotinic acid
CAS No: 2942-59-8 (p. 24)
Synonyms: Reg No. 107371
SMILES string:

Primary Reviewer: Joan Gaidos
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Company Code: [for PMRA]
Active Code: [for PMRA]
Use Site Category: [for PMRA]
EPA PC Code: 128008

CITATION: Ebert, D. and U. Harder. 2000. Degradation of ^{14}C-chloronicotinic acid in soil under aerobic conditions. Unpublished study performed by BASF Aktiengesellschaft, Ecology and Environmental Analytics, Limburgerhof, Germany, and submitted by BASF Corporation, Research Triangle Park, NC. BASF Registration Document Number 2000/1013280; Laboratory Project Identification 54518. Study initiated September 28, 1998 and completed August 9, 2000 (p. 10).

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EXECUTIVE SUMMARY

The biotransformation of radiolabeled [pyridine-3-^{14}C]-2-chloronicotinic acid was studied in a German sandy loam soil (pH 7.6, organic carbon 1.9%) for 14 days under aerobic conditions in darkness at 20 ± 1°C and a soil moisture content of 40% of the maximum water holding capacity. [^{14}C]Chloronicotinic acid was applied at a nominal rate of 0.250 mg a.i./kg soil. This experiment was conducted in accordance with SETAC Procedures for assessing the environmental fate and ecotoxicity of pesticides and in compliance with Good Laboratory Practice Regulations of the Federal Republic of Germany (1994). The test system consisted of glass dishes containing treated soil that were maintained in a flow-through apparatus with traps for the collection of CO_2 and volatile organics. Single treated samples were collected after 0, 1, 3, 7, and 14 days of incubation. Soil samples were sequentially extracted three times with methanol and three times with methanol:water (1:1, v:v). Extracts and extracted soil were analyzed for total radioactivity using LSC. Extracts and extracted soil were analyzed for total radioactivity using LSC. Soil extracts were analyzed by HPLC. The method used to identify the peak associated with 2-chloronicotinic acid was not described; no attempt was made to identify other peaks.

The overall material balance was 94.0 ± 5.4% of the applied, decreasing from 100.0% at day 0 to 88.2-88.8% by days 7 and 14 posttreatment. [Pyridine-3-^{14}C]-2-chloronicotinic acid decreased from 98.0% of the applied at day 0 to 68.1% at 3 days posttreatment, 13.0% at 7 days, and 0.4% at 14 days (final sampling interval). The calculated half-life (first-order linear regression) was 1.7 days; the DT50 (nonlinear) was 3.3 days.

Volatilized $^{14}CO_2$ was the only major transformation product detected, at 27.6% of the applied, and bound residues were high, comprising 55.5% of the applied at the final sampling interval. All other transformation products were minor and not characterized; extractable [^{14}C]residues other than chloronicotinic acid totaled ≤5.1% of the applied radioactivity at all sampling intervals. Organic volatiles were not detected at any sampling interval.

A biotransformation pathway was not proposed by the registrant. No transformation products were identified. Based on the study results, the final products of chloronicotinic acid are CO_2 and soil bound residues.

Results Synopsis:

Soil type: Sandy loam (German)
Half-life: 1.7 days (first-order linear regression; affected by a high level of bound residues)
DT50: 3.3 days (nonlinear)
DT90: 11.1 days (nonlinear)
Major transformation product: CO_2
Minor transformation products: Minor transformation products were not identified.

Study Acceptability: This study is scientifically valid and provides supplemental information on parent BAS 510 F only by providing information on the degradation of 2-chloronicotinic acid, a possible degradate of BAS 510 F.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with SETAC Procedures for assessing the environmental fate and ecotoxicity of pesticides. The only significant deviations from USEPA Subdivision N Guideline §162-1 are:

The study was conducted using 2-chloronicotinic acid, a proposed degradate of BAS 510 F. This does not affect the validity of the study.

The soil moisture content was 40% of the maximum water holding capacity, rather than the recommended 75% of field capacity at 1/3 bar. This does not affect the validity of the study.

COMPLIANCE: This study was conducted in compliance with the Good Laboratory Practice Regulations of the Federal Republic of Germany (1994). Signed and dated GLP, Data Confidentiality, Quality Assurance and Study Certification statements were provided (pp. 2-5).

A. MATERIALS:

1. Test Material [Pyridine-3-^{14}C]-labeled 2-Chloronicotinic acid

Chemical Structure:

Description: Not reported

Purity: [Pyridine-3-^{14}C] label: Radiochemical purity: >99% (p. 23)
Chemical purity: >98%
Batch No.: 640-2039
Specific activity: 680,000 dpm/µg (206,738 dpm/µg after dilution, p. 11)
Position of label: 3 carbon on the pyridine ring

Unlabeled: Chemical Purity: 99.7% (p. 24)

Lot No.: 01174-232

Storage conditions of test chemicals: In darkness at a low (unspecified) temperature (p. 23).

Table 1. Physico-chemical properties of chloronicotinic acid.

Parameter	Values	Comments
Molecular weight	157.55 g/mol	
Molecular Formula	$C_6H_4ClNO_2$	
Water solubility	Not reported.	
Vapor pressure/volatility	Not reported.	
UV absorption	Not reported.	
pK_a	Not reported.	
K_{ow}/log K_{ow}	Not reported.	
Stability of compound at room temperature	Not reported.	

Data obtained from p. 23 of the study report.

2. Soil Characteristics

Table 2: Description of soil collection and storage.

Description	Details
Geographic location	Limburgerhof, Germany (Bruch West)
Collection Date	Not reported. Soils received at the lab December 6, 1999.
Pesticide use history at the collection site	Not reported.
Collection procedures	Not reported.
Sampling depth (cm)	Not reported.
Storage conditions	Not reported.
Storage length	Not reported.
Soil preparation (eg: 2 mm sieved; air dried etc.)	2-mm sieved

Data obtained from pp. 12 and 26 of the study report.

Table 3: Properties of the soils.

Property	Details
Soil texture	Sandy loam
% sand	59.3
% silt	29.8
% clay	10.8
pH ($CaCl_2$)	7.6
Organic carbon (%)	1.9
CEC (meq/100 g)	18.7
Moisture at 1/3 atm (%)	Not reported.
Maximum Water Holding Capacity (g/100 g dry wt)	Not reported.
Bulk density (g/cm^3)	Not reported.
Soil Taxonomic classification	Not reported.
Soil Mapping Unit (for EPA)	Not reported.

Data obtained from p. 26 of the study report.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: No preliminary experiments were conducted.

2. Experimental conditions:

Table 4: Experimental design.

Parameter		Details
Duration of the test		14 days
Soil condition (Air dried/fresh)		Not reported.
Soil (g/replicate)		100 g dry wt
Application rate		0.254 mg a.i./kg dry soil (p. 18); 0.200 kg a.i./ha*
Control conditions, if used		No controls were used.
No. of Replication	Controls, if used	No controls were used.
	Treatments	Single samples were collected at each sampling interval.

Parameter		Details
Test apparatus (Type/material/volume)		Treated soil samples (100 g) were weighed into glass dishes (not described). The dishes were placed on metal trays (5 per tray), and the trays were placed in stainless steel incubation tubes. Each tube was equipped with inlet/outlet ports. The tubes were housed within an incubation chamber. The test apparatus is illustrated in Appendix 5, p. 27.
Details of traps for CO_2 and organic volatile, if any		Humidified CO_2-free air was drawn (flow rate not specified) through each incubation tube, then sequentially through 0.5 M sodium hydroxide, 0.5 M sulfuric acid, and ethylene glycol trapping solutions (50 mL/solution).
If no traps were used, is the system closed/open		Volatiles traps were used.
Co-solvent.	Identity:	Methanol.
	Final concentration:	~0.05%
Test material application	Volume of test solution used/treatment	985 µL of 0.450 mg/mL test solution per 1800 g soil (dry weight)
	Application method	The test solution was pipetted onto soil surface, then the soil was mixed. The mixing procedure was not described.
	Is the co-solvent evaporated?	No.
Microbial biomass/Microbial population of test soil		Initial: 31.1 mg C/100 g dry soil.
	Actinomycetes	Individual CFUs were not measured.
	Fungi	Individual CFUs were not measured.
	Bacteria	Individual CFUs were not measured.
Microbial biomass/microbial population of treated soil, if provided		Not determined.
Any indication of the test material adsorbing to the walls of the test apparatus		Not determined.
Experimental conditions	Temperature (°C)	20 ± 1°C
	Moisture content Moisture maintenance method:	40% of maximum water holding capacity. Water content of the soil was checked periodically by weighing the glass dishes and readjusted if necessary.

Parameter		Details
	Continuous darkness (Yes/No):	Yes.
Other details, if any		None.

Data obtained from pp. 11-12 and 26 of the study report.
* Field application rate equivalents determined by the study author assuming a soil depth of 5 cm and a bulk density of 1.5 g/cm^2 (p. 12).

3. Aerobic conditions: It was reported that the metal tubes containing the treated soil samples were purged with humidified CO_2-free air during the study (p. 12). No measurements were made to establish the oxygen content of the soils.

4. Supplementary experiments: No supplemental experiments were conducted.

5. Sampling:

Table 5: Sampling details.

Parameters	Details
Sampling intervals (days)	0, 1, 3, 7, and 14 days
Sampling method for soil samples	One dish (100 g soil) was collected at each interval; 50 g of each sample was extracted and 50 g was frozen.
Method of collection of CO_2 and volatile organic compounds	Trapping solutions were collected and replaced at each sampling interval (p. 18).
Sampling intervals/times for: Sterility check, if sterile controls are used: Moisture content: Redox potential/other:	 Sterile controls were not used. Not reported. Not determined.
Sample storage before analysis:	Soil samples were analyzed "as soon as possible" during the study, usually within a few days after generation. All samples and extracts were stored in refrigerator or freezer. (p. 15)
Other observations, if any	None

Data obtained from pp. 12, 13, and 15 of the study report.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: Each soil sample (50 g) was sequentially extracted three times with 100 mL of methanol and three times with 100 mL of methanol:water by shaking on a rotary shaker at room temperature (1:1, v:v; p. 12). Like extracts were combined

and aliquots were analyzed for total radioactivity by LSC. The remaining extracts were dried by rotary evaporation (<35°C), and the resulting residues were dissolved in methanol for HPLC analysis.

Nonextractable residue determination: Portions of the extracted soils were analyzed for total radioactivity using LSC following combustion (p. 13).

Volatile residue determination: The method for analyzing the trapping solutions was not reported. Aliquots of the NaOH trapping solutions were precipitated with barium chloride and the resulting supernatant analyzed using LSC; this suggests that the NaOH solution was analyzed using LSC prior to precipitation (p. 12). The other trapping solutions were analyzed because the study author reports that other volatiles were not detected (p. 16).

Total ^{14}C measurement: Prior to extraction, three subsamples of each soil sample were analyzed by LSC following combustion (p. 12). It is noted, however, that the total [^{14}C]residue data reported by the study author (Table 1, p. 18) are the sums of the residues in the soil extracts, extracted soil, and volatile trapping solutions.

Derivatization method, if used: A derivatization method was not used.

Identification and quantification of parent compound: Extracts were analyzed by HPLC with a Spherisorb ODS II column (250mm x 4.0 mm, 5 µm particle size). The mobile phase consisted of (A) water:formic acid (1000:2, v:v) and (B) acetonitrile:water:formic acid (950:50:2, v:v:v) [percent A:B at 0 min.100:0 (%), 20 min. 100:0, 35 min. 0:100, 45 min 100:0], injection volume not specified, flow rate 1 mL/minute, Kontron UV detector 430 and Berthold 507 A radiodetector (pp 13-14). It could not be determined if [^{14}C]chloronicotinic acid was identified by co-chromatography or R_f value.

Identification and quantification of transformation products: Transformation products were isolated by HPLC as described. There was no apparent attempt at identification.

Detection limits (LOD, LOQ) for the parent compound: LSC values of less than two-fold background levels were considered below the limit of accurate detection. The detection limits for HPLC were not reported.

Detection limits (LOD, LOQ) for the transformation products: The detection limits for HPLC were not reported.

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS: It was reported that aerobicity, moisture, temperature and other environmental conditions were maintained throughout the study. No supporting records were provided.

B. MATERIAL BALANCE: Overall recoveries of radiolabeled material averaged 94.0 ± 5.4% of applied radioactivity during the study (p. 18). Recoveries decreased with time, from 100.0% at day 0 to 95.4% at 3 days and 88.2-88.6% at 7 and 14 days.

Table 6: Biotransformation of ^{14}C-chloronicotinic acid, expressed as percentage of applied radioactivity (mean ± s.d), in sandy loam soil under aerobic conditions.*

Compound	Sampling times (days after treatment)				
	0	1	3	7	14
[^{14}C]Chloronicotinic acid	98.0	88.6	68.1	13.0	0.4
Others	N/R	N/R	N/R	5.1	4.7
Total extractable residues	98.0	89.0	68.1	18.1	5.1
Total volatile organics	---	ND	ND	ND	ND
CO_2	---	1.2	5.6	18.5	27.6
Nonextractable residues	2.0	7.9	21.7	52.0	55.5
Total % recovered	100.0	98.0	95.4	88.6	88.2

Data obtained from Tables 1 and 2, p. 18 of the study report.
* Only one sample was collected at each sampling interval.
ND - Not detected. No quantitative data for organic volatiles are reported; the space for this value was left blank in the MRID data table. The study author reported that other volatiles could not be detected (p. 16, paragraph 2).

C. TRANSFORMATION OF PARENT COMPOUND: [^{14}C]Chloronicotinic acid decreased from 98.0% at time 0 to 68.1% of the applied at 3 days posttreatment and 0.4% at 14 days (Table 2, p. 18).

HALF-LIFE: The half-life for BAS 510 F was determined by the reviewer using linear regression analysis based on first-order kinetics as calculated by Excel 2000. DT50 and DT 90 values were determined by the study author using a non-linear analysis based on multi-compartment models as calculated by ModelMaker v. 3 (pp. 14, 16, 22).

Table 7: Half-life and DT 50/DT90 values of BAS 510 F in aerobic sandy loam soil.

Soil type	First order Linear			DT50 (days)	DT90 (days)
	Half-life (days)	Regression equation	r^2		
Sandy loam	1.7	Linear form y = mx + b as $\ln C = -kt + \ln C_0$; $\ln C_0$ is initial concentration (b = y intercept), lnC is concentration at time t (y), k is the slope (m), t is time (x) or $kt = \ln C_0 - \ln C$. Half-life (t ½) = -(ln 2/k).	0.9727	3.3	11.1

[1]Half-life calculated using data obtained from data obtained from Table 2, p. 18. DT50 and DT90 data were calculated by the study author using a nonlinear model.

TRANSFORMATION PRODUCTS: HPLC chromatograms from 7 and 14 days show up to four unidentified peaks (pp. 19-21). There was no attempt made to identify transformation products in the soil extracts. The concentration of extractable compounds other than chloronicotinic acid totaled ≤5.1% of the applied (Table 2, p. 18).

NONEXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues decreased from 98.0% at 0 days posttreatment to 5.1% of the applied radioactivity at 14 days. Nonextractable [^{14}C]residues increased from 2.0% at time 0 to 55.5% of the applied radioactivity at 14 days (p. 16).

VOLATILIZATION: Volatilized $^{14}CO_2$ totaled 27.6% of the applied radioactivity at 14 days posttreatment (Table 1, p. 18). Other volatiles were not detected (p. 16).

TRANSFORMATION PATHWAY: No transformation pathway was proposed. No transformation products were identified. Based on the study results, the final products of chloronicotinic acid are CO_2 and soil bound residues.

Table 8: Chemical names and CAS numbers for the transformation products of ^{14}C-Chloronicotinic acid.

Applicant's Code Name	CAS Number	CAS and/or IUPAC Chemical Name(s)	Chemical formula	Molecular weight	SMILES string
		No transformation products were identified.			

D. SUPPLEMENTARY EXPERIMENT-RESULTS: Supplemental experiments were not performed.

III. STUDY DEFICIENCIES: This study was conducted in accordance with SETAC Procedures for assessing the environmental fate and ecotoxicity of pesticides. The soil moisture content was 40% of the maximum water holding capacity, rather than the recommended 75% of field capacity at 1/3 bar. The study author did not report either the maximum water holding capacity of the soil or the field capacity at 1/3 bar, so the precise relationship between the two values could not be calculated. Therefore, the soil contained more water and less oxygen in its pore space than recommended by Subdivision N Guidelines, which were intended to provide for sub-optimal conditions in the soil. However, this moisture level likely had only a minimal effect on the microbial metabolic rate and this deviation should not have had a significant impact on the study results. Since the study is scientifically valid and no other deviations from Subdivision N Guidelines were noted, this study can be used to provide supplemental information on BAS 510 F by providing information on the aerobic soil metabolism of the proposed degradate, chloronicotinic acid.

IV. REVIEWER'S COMMENTS:

1. In MRID 45405208, degradates of BAS 510 F were identified and a degradation pathway for the compound in aerobic soil was proposed. The reviewer notes that 2-chloronicotinic acid was not identified as a possible aerobic transformation product in that study.

2. The study author stated that the samples were "purged" with humidified air in order to collect volatiles. Purged usually describes a volatile trapping system in which air is drawn intermittently through the samples, often just before sample collection. However, the volatile trapping system illustrated in this study is sophisticated and appears to be designed for continuous aeration. It should be noted that several terms were used incorrectly in the study writeup. Whether continuous or intermittent, the collection system appears to have been adequate for trapping volatiles.

3. The method of analysis for the volatile trapping solutions was not reported. It was assumed that they were analyzed for total radioactivity using LSC.

4. Only one sample was collected for each treatment at each sampling interval. Replicate sampling is preferred, so that normal variability can be quantified and outliers can be identified.

5. For HPLC analyses, it was not specified how chloronicotinic acid was identified in the soil extracts. The registrant should specify whether chloronicotinic acid was identified by co-chromatography with a labeled reference standard and/or comparison to the retention time of an unlabeled reference standard, and provide supporting chromatograms.

6. The detection limits for HPLC were not reported.

7. The author stated that the water content of the soil was checked periodically, but did not specify the intervals.

8. The treated soils were reported to have been incubated at 20 ± 1°C during the study. Supporting data were not provided.

9. Oxidizer recovery was determined by carbon ^{14}C-standards and always exceeded 92%. Radioactivity measurements were corrected for oxidizer efficiency (p. 13, 14).

V. REFERENCES: The following reference was cited in the study.

1. Walker, A. and N. Crout. 1997. ModelMaker User Manual, version 3; Cherwell Scientific Publishing Limited, Oxford, U.K.

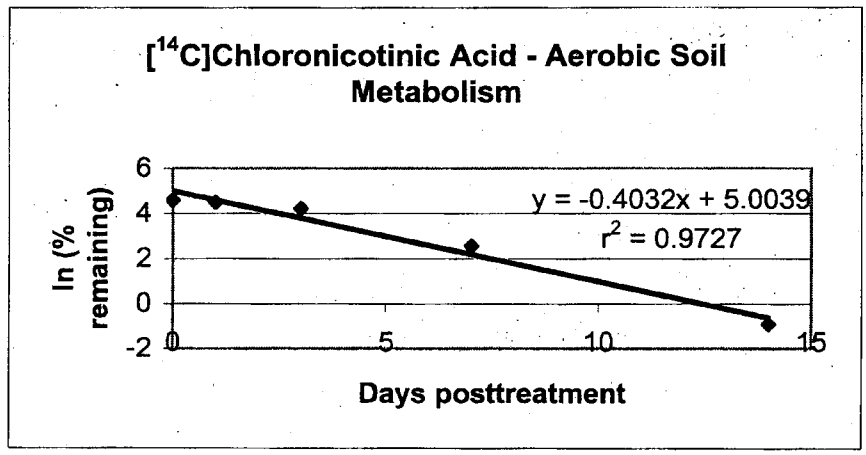
Attachment 1

Excel Spreadsheets

162-1, Aerobic biotransformation of chloronicotinic acid
PC Code 128008
MRID 45405209
Sandy loam soil

Half-life (days) =	1.72	DT90 (days) =	5.71

Days posttreatment	%remaining	ln(%remaining)
0	98	4.584967479
1	88.6	4.484131858
3	68.1	4.220977213
7	13	2.564949357
14	0.4	-0.916290732



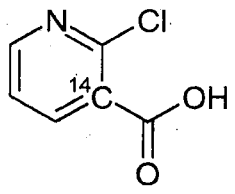
Constant	5.0039
r2	0.97
x (slope)	-0.4032

Attachment 2

Structure of Parent

[Pyridine-3-^{14}C]-labeled 2-chloronicotinic acid-

CAS No: 2942-59-8 (p. 24)
Synonyms: Reg No. 107371



Attachment 3

Diagram of Metabolism Apparatus

RIN-0333-06

MRID NO. 45405209

Page 18 is not included in this copy.

Pages ___ through ___ 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) _____.

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