Data Evaluation Report on the aerobic biotransformation of halcomid in soil

PMRA Submission Number {......} EPA MRID Number 45369736

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

Test material:

Common name:	Halcomid.
Chemical name	
IUPAC:	N,N-Dimethyldecanoic acid amide.
CAS name:	Not reported.
CAS No:	Not reported.
Synonyms:	Not reported.
SMILES string:	O=C(CCCCCCCCC)N(C)C.

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EXECUTIVE SUMMARY

The biotransformation of [1-^{14}C]N,N-dimethyldecanoic acid amide (halcomid) was studied in Hoefchen silt loam soil (pH 6.0, organic carbon 2.4%), Speyer 2.2 loamy sand soil (pH 6.6, organic carbon 2.29%), and Laacherhof silt loam soil (pH 8.1, organic carbon 0.9%) from Germany for 50 days under aerobic conditions in darkness at 20 ± 2°C and a soil moisture content of 40% of maximum water holding capacity. Halcomid was applied at a nominal rate of 0.8 mg a.i./kg soil, (equivalent to 600 g a.i./ha assuming incorporation to 5 cm depth and bulk density of 1.5 g/cm^3). This experiment was conducted in accordance with German Guidelines and in compliance with OECD, Swiss and USEPA GLP Standards. The test system consisted of glass metabolism flasks containing treated soil (100 g dry wt) that were incubated in a dark air-conditioned room. The flasks were connected to a flow-through volatile trapping system using humidified, CO_2-free air and consisting of two flasks of 2N NaOH and one flask of ethylene glycol. For the Speyer 2.2 and Laacherhof soils, single soil samples (duplicate at 4 and 16 days) were collected after 0, 1, 2, 8, and 50 days of incubation. For the Hoefchen soil, single samples were collected at 0, 1, 2, 4, 8, 50 days (duplicates at 16 days). Soil samples were extracted with acetonitrile, acetonitrile:water (1:1), and water until <5% of the applied was detected in the extract; details of the extraction procedure were not provided. The soil extracts, extracted soil, and volatile trapping materials were analyzed for total radioactivity using LSC. The soil extracts were analyzed for [^{14}C]halcomid and its transformation products by TLC; [^{14}C]compounds were identified by comparison to reference standards.

The test conditions outlined in the study protocol were reportedly maintained throughout the study; supporting data were not provided.

The overall recoveries of radiolabeled material throughout the study averaged 98.6 ± 5.2% (range 92.1-110.0%) of the applied in the Hoefchen silt loam soil, 98.3 ± 5.0% (range 94.3-111.3%) in the Speyer 2.2 loamy sand soil, and 100.0 ± 3.6% (range 96.5-108.0%) in the Laacherhof silt loam soil. None of the soil systems evidenced a loss of radioactive residues over time.

In Hoefchen silt loam soil, [^{14}C]halcomid decreased from 104.6% of the applied at 0 days posttreatment to 9.2% at 1 day, 1.6% at 8 days, and 0.9% at 50 days. The only major transformation product was N,N-dimethylsuccinic acid (M3; WAK 6747), which was a maximum of 26.8% of the applied at 1 day posttreatment, declined to 5.7% at 2 days, and was ≤0.1% at 8-50 days. The only identified minor transformation product was N,N-dimethylmalonic acid (M2; Acid of WAK 7034) at a maximum of 8.5% of the applied. [^{14}C]Residues remaining at the origin totaled a maximum of 7.5% of the applied at 1 day posttreatment and other unidentified regions of radioactivity were ≤2.5%. Extractable [^{14}C]residues decreased from 104.6% of the applied at 0 days posttreatment to 3.7% at 50 days, while nonextractable residues increased to a maximum of 12.2% at 2 days and were to 10.1% at 16-50 days. $^{14}CO_2$ accounted for 32.9% of the applied by 1 day posttreatment, 76.6% by 4 days, and was 83.7% by 50 days. Volatile organics totaled <0.1% of the applied at study termination.

In Speyer 2.2 loamy sand soil, [^{14}C]halcomid decreased from 108.5% of the applied at 0 days posttreatment to 8.7% at 1 day, 1.8% at 8 days, and 0.9% at 50 days. The major transformation products were M3 and M2. M3 was a maximum of 26.1% of the applied at 1 day posttreatment, declined to 1.3% at 2 days, and was ≤0.2% at 4-50 days. M2 was a maximum of 13.7% of the applied at 1 day, declined to 6.4% at 2 days, and was ≤0.6% at 4-50 days. No minor transformation products were identified. [^{14}C]Residues remaining at the origin totaled a maximum of 10.8% of the applied at 2 days posttreatment and other unidentified regions of radioactivity were ≤5.1%. Extractable [^{14}C]residues decreased from 108.5% of the applied at 0 days posttreatment to 3.2% at 50 days, while nonextractable residues increased to an average maximum of 11.2% at 4 days and were 9.4% at 50 days. $^{14}CO_2$ accounted for 30.1% of the applied by 1 day posttreatment, averaged 75.5% by 4 days, and was 84.9% by 50 days. Volatile organics totaled <0.1% of the applied at study termination.

In Laacherhof silt loam soil, [^{14}C]halcomid decreased from 104.6% of the applied at 0 days posttreatment to 15.2% at 1 day, 2.6% at 8 days, and 2.5% at 50 days. The only major transformation product was M3, which was a maximum of 61.4% of the applied at 1 day posttreatment, declined to 34.0% at 2 days, and was ≤1.1% at 4-50 days. The only identified minor transformation product was M2 at a maximum of 0.6% of the applied at 1 day posttreatment. [^{14}C]Residues remaining at the origin totaled a maximum of 6.6% of the applied at 4 days posttreatment and other unidentified regions of radioactivity were ≤0.8%. Extractable [^{14}C]residues decreased from 104.6% of the applied at 0 days posttreatment to 5.6% at 50 days, while nonextractable residues increased to 12.3% at 8 days and were 10.8% at 50 days. $^{14}CO_2$ accounted for 15.6% of the applied by 1 day posttreatment, averaged 72.5% by 4 days, and was 83.0% by 50 days. Volatile organics totaled <0.1% of the applied at study termination.

In all three soils, the observed DT50 was <1 day for halcomid and <1 day for its major transformation product N,N-dimethylsuccinic monoamide (M3). Precise half-lives could not be calculated because >80% of the applied halcomid degraded between the first and second samplings, and ≥*ca.* 50% of the measured N,N-dimethylsuccinic acid monoamide degraded between the day of measured maximum concentration and the next sampling interval.

A transformation pathway was proposed by the study author. Halcomid degraded to N,N-dimethylsebacic acid monoamide via oxidation of the decanoic acid. Degradation continued via fatty acid oxidation to the monoamides of the N,N-dimethylsuccinic acid and N,N-dimethylmalonic acid with the ultimate formation of CO_2.

Results Synopsis:

Soil type: Hoefchen Silt loam.
DT50: <1 day (observed).
Major transformation products:
  N,N-Dimethylsuccinic acid monoamide (M3; WAK 6747).
  CO_2.
Minor identified transformation products:
  N,N-Dimethylmalonic acid monoamide (M2; Acid of WAK 7034).

Soil type: Speyer 2.2 loamy sand loam.
DT50: <1 day (observed).
Major identified transformation products:
  N,N-Dimethylsuccinic acid monoamide (M3; WAK 6747).
  N,N-Dimethylmalonic acid monoamide (M2; Acid of WAK 7034).
  CO_2.
Minor identified transformation products:
  None.

Soil type: Laacherhof Silt loam.
DT50: <1 day (observed).
Major transformation products:
  N,N-Dimethylsuccinic acid monoamide (M3; WAK 6747).
  CO_2.
Minor identified transformation products:
  N,N-Dimethylmalonic acid monoamide (M2; Acid of WAK 7034).

Study Acceptability: This study is classified as **supplemental**. It is scientifically valid but does not meet the requirement for an aerobic soil metabolism study because the sampling intervals were inadequate to establish the pattern of decline for halcomid, >80% of the applied halcomid degraded between time 0 and the first sampling interval (day 1).

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with Richtlinie Teil IV, 4-1 BBA der Bundesrepublik Deutschland (December 1986, p. 15). The following significant deviation from USEPA Subdivision N Guideline §162-1 was noted.

> The sampling intervals were inadequate to accurately establish the degradation half-life of halcomid. Halcomid decreased from 104.6-108.5% of the applied at 0 days posttreatment to 8.7-15.2% at 1 day; it is preferred that no more that *ca.* 10% of the applied dissipate between successive sampling intervals. This does not affect the validity of the study.

COMPLIANCE: This study was conducted in compliance with OECD, Swiss, and USEPA GLP Standards (p. 15). Signed and dated Data Confidentiality, GLP, and Quality Assurance Statements were provided (pp. 2-7). A Certification of Authenticity was not provided.

A. MATERIALS:

1. Test Material: [1-^{14}C]Halcomid (p. 21).

Chemical Structure: See DER Attachment 2.

Description: Colorless liquid (p. 20).

Purity:
Radiochemical purity: 100.0% (p. 21).
Batch: A 387.
Analytical purity: Not reported.
Specific activity: 100.5 mCi/g (3.72 MBq/mg).
Location of the radiolabel: 1 Carbon (carbonyl carbon).

Storage conditions of test chemicals: The test material was stored in the dark in a freezer (p. 21).

Table 1: Physico-chemical properties of halcomid.

Parameter	Values	Comments
Molecular weight:	199.4 g/mol	
Molecular formula:	$C_{12}H_{25}NO$	
Water solubility:	270 mg/L	In water at 20°C and pH 5.5
Vapor pressure/volatility (Pa):	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. 20 of the study report.

2. Soil Characteristics:

Table 2: Description of soil collection and storage.

Description	Hoefchen	Speyer 2.2	Laacherhof
Geographic location:	Hoefchen, Germany	LUFA, Speyer, Germany	Laacherhof, Germany
Pesticide use history at the collection site:	Not reported.	None since arrival at test facility.	Not reported.
Collection date:	Not reported.	Obtained January 1991.	Not reported.
Collection procedures:	Not reported.		
Sampling depth:	Not reported.		
Storage conditions:	Not reported.	Stored in concrete cylinders outdoors.	Not reported.
Storage length:	Not reported.		
Preparation:	Sieved (2.0 mm).		

Data obtained from p. 18 of the study report.

Table 3: Properties of the soil.

Property			**Hoefchen**	**Speyer 2.2**	**Laacherhof**
Soil texture:[1]			Silt loam	Loamy sand	Silt loam
% sand (>0.063-2.0 mm):			2.5	82.0	35.9
% silt (0.002-0.063 mm):			81.9	13.0	52.9
% clay (<0.002 mm):			15.6	5.1	11.2
pH 0.1M $CaCl_2$: water:			5.8 6.0	6.2 6.6	7.3 8.1
Organic carbon (%)			2.4	2.29	0.9
CEC (meq/100 g):			10.0	9.7	10.0
Water holding capacity (%):			55.0	44.3	35.0
40% Maximum water holding capacity:			22.0	17.7	14.0
Bulk density (g/cm^3):			Not reported.		
Microbial biomass (mg C/100 g):	Start		52.3	36.9	30.1
	Completion	Untreated	54.5	39.7	24.4
		Treated	52.0	33.6	26.3
Soil Taxonomic classification:			Not reported.		
Soil Mapping Unit:			Not reported.		

Data obtained from Table 1, p. 40 of the study report.

[1] The registrant did not characterize the soils according to the USDA Soil Textural Classification system, in which sand particles are 0.05-2.0 mm in diameter, silt 0.002-0.05 mm, and clay <0.002 mm.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: No preliminary studies were described.

2. Experimental conditions:

Table 4: Study design.

Criteria		Hoefchen	Speyer 2.2	Laacherhof
Duration of the test		50 days.		
Soil condition (air dried/fresh):		Moist (not further specified).		
Soil (g/replicate)		100 g dry wt equivalent.		
Test concentrations (mg a.i./kg soil and equivalent kg a.i./ha):	Nominal:	0.80 mg a.i./kg dry soil, equivalent to 600 g a.i./ha assuming incorporation to 5 cm depth and bulk density of 1.5 g/cm^3.		
	Actual:	0.808 mg a.i./kg dry soil (606 g a.i./ha).		
Control conditions, if used		Sterile controls were not used.		
No. of Replications:	Controls	Sterile controls were not used.		
	Treatments	Single samples, except duplicate on days 4 and 16.		
Test apparatus (Type/material/volume):		Glass metabolism flasks (not further described) containing treated soil (100 g dry wt equivalent) were attached to an individual volatile trapping apparatus and incubated in a dark air-conditioned room. The test apparatus is illustrated in Scheme 1, p. 52.		
Details of traps for CO_2 and organic volatiles, if any:		Humidified, CO_1-free air was forced (30-60 mL/min) through a sample flask, then sequentially through two flasks of 2N NaOH and one flask of ethylene glycol trapping solution. The number of NaOH traps was reduced to one after 8 days posttreatment.		
If no traps were used, is the system closed/open?		Volatile traps were used.		
Identity and concentration of co-solvent:		Acetone (*ca.* 0.5% by volume, reviewer-calculated).		
Test material application:	Vol. of test solution used/treatment:	580 µL/100 g soil (dry wt. equivalent).		
	Application method:	The test substance was applied dropwise onto the soil using a Hamilton syringe, then the soil was mixed by shaking and the moisture content adjusted.		
	Co-solvent evaporated:	No.		
Any indication of the test material adsorbing to the walls of the test apparatus?		None.		
Microbial biomass of controls (mg C/kg):		Sterile controls were not used.		
Microbial biomass of the treated soil: (mg C/100 g)		Initial: 52.3 Final: 52.0 (treated) 54.5 (untreated)	Initial: 36.9 Final: 33.6 (treated) 39.7 (untreated)	Initial: 30.1 Final: 26.3 (treated) 24.4 (untreated)

Criteria		Hoefchen	Speyer 2.2	Laacherhof
Experimental conditions:	Temperature (°C):	20 ± 2°C		
	Moisture content:	40% of the maximum moisture holding capacity.		
	Moisture maintenance method:	The soils were weighed weekly and re-moistened with bidistilled water as needed.		
	Continuous darkness:	Yes		
Other details, if any:		None.		

Data obtained from pp. 16, 18, 19, 22, 23, Table 1, p. 40, Table 5, p. 44; Table 7, p. 46; Table 9, p. 48; Scheme 1, p. 52 of the study report.

3. Aerobic conditions: Humidified, CO_2-free air was continuously forced (30-60 mL/min) through the sample flasks (p. 23; Scheme 1, p. 52). No measurements such as redox potentials were made to verify that the soil remained aerobic throughout the study.

4. Supplementary experiments: To produce sufficient material to identify the radioactive fraction M2, eight additional flasks containing 106.2 g (dry weight) of Speyer 2.2 loamy sand soil were treated with halcomid (Batch No. A387/1; p. 21) at 97 mg/kg and incubated as described in the definitive study for 21.25 hours (p. 25). The soil was extracted sequentially with acetonitrile, acetonitrile:water (1:1, v:v), and water. The acetonitrile:water extracts were combined, concentrated, reconstituted in methanol and chromatographed using TLC (solvent system SS2). The relevant radioactive region was isolated, eluted from the silica with methanol and analyzed by GC/MS. M2 was derivatized with trimethyl anilinium hydroxide and analyzed by GC/MS with positive ion detection in the electron impact and the chemical ionization mode (p. 30). Methane was the ionization gas. The synthetic reference (WAK 7034) was already methylated (methyl ester of N,N-dimethylmalonic acid monoamide).

5. Sampling:

Table 5: Sampling details.

Criteria	Details
Sampling intervals (days):	0, 1, 2, 4, 8, 16, and 50 days.
Sampling method:	For Speyer 2.2 and Laacherhof soils: Duplicate flasks were collected at 4 and 16 days; single flasks were collected at 0, 1, 2, 8, and 50 days. For Hoefchen soil: Duplicate flasks were collected at 16 days; single flasks were collected at 0, 1, 2, 4, 8, and 50 days.
Method of collection of CO_2 and volatile organic compounds:	Trapping solutions were collected and replaced at each sampling interval or weekly, whichever was shorter, through 28 days. After 28 days, trapping solutions were collected at each sampling interval or every two weeks.
Sampling intervals/times for:	
Sterility check:	Sterile controls were not used.
Moisture content:	The moisture content was measured weekly.
Redox potential/other:	Not determined.
Sample storage before analysis:	Samples were extracted on the day of sampling. The sample extracts were generally analyzed by TLC on the day of extraction; if analysis was not conducted within 2-3 days, samples were stored at *ca.* -20°C for a few days.
Other observations, if any:	None.

Data obtained from pp. 23-25 of the study report.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: At sampling, the soil was extracted up to 2 times with acetonitrile, 1-4 times with acetonitrile:water (1:1, v:v), and 1 time with water; additional information about the extraction procedure (i.e., shaking, ambient temperature) was not provided (p. 25, Scheme 2, p. 53). Extractions with the same solvent were continued until <5% of the applied was recovered in the extract. The extracts were combined and aliquots were analyzed using LSC. The remaining extracts were concentrated using rotary evaporation and analyzed using TLC.

Nonextractable residue determination: Subsamples (*ca.* 1 g) of the dried extracted soils were analyzed for total radioactivity by LSC following combustion (p. 26).

Volatile residue determination: Aliquots (0.5 mL) of the trapping solutions were analyzed for total radioactivity using LSC (pp. 24, 26). The presence of $^{14}CO_2$ in the NaOH traps was confirmed using precipitation with barium hydroxide.

Total ^{14}C measurement: Total ^{14}C residues were determined by summing the concentration of residues measured in the soil extracts, extracted soil, and volatile traps (Table 7, p. 46).

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

Identification and quantification of parent compound: [^{14}C]Residues in the concentrated sample extracts were separated, quantified, and identified by one-dimensional TLC on silica gel plates (5 cm x 20 cm or 20 x 20 cm; 0.25 mm thickness; 60 F_{254}) developed in (SS2) chloroform:acetonitrile:acetic acid (50:50:2, v:v:v; p. 27). The samples were cochromatographed with an unlabeled reference standard of halcomid (Purity 98.8%; R_f 0.88; p. 20; Table 3, p. 42). The plates were visualized by exposure in an iodine chamber after being sprayed with a mixture of bromocresol green, bromocresol blue, potassium permanganate, and sodium carbonate; radioactive areas were located and quantified using a linear analyzer (pp. 27-28).

To confirm the results of the TLC analyses using SS2, sample extracts were also analyzed using reverse-phase one-dimensional TLC on RP-18 F_{254}S plates developed in (SS4) chloroform:methanol (98:2, v:v; p. 27). The RP-18 plates were visualized using the spray reagent followed by exposure to NH_3 vapors. Halcomid Rf 0.88 (Table 3, p. 42).

Confirmation of the TLC soil extract residue identification using HPLC was attempted; however, substantial losses of radioactivity from the samples during preparation were observed. HPLC was used to elucidate the purity of the [^{14}C] halcomid test material (pp. 28-29).

Identification and quantification of transformation products: Transformation products were quantified and identified using the methods described for the parent. Also, TLC zones M2 and M3 on the silica plates were scraped from the plates, eluted with methanol, and reanalyzed on the RP-18 plates using chloroform:methanol (50:50; v:v) as the developing solvent (p. 27). The reference standards used during TLC were:

- N,N-dimethyloctanoic acid amide (A; Rf 0.85 in SS2 and SS4, purity 97.0%);
- N,N-dimethylsuccinic acid monoamide (M3; Rf 0.30 in SS2, 0.34 in SS4, purity 92.8%);
- N,N-dimethylmalonic acid monoamide (M2; Rf 0.13 in SS2, 0.25 in SS4, purity 100%);
- decanoic acid (B; Rf 0.84 in SS2, 0.86 in SS4, purity >98%);
- pentanedioic acid (M; Rf 0.32 in SS2, 0.40 in SS4, purity >99%); and
- butanedioic acid (O; Rf 0.23 in SS2, 0.31 in SS4, purity >99%; Tables 2-3, pp. 41-42).

Detection limits (LOD, LOQ) for the parent compound: The Limits of Detection were 0.1% of the applied (0.8 µg/kg soil) for TLC and 0.5% (4.0 µg/kg) in HPLC (pp. 28, 29). The Limits of Quatification were not reported.

Detection limits (LOD, LOQ) for transformation products: The LODs were the same as for the parent. The LOQs were not reported.

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS: The aerobicity, soil moisture content, and other environmental conditions were reportedly maintained throughout the study; however, no supporting data were provided.

B. MATERIAL BALANCE: Total [^{14}C]residue recoveries averaged 98.6 ± 5.2% (range 92.1-110.0%) of the applied in the Hoefchen silt loam soil, 98.3 ± 5.0% (range 94.3-111.3%) in the Speyer 2.2 loamy sand soil, and 100.0 ± 3.6% (range 96.5-108.0%)in the Laacherhof silt loam soil (Table 5, p. 44, Table 7, p. 46, Table 9, p. 48). There was no pattern of loss of material with time in any of the soils.

Table 6a: Biotransformation of [^{14}C]halcomid in Hoefchen silt loam soil, expressed as percentage of applied radioactivity (mean ± s.d when n = 2), under aerobic conditions.

Compound	**Sampling times (days)**						
	0	1	2	4	8	16	50
Parent (Halcomid, Rf 0.88)	104.6	9.2	5.6	4.0	1.6	1.4 ± 0.3	0.9
Origin	<0.1	7.5	7.1	5.5	4.9	4.1 ± 0.3	2.8
M2 N,N-Dimethylmalonic acid monoamide (Acid of WAK 7034, Rf 0.13)	<0.1	8.5	4.6	0.6	<0.1	<0.1	<0.1
M3 N,N-Dimethylsuccinic acid monoamide (WAK 6747, Rf 0.30)	<0.1	26.8	5.7	0.6	0.1	<0.1	<0.1
Rest (other unidentified radioactivity)[1]	<0.1	2.5	2.2	0.3	0.3	<0.1	<0.1
Total extractable residues	104.6	54.5	25.2	11.0	6.9	5.5 ± 0.0	3.7
Nonextractable residues	5.4	11.4	12.2	11.9	10.9	10.1 ± 0.0	10.1
CO_2	—[2]	32.9	61.2	76.6	79.2	78.3 ± 2.5	83.7
Volatile organics	—[2]	<0.1					
Total % recovery	110.0	98.8	98.6	99.5	97.0	93.9 ± 2.5	97.5

Data obtained from Table 5, p. 44 of the study report. Means and standard deviations for day 16 (only interval with duplicate sampling) were calculated by the reviewer.

[1] Other unidentified radioactivity consisted of ≤3 unknown radioactive fractions ≤1.5% of the applied each, Figure 13A, p. 66.

[2] Volatiles were not collected from day 0 samples.

Table 6b: Biotransformation of [^{14}C]halcomid in Speyer 2.2 loamy sand soil, expressed as percentage of applied radioactivity (mean ± s.d when n = 2), under aerobic conditions.

Compound	**Sampling times (days)**						
	0	1	2	4	8	16	50
Parent (Halcomid, Rf 0.88)	108.5	8.7	4.0	3.9 ± 0.5	1.8	1.1 ± 0.1	0.9
Origin	<0.1	8.3	10.8[3]	5.5 ± 0.2	3.9	3.7 ± 0.1	2.1
M2 N,N-Dimethylmalonic acid monoamide (Acid of WAK 7034, Rf 0.13)	<0.1	13.7	6.4	0.6 ± 0.1	<0.1	<0.1	<0.1
M3 N,N-Dimethylsuccinic acid monoamide (WAK 6747, Rf 0.30)	<0.1	26.2	1.3	0.2 ± 0.0	<0.1	<0.1	<0.1
Rest (other unidentified radioactivity)[1]	<0.1	5.1	2.3	0.6 ± 0.1	0.1	<0.1	<0.1
Total extractable residues	108.5	62.0	24.8	10.6 ± 0.3	5.8	4.8 ± 0.1	3.2
Nonextractable residues	2.8	2.2	11.0	11.2 ± 0.3	10.5	10.6 ± 0.5	9.4
CO_2	—[2]	30.1	61.4	75.5 ± 0.4	81.6	80.9 ± 0.9	84.9
Volatile organics	—[2]	<0.1					
Total % recovery	111.3	94.3	97.2	97.3 ± 0.4	97.9	96.2 ± 0.4	97.5

Data obtained from Table 7, p. 46 of the study report. Means and standard deviations for days 4 and 16 (only intervals with duplicate sampling) were calculated by the reviewer.

[1] Other unidentified radioactivity consisted of ≤3 unknown radioactive fractions ≤2.5% of the applied each, Figure 16B, p. 69.

[2] Volatiles were not collected from day 0 samples.

[3] Quantification of the origin on day 2 was based on silica gel plates re-developed in a more polar solvent system using chloroform:methanol (50:50, v:v) and could be separated into at least two fractions. One fraction with 1.8% remained at origin, and at least one or more additional radioactive zones totaled 9.0% (p. 37; Figure 18B, p. 71).

Table 6c: Biotransformation of [^{14}C]halcomid in Laacherhof silt loam soil, expressed as percentage of applied radioactivity (mean ± s.d when **n** = 2), under aerobic conditions.

Compound	**Sampling times (days)**						
	0	1	2	4	8	16	50
Parent (Halcomid, Rf 0.88)	104.6	15.2	9.9	4.9 ± 1.1	2.6	3.2 ± 0.1	2.5
Origin	<0.1	6.0	5.5	6.6 ± 1.3	5.6	4.1 ± 0.4	2.7
M2 N,N-Dimethylmalonic acid monoamide Acid of WAK 7034, Rf 0.13)	<0.1	0.6	0.5	0.4 ± 0.1	<0.1	<0.1	<0.1
M3 N,N-Dimethylsuccinic acid monoamide (WAK 6747, Rf 0.30)	<0.1	61.4	34.0	1.1 ± 1.1	<0.1	<0.1	<0.1
Rest (other unidentified radioactivity)[1]	<0.1	<0.1	0.8	0.2 ± 0.1	0.1	<0.1	<0.1
Total extractable residues	104.6	83.2	50.7	13.1 ± 1.7	8.3	7.2 ± 0.3	5.6
Nonextractable residues	3.4	5.1	9.3	11.9 ± 0.8	12.3	12.1 ± 0.1	10.8
CO_2	—[2]	15.6	38.5	72.5 ± 2.4	77.2	79.4 ± 0.5	83.0
Volatile organics	—[2]	<0.1					
Total % recovery	108.0	103.9	98.5	97.6 ± 1.5	97.9	98.6 ± 0.1	99.4

Data obtained from Table 9, p. 48 of the study report. Means and standard deviations for days 4 and 16 (only intervals with duplicate sampling) were calculated by the reviewer.

1 Other unidentified radioactivity consisted of ≤2 unknown radioactive fractions.

2 Volatiles were not collected from day 0 samples.

C. TRANSFORMATION OF PARENT COMPOUND: In Hoefchen silt loam soil, [^{14}C]halcomid decreased from 104.6% of the applied at 0 days posttreatment to 9.2% at 1 day, 1.6% at 8 days, and 0.9% at 50 days (Table 5, p. 44).

In Speyer 2.2 loamy sand soil, [^{14}C]halcomid decreased from 108.5% of the applied at 0 days posttreatment to 8.7% at 1 day, 1.8% at 8 days, and 0.9% at 50 days (Table 7, p. 46).

In Laacherhof silt loam soil, [^{14}C]halcomid decreased from 104.6% of the applied at 0 days posttreatment to 15.2% at 1 day, 2.6% at 8 days, and 2.5% at 50 days (Table 9, p. 48).

HALF-LIVES: An accurate half-life for halcomid in the three soils could not be calculated because >80% of the applied halcomid dissipated between the first two sampling intervals. In all three soils, the observed DT50 is <1 day. The study author, using 1.5 and 2nd order kinetics models, calculated DT50 values of 0.02-0.27 days; DT90 values 0.65-2.46 (pp. 33-34, 37, Table 12, p. 51).

Half-life/DT50 values:

Soil	First order Linear			DT50 (days)	DT90 (days)
	Half-life	Regression equation	r^2		
Hoefchen silt loam	<1 day; could not be accurately calculated using the data provided			0.02	0.65
Speyer 2.2 sandy loam	<1 day; could not be accurately calculated using the data provided			0.04	1.14
Laacherhof silt loam	<1 day; could not be accurately calculated using the data provided			0.27	2.46

Registrant-calculated DT50/DT90 values obtained from Table 12, p. 51 of the study report.

TRANSFORMATION PRODUCTS: In the Hoefchen silt loam soil, the only major transformation product was N,N-dimethylsuccinic acid (M3; WAK 6747), which was a maximum of 26.8% of the applied at 1 day posttreatment, declined to 5.7% at 2 days, and was ≤0.1% at 8-50 days (Table 5, p. 44). The only identified minor transformation product was N,N-dimethylmalonic acid (M2; Acid of WAK 7034) at a maximum of 8.5% of the applied at 1 day posttreatment; it was 4.6% at 2 days and <0.6 at and after 4 days. [^{14}C]Residues remaining at the origin totaled a maximum of 7.5% of the applied at 1 day posttreatment and other unidentified regions of radioactivity were ≤2.5%.

In the Speyer 2.2 loamy sand soil, the major transformation products were M3 and M2 (Table 7, p. 46). M3 was a maximum of 26.1% of the applied at 1 day posttreatment, declined to 1.3% at 2 days, and was ≤0.2% at 4-50 days. M2 was a maximum of 13.7% of the applied at 1 day, declined to 6.4% at 2 days, and was ≤0.6% at 4-50 days. No minor transformation products were identified. [^{14}C]Residues remaining at the origin totaled a maximum of 10.8% of the applied at 2 days posttreatment and other unidentified regions of radioactivity were ≤5.1%.

In the Laacherhof silt loam soil, the only major transformation product was M3, which was a maximum of 61.4% of the applied at 1 day posttreatment, declined to 34.0% at 2 days, and was ≤1.1% at 4-50 days (Table 9, p. 48). The only identified minor transformation product was M2 at a maximum of 0.6% of the applied at 1 day posttreatment. [^{14}C]Residues remaining at the origin totaled a maximum of 6.6% of the applied at 4 days posttreatment and other unidentified regions of radioactivity were ≤0.8%.

NONEXTRACTABLE AND EXTRACTABLE RESIDUES: In Hoefchen silt loam soil, extractable [^{14}C]residues decreased from 104.6% of the applied at 0 days posttreatment to 3.7% at 50 days, while nonextractable residues increased to a maximum of 12.2% at 2 days and were to 10.1% at 16-50 days (Table 5, p. 44).

In Speyer 2.2 loamy sand soil, extractable [^{14}C]residues decreased from 108.5% of the applied at 0 days posttreatment to 3.2% at 50 days, while nonextractable residues increased to an average maximum of 11.2% at 4 days and were 9.4% at 50 days (Table 7, p. 46).

In Laacherhof silt loam soil, extractable [^{14}C]residues decreased from 104.6% of the applied at 0 days posttreatment to 5.6% at 50 days, while nonextractable residues increased to 12.3% at 8 days and were 10.8% at 50 days (Table 9, p. 48).

VOLATILIZATION: In the Hoefchen silt loam soil, $^{14}CO_2$ accounted for 32.9% of the applied by 1 day posttreatment, 76.6% by 4 days, and was 83.7% by 50 days (Table 5, p. 44). Volatile organics totaled <0.1% of the applied at study termination.

In the Speyer 2.2 loamy sand soil, $^{14}CO_2$ accounted for 30.1% of the applied by 1 day posttreatment, averaged 75.5% by 4 days, and was 84.9% by 50 days (Table 7, p. 46). Volatile organics totaled <0.1% of the applied at study termination.

In the Laacherhof silt loam soil, $^{14}CO_2$ accounted for 15.6% of the applied by 1 day posttreatment, averaged 72.5% by 4 days, and was 83.0% by 50 days (Table 9, p. 48). Volatile organics totaled <0.1% of the applied at study termination.

TRANSFORMATION PATHWAY: A transformation pathway was proposed by the study author (p. 38, Figure 30, p. 83). It was proposed that halcomid (N,N-dimethyldecanoic acid amide) degraded to N,N-dimethylsebacic acid monoamide, which was not identified in this study, via oxidation of the decanoic acid. N,N-dimethylsebacic acid monoamide degraded to N,N-dimethylsuccinic acid monoamide (M3) which in turn degraded to N,N-dimethylmalonic acid monoamide (M2) via fatty acid oxidation. The ultimate endproduct was CO_2.

Table 7: Chemical name for the transformation product of halcomid

Applicant's Code	CAS Number	Chemical Name	Chemical formula	Molecular weight (g/mol)	SMILES string
M3	--	N,N-dimethylsuccinic acid	$C_6H_{11}NO_3$	--	--
M2	--	N,N-dimethylmalonic acid	$C_5H_9NO_3$	--	--

Data obtained from Table 2, p. 41 of the study report.
-- Not reported.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: The M2 fraction was determined to be N,N-dimethylsuccinic acid monoamide by analysis using GC/MS (p. 37; Figure 29, p. 82).

III. STUDY DEFICIENCIES: The concentration of halcomid decreased from 104.6-108.5% of the applied at day 0 to 8.7-15.2% at day 1 (first sampling interval). Since no data are available for the period during which the majority of the degradation occurred, it is not possible to confidently calculate a precise half-life for halcomid. Similarly, in all three soils, the majority of the measured N,N-dimethylsuccinic acid monoamide (M3) degraded between day 1 (maximum concentration) and day 2. It is preferred that no more that 10% of the applied decrease between successive sampling intervals; several samplings should have been scheduled during 0 and 2 days posttreatment.

IV. REVIEWER'S COMMENTS:

1. The study author identified the solvents that were used to extract the soil, but did not described the extraction procedure. Based on the similarity to other studies in this data package, the reviewer believes that the soils were extracted with acetonitrile, acetonitrile:water, and water by shaking at ambient temperatures.

2. In the Speyer 2.2 loamy sand soil at 2 days posttreatment, radioactivity remaining at the origin totaled 10.8% of the applied (Table 7, p. 46). The study author stated that the extract was re-analyzed at a later time using chloroform:methanol (50:50, v:v; p. 37). In the second analysis, 1.8% of the applied remained at the Origin and 9.0% was an unknown peak (Figure 18B, p. 71). However, it was not demonstrated that the extracts remained stable during storage.

3. Following concentration of the extracts, losses of radioactivity were <10% (p. 25).

4. Only one sample was collected from each soil at most sampling intervals. Replicate sampling at each sampling interval is preferred, so that normal variability between replicate samples can be quantified and outliers identified.

5. The soils were maintained at 40% of maximum water holding capacity rather than at 75% of 1/3 bar as recommended in Subdivision N guidelines. Since the water holding capacity of

these soils at 1/3 bar was not reported, it could not be determined if the soils were wetter or drier than recommended. Since it is certain that the soils were not exceptionally dry or waterlogged, this is not expected to affect the outcome of the study.

6. Data supporting the reported temperature, soil moisture content, and soil aerobicity were not provided.

7. Physico-chemical properties such as vapor pressure and UV absorption were not reported.

8. The three soils were from Germany. The Hoefchen silt loam and Laacherhof silt loam were reportedly classified according to the USDA textural classifications, but could not be confirmed because the particle size scale ranges presented (DIN) differ from those used by the USDA soil classification system. According to DIN soil classification guidelines, particles in the range of 2.0-0.063 mm are categorized as sand, 0.063-0.002 mm as silt and <0.002 mm as clay. The Speyer 2.2 soil was classified according to the DIN Soil Textural Classification system. The soils were not formally compared to soils from the US. The physical properties of the soil appear to be typical of US soils.

9. The particle size distribution for the Speyer 2.2 loamy sand equaled 100.1% (Table 1, p. 40).

10. The field rate for the application of halcomid was reported as 600 g a.i./ha (equivalent to 0.8 mg a.i./kg dry soil assuming incorporation to 5 cm depth and bulk density of 1.5 g/cm^3; p. 23).

11. In MRID 45369735, the biotransformation of halcomid in a sandy loam soil from California was studied for 154 days under aerobic conditions in darkness at 20°C and a soil moisture content of 75% at 1/3 bar. [^{14}C]Halcomid decreased from an average of 101.0% of the applied immediately posttreatment to 20.9% at 1 day, 10.2-11.9% at 3-7 days, 4.4% at 28 days and 1.7% at 154 days. The only major transformation product was N,N-dimethylsuccinic acid monoamide (M3, WAK 6747), which averaged a maximum of 22.1% of the applied at 1 day posttreatment, decreased to 0.6% at 2 days, and was not detected at and after 3 days. The only identified minor transformation product was N,N-dimethylmalonic acid monoamide (M2; Acid of WAK 6747), which averaged a maximum of 2.2% of the applied at 1 day posttreatment. $^{14}CO_2$ averaged 33.5% of the applied by 1 day posttreatment, 70.6% by 3 days, and 83.3-83.5% at 77-154 days. Volatile organics totaled <0.1% of the applied at study termination.

V. REFERENCES:

1. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 162-1. Aerobic soil metabolism. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738-R-93-010.

Attachment 1

Excel Spreadsheets

Chemical: Halcomid
PC: 999999
MRID: 45369736
Guideline: 162-1
Soil: Hoefchen

Mass Balance

0	110.0
1	98.8
2	98.6
4	99.5
8	97.0
16	95.6
16	92.1
50	97.5
average	98.6
SD	5.2

Table 5, p. 44 of the study report.

Day	Halcomid	Origin	CO2	Recovery
16	1.6	3.9	80.0	95.6
16	1.2	4.3	76.5	92.1
average	1.4	4.1	78.3	93.9
SD	0.3	0.3	2.5	2.5

Chemical: Halcomid
PC: 999999
MRID: 45369736
Guideline: 162-1
Soil: Speyer 2.2

Mass Balance

0	111.3
1	94.3
2	97.2
4	97.6
4	97.0
8	97.9
16	95.9
16	96.4
50	97.5
average	98.3
SD	5.0

Table 7, p. 46 of the study report.

Day	Halcomid	Origin	M2	Extract	Non-extract	CO2	Recovery
4	4.2	5.3	0.6	10.8	11.0	75.8	97.6
4	3.5	5.6	0.5	10.4	11.4	75.2	97.0
average	3.9	5.5	0.6	10.6	11.2	75.5	97.3
SD	0.5	0.2	0.1	0.3	0.3	0.4	0.4
16	1.2	3.6		4.8	10.9	80.2	95.9
16	1.0	3.7		4.7	10.2	81.5	96.4
average	1.1	3.7		4.8	10.6	80.9	96.2
SD	0.1	0.1		0.1	0.5	0.9	0.4

Chemical: Halcomid
PC: 999999
MRID: 45369736
Guideline: 162-1
Soil: Laacherhof

Mass Balance

0	108.0
1	103.9
2	98.5
4	96.5
4	98.6
8	97.9
16	98.5
16	98.7
50	99.4
average	100.0
SD	3.6

Table 9, p. 48 of the study report.

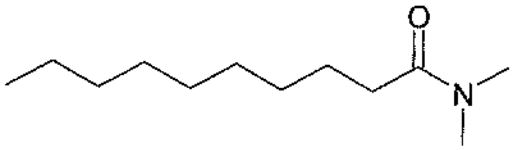
Day	Halcomid	Origin	M2	M3	Extract	Non-extract	CO2	Recovery
4	4.1	7.5	0.5	1.9	14.3	11.3	70.8	96.5
4	5.6	5.6	0.3	0.3	11.9	12.4	74.2	98.6
average	4.9	6.6	0.4	1.1	13.1	11.9	72.5	97.6
SD	1.1	1.3	0.1	1.1	1.7	0.8	2.4	1.5
16	3.1	4.3			7.4	12.1	79.0	98.5
16	3.2	3.8			7.0	12.0	79.7	98.7
average	3.2	4.1			7.2	12.1	79.4	98.6
SD	0.1	0.4			0.3	0.1	0.5	0.1

Attachment 2

Structures of Parent and Transformation Products

Halcomid

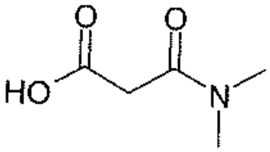
IUPAC name: N,N-Dimethyldecanoic acid amide.
CAS name: Not reported.
CAS No: Not reported.
SMILES string: O=C(CCCCCCCCC)N(C)C



Identified Compounds

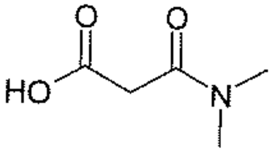
Acid of WAK 7034

IUPAC name:	Not reported.
CAS name:	N,N-dimethylmalonoic acid monoamide.
CAS No:	Not reported.



Acid of WAK 7034

IUPAC name:	Not reported.
CAS name:	N,N-dimethylmalonoic acid monoamide.
CAS No:	Not reported.



Unidentified Reference Compounds

N,N-Dimethyloctanoic acid amide

Structure not provided.

Decanoic acid

Structure not provided.

Pentanedioic acid

Structure not provided.

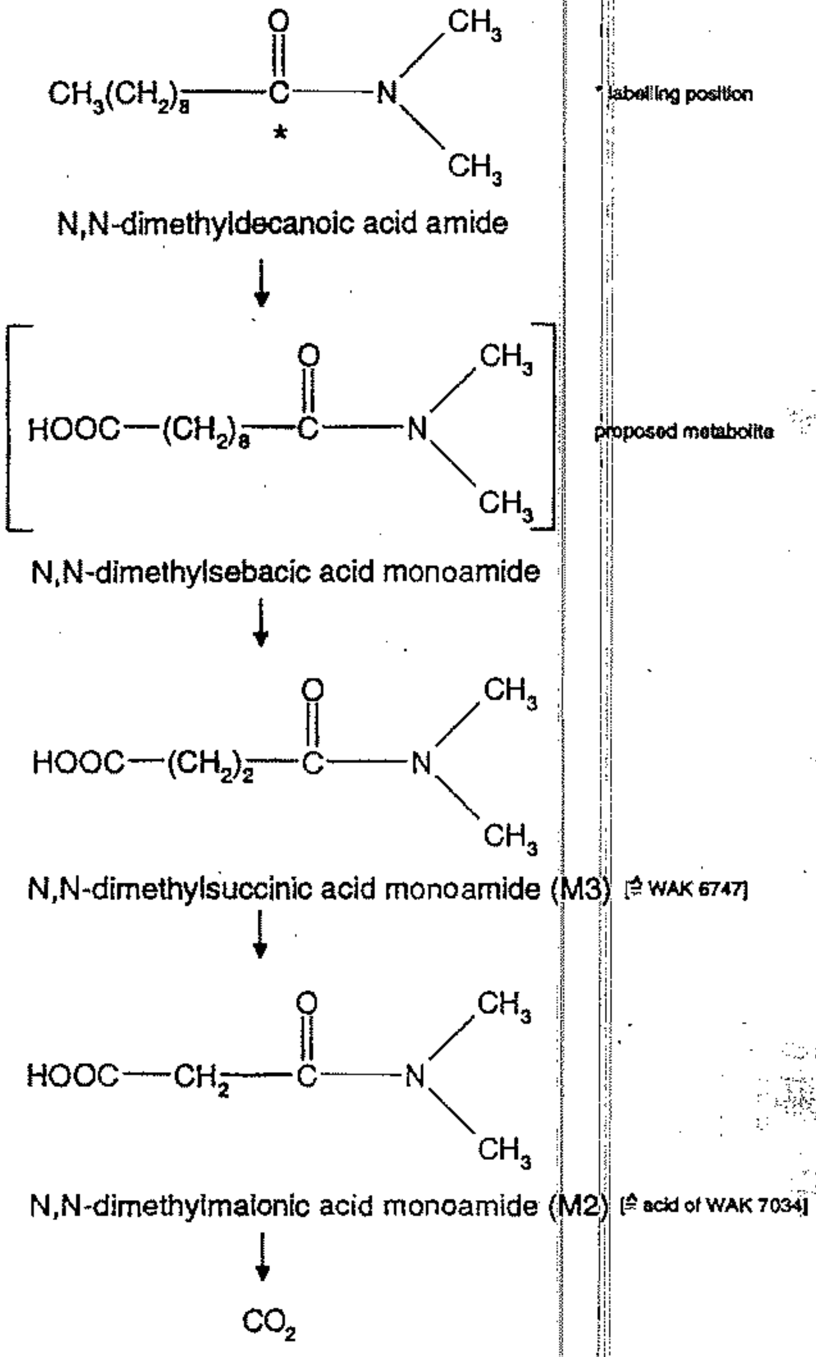
Butanedioic acid

Structure not provided.

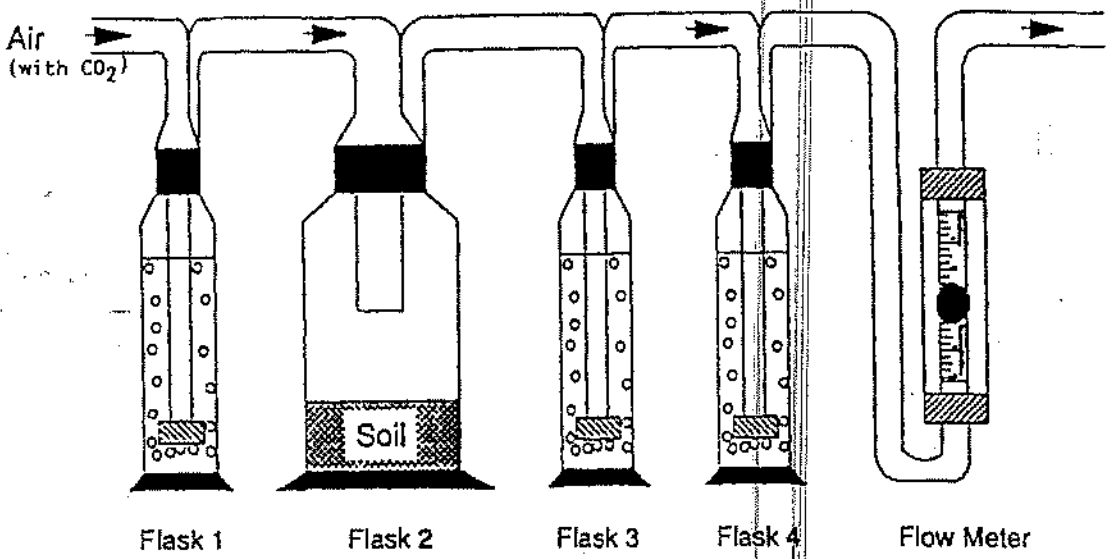
Attachment 3

Transformation Pathway Presented by Registrant
Illustration of Test System

Figure 30: Proposed scheme for the degradation pathway



Scheme 1: Soil degradation apparatus.



Each apparatus consisted of the following components:

1 Flask 1 containing bidistilled water to moisten the incoming air.

2 Flask 2 containing 100 g (dry weight basis) of the soil samples.

3 Flask 3 containing 50 ml of a 2N NaOH solution to trap CO_2 and $^{14}CO_2$ (only one of two flasks shown).

4 Flask 4 containing 50 ml of ethylene glycol to trap volatiles other than carbon dioxide.

5 A flow meter to control the air flow rate (not permanently installed).