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 HEALTH EFFECTS DIVISION
 SCIENTIFIC DATA REVIEWS
 EPA SERIES 361

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Dichlormid

Summary of Analytical Chemistry and Residue Data

Barcode: D318075

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
 PREVENTION, PESTICIDES
 AND TOXIC SUBSTANCES

MEMORANDUM

Date: September 14, 2005

Subject: Dichlormid. Petition for the Use On Corn. Additional Crop Field Trials and
 Confined Rotational Crop Study. Summary of Analytical Chemistry and Residue
 Data. Petition Number 4F6950

DP Number: D318075
 PC Code: 900497
 40 CFR 180.469

Decision Number: 357398
 MRID Numbers: 46353807, 46353808
 Chemical Class: Inert, Herbicide Safener

From: Debra Rate, PhD, Biologist
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Executive Summary

Dichlormid, N,N-diallyl dichloroacetamide, is a herbicide safener used in pesticide formulations with the active ingredient, acetochlor, for the control of grass and broadleaf weeds. Products containing dichlormid are conditionally registered in the U.S. to Dow AgroSciences, LLC under the trade names Surpass[®] EC, Keystone[®], TopNotch[®], Surpass[®] 20G, FulTime, Surpass[®] 7 E, and Keystone[®] LA. Currently, it is used in the treatment of corn (field, sweet and pop) raw agricultural commodities (RACs). Dichlormid is an emulsifiable concentrate that was prepared by blending dichlormid (98.0% purity X 12.04% of formulation) with acetochlor technical. The herbicide/safener formulations are typically applied as pre-emergence soil or early post-emergence foliar applications using broadcast ground equipment. The herbicide/safener may be applied both in the spring and fall, but the total applied must not exceed the maximum labeled rate for corn in that type of soil. The application must also be made within 14 days of planting when applied by conventional tillage systems and up to 40 days before planting in no-till systems. The application rate of dichlormid ranges from 0.30-0.54 lbs a.i./A.

The nature of the residue in corn, live stock and rotational crops has not been adequately described to determine the residues of concern. Residues > 10% of the total radioactive residues (TRR) have not been identified in many of these matrices. The data submitted by the registrant for metabolism of dichlormid in corn comprises two pathways of breakdown and suggests that the route of metabolism is the same for all of the matrices in corn. One pathway involves the dechlorination of the parent compound, dichlormid, to form an unknown intermediate (possibly N,N-diallylglyoxylamide) which is oxidized to form N,N-diallyl glycolamide. In the second route, dichlormid loses an allyl group, forming one or more unknown intermediates, one of which is oxidized to form dichloroacetic acid. Dichlormid residues are not detected above the limit of quantitation (LOQ) in the submitted crop field trials. An enforcement method of determining the level of dichlormid residues in field corn, grain and fodder was previously submitted and reviewed (MRID 427735, Memo, G. Kramer, 04/28/94). The registrant must submit the revised method to the Agency for review. The multiresidue method of testing for dichlormid residues used Protocols C, D, and E. Protocol C demonstrated that dichlormid could be detected by electron capture, nitrogen/phosphorous and electrolytic conductivity detectors. Method validation recoveries ranged from 79.2% for lettuce fortified at 0.1 ppm (Protocol D) to 41.4% (Protocol E) and 38.3% from soybean samples fortified with 0.1 ppm (Protocol E).

Dichlormid time-limited tolerances for field corn RACs including corn forage, stover, and grain; sweet corn RACs including corn, forage, stover, and grain; and pop corn RACs including grain and stover have been previously established at 0.05 ppm and will expire on December 31, 2005. No new tolerances are being requested at this time. However, the registrant is requesting that each of the time-limited tolerances of 0.05 ppm be converted into permanent tolerances for the field corn, sweet corn and pop corn RACs listed above. The residues recovered from the confined field trials for the parent dichlormid were greatly below the limit of quantitation (LOQ) ~0.01 ppm when used at a 1X usage rate. Decline studies for dichlormid were not submitted, due to the low levels of residue detected. Based on the submitted confined

rotational crop study (spring wheat, carrot, and soybean), the label crop rotation restriction interval for all crops should be established at one year, because residues >0.01 ppm were found at 30 DAA and 120 DAA in all three rotational crops. The data presented on dichlormid metabolites was not sufficient for establishing residues of concern beyond that of the parent. The data can be used for dietary risk assessment provided that it be updated when/if other metabolites of concern are identified.

Regulatory Recommendations and Residue Chemistry Deficiencies

For the purpose of establishing permanent tolerances for field corn (forage, grain, stover), sweet corn (forage, grain, stover) and pop corn (grain, stover) at 0.05 ppm, the studies provided are not adequate. For permanent tolerances to be set for the use of dichlormid, the following deficiencies need to be addressed:

1. 860.1300: Nature of the Residue - Plants

The studies submitted (MRID No. 46015801) for the purpose of fulfilling the Guideline 860.1300 is scientifically unacceptable and does not satisfy the requirements. It may be upgraded if additional metabolites are identified, including unknown A, to allow a more complete characterization of the nature of the residue of dichlormid in corn.

2. 860.1300: Nature of the Residue - Livestock

The studies submitted (MRID No. 46015802, 46015803) for the purpose of fulfilling the Guideline 860.1300 are scientifically unacceptable and do not satisfy the requirements. The studies do not adequately define the nature of the residues or the residue(s) of concern for dichlormid. They may be upgraded upon further identification of residues representing $\geq 10\%$ and/or 0.05 ppm.

3. 860.1340: Residue Analytical Methods

The requested revised method has not yet been received.

4. 860.1900: Field Accumulation in Rotational Crops

No studies in field accumulation in rotational crops have been submitted. **The registrant should submit a field accumulation in rotational crop study in accordance with OPPTS Guideline, 860.1900.**

There are no new tolerances requested in this petition, only the conversion of time-limited tolerances to permanent tolerances. Based on the submitted studies (MRID No. 46353807 and 46353808) and previously submitted data on corn, the TRB can only recommend for an extension to the current time-limited tolerances of 0.05 ppm for the use of dichlormid on field corn (forage, grain, stover), sweet corn (K+CWHR, forage and stover) and pop corn (grain, stover) pending completion of a human health risk assessment.

Dichlormid

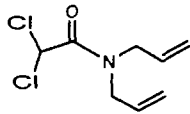
Summary of Analytical Chemistry and Residue Data

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Current tolerances that HED is supporting for the use of the herbicide safener, dichlormid, are the following:

<u>Commodity:</u>	<u>ppm</u>	<u>Expiration/ Revocation:</u>
Corn, field, forage.....	0.05	12/31/05
Corn, field, grain.....	0.05	12/31/05
Corn, field, stover.....	0.05	12/31/05
Corn, pop, grain.....	0.05	12/31/05
Corn, pop, stover.....	0.05	12/31/05
Corn, sweet, forage.....	0.05	12/31/05
Corn, sweet, grain.....	0.05	12/31/05
Corn, sweet, stover.....	0.05	12/31/05

Background

Compound	
Common name (proposed)	Dichlormid
Company experimental name	Dichlormid
IUPAC name	<i>N,N</i> -diallyl-2,2-dichloroacetamide
CAS name	2,2-dichloro- <i>N,N</i> -di-2-propenylacetamide
CAS #	37764-25-3
End-use product/EP	Surpass™ EC [62719-367], Keystone™ [62719-368]; TopNotch™ [62719-369]; Surpass™ 20G [62719-370]; FullTime™ [62719-371]; Surpass™ 7 E [62719-372], Keystone™ LA [62719-479].

Parameter	Value	Reference
Melting point/range	Not Available	
pH	6.9	MRID 42773501
Density	1.1963 g / ml	MRID 42773501
Water solubility	4388 mg / L at 25 °C	MRID 42773501
Vapor pressure	6.3 x 10 ⁻³ mm Hg at 25 °C	MRID 42773501
Dissociation constant, pK _a	Dichlormid is neither an acid nor a base, so the determination of a dissociation constant is waived.	MRID 42773501

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Parameter	Value			Reference
Octanol/water partition coefficient, Log(K _{ow})	pH	dissociation constant	Log(K _{ow})	MRID 42773501
	(unbuffered)	69	1.839	

860.1200 Directions for Use

Formulation [EPA Reg. No.]	Applic. Timing, Type, and Equip.	Applic. Rate: Dichlormid (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate: Dichlormid (lb ai/A)	PHI (days)	Use Directions and Limitations
Field Corn, Pop Corn, Sweet Corn and RACs¹						
Surpass™ EC [62719-367] Emulsifiable Concentrate	Pre-plant or pre-emergence Post-emergence up to 11"high Broadcast Ground equipment	0.216-0.54	1 - 2	0.54	within 30 days of planting: early pre-plant within 14 days of planting: pre-plant	Do not apply to following soils if ground water is below 30ft.: sand with <3% organic, loamy sand with <2% organic, sandy loam with <1% organic. Do not apply through irrigation or aerial.
Keystone™ [62719-368] Suspo-Emulsion	Pre-plant or pre-emergence Post-emergence up to 11"high Broadcast Ground equipment	0.275 - 0.43	1 - 2	0.43	within 30 days of planting: early pre-plant within 14 days of planting: pre-plant	Do not apply to following soils if ground water is below 30ft.: sand with <3% organic, loamy sand with <2% organic, sandy loam with <1% organic. Do not apply through irrigation or aerial.

Dichlormid

Summary of Analytical Chemistry and Residue Data

Barcode: D318075

Table 3. Summary of Directions for Use of Dichlormid.						
Formulation [EPA Reg. No.]	Applic. Timing, Type, and Equip.	Applic. Rate: Dichlormid (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate: Dichlormid (lb ai/A)	PHI (days)	Use Directions and Limitations
Field Corn, Pop Corn, Sweet Corn and RACs¹						
TopNotch™ [62719-369] Micro- Encapsulate	Pre-plant or pre- emergence Broadcast Ground equipment	0.26 - 0.43	1 - 2	0.43	within 40 days of planting: early pre- plant within 14 days of planting: pre-plant	Do not apply to following soils if ground water is below 30ft.: sand with <3% organic, loamy sand with <2% organic, sandy loam with <1% organic. Do not apply through irrigation or aerial.
Surpass™ 20G [62719-370] Granular	Pre-plant or pre- emergence Broadcast Ground equipment	0.3 - 0.52	1 - 2	0.52	within 30 days of planting: early pre- plant within 14 days of planting	Do not apply to following soils if ground water is below 30ft.: sand with <3% organic, loamy sand with <2% organic, sandy loam with <1% organic. Do not apply through irrigation or aerial.
FulTime™ [62719-371] Micro- Encapsulate	Pre-plant or pre- emergence Post- emergence up to 11"high Broadcast Ground equipment	0.25 - 0.51	1 - 2	0.51	within 40 days of planting: early pre- plant within 14 days of planting: pre-plant	Do not apply to following soils if ground water is below 30ft.: sand with <3% organic, loamy sand with <2% organic, sandy loam with <1% organic. Do not apply through irrigation or aerial.
Surpass™ 7 E [62719-372] Soluble Concentrate	Pre-plant or pre- emergence Post- emergence up to 11"high Broadcast Ground equipment	0.25 - 0.43	1 - 2	0.43	within 30 days of planting: early pre- plant within 14 days of planting: pre-plant	Do not apply to following soils if ground water is below 30ft.: sand with <3% organic, loamy sand with <2% organic, sandy loam with <1% organic. Do not apply through irrigation or aerial.

Dichlormid

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Table 3. Summary of Directions for Use of Dichlormid.						
Formulation [EPA Reg. No.]	Applic. Timing, Type, and Equip.	Applic. Rate: Dichlormid (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate: Dichlormid (lb ai/A)	PHI (days)	Use Directions and Limitations
Field Corn, Pop Corn, Sweet Corn and RACs¹						
Keystone™ LA [62719-479] Suspo- Emulsion	Pre-plant or pre- emergence Post- emergence up to 11" high Broadcast Ground equipment	0.20 - 0.49	1 - 2	0.49	within 30 days of planting: early pre- plant within 14 days of planting: pre-plant	Do not apply to following soils if ground water is below 30ft.: sand with <3% organic, loamy sand with <2% organic, sandy loam with <1% organic. Do not apply through irrigation or aerial.

¹RAC = Raw Agriculture Commodity

The proposed use directions are adequate.

860.1300 Nature of the Residue - Plants

MRID 46015801, D. Rate, 09/13/04.

A plant metabolism study was conducted using the herbicide safener dichlormid (N,N-diallyl-2,2-dichloroacetamide, 99.8% a.i., [¹⁴C]-labeled at the carbonyl carbon). Dichlormid, applied either pre-emergence (on soil) or post-emergence (foliarly) to corn at 10X the maximum agricultural use rate (5.60 kg a.i./ha), yielded sufficient TRRs in corn matrices (0.027-0.272 mg/kg) for analysis of residues by solvent extraction and TLC. Extractability of TRR was substantial for young forage (63.0%) and stover (53.1-53.8%) but was poor for grain and cobs (6.8-7.8%), and the latter were not further characterized. Identified components in young forage were dichlormid and the metabolites N,N-diallyl glycolamide and dichloroacetic acid (4.2%, 4.9%, and 2.5% TRR, respectively), but the rest of the TRR, including metabolite A (15.0% TRR; 0.16 mg/kg), was not identified. The stover contained dichloroacetic acid (5.3-5.9% TRR) and unknown metabolite A (14.0-16.6% TRR) and post-emergence stover also had low levels of dichlormid and N,N-diallyl glycolamide (0.9-1.2% TRR). Enzyme and acid hydrolysis of stover unextractable debris released residues that were not identified. The results indicate that dichlormid metabolism is qualitatively similar in all corn matrices and involves two routes: a de-chlorination followed by oxidation to form N,N-diallyl glycolamide, and loss of an allyl group followed by oxidation to form dichloroacetic acid.

The residue(s) of concern in corn for dichlormid were not defined with certainty in this study. The identified residues represent a small fraction (<12%) of the TRR in each matrix, and a metabolite (unknown A) that represented >10% TRR in stover and young forage was not identified. The relatively low residue levels in the 10X samples may have contributed to the low percent identification of residues in the various corn matrices. It is possible that the identified residues, which each represented ≤0.010 mg/kg at the 10X treatment rate (unknown A was

≤ 0.045 mg/kg), would not be detected in plants treated at the 1X treatment rate.

The plant metabolism data are classified as scientifically **Unacceptable/Guideline** and does not satisfy OPPTS 860.1300. However, the study may be upgraded if additional metabolites are identified, including unknown A, to allow a more complete characterization of the nature of the residue of dichlormid in corn.

It is notable that the corn matrices had low TRR (≤ 0.27 mg/kg), as did the individual components (≤ 0.010 mg/kg for identified residues; ≤ 0.045 mg/kg for unknown A) at the 10X treatment rate, and these may not be detectable at the 1X treatment rate. The study is acceptable for the purpose of extending time-limited tolerances.

860.1300 Nature of the Residue - Livestock

MRID 46015802, D. Rate, 09/13/04.

MRID 46015803, D. Rate, 09/13/04.

Lactating Goat:

In a goat metabolism study, the herbicide safener dichlormid (N,N-diallyl-2,2-dichloroacetamide, 99.8% a.i., [^{14}C]-labeled at the carbonyl carbon) (14.36-14.88 mg/day; 11.59 - 13.89 ppm) was given to one goat by gavage once/day for 5 days. The majority of each dose was excreted within 24 hours of administration; the total excreted radioactivity 23 hours after the last dose accounted for ~82% of the administered dose. The milk, liver, kidneys, and muscle each contained <1% of the administered radioactivity, the majority of which was solubilized by solvent extraction and enzyme hydrolysis. Only 0.6- 22.7% of the radioactive residues present in any given matrix were identified by HPLC, precluding a complete characterization of the nature of the residues, or residues of concern, of dichlormid in goat milk and tissues.

Dichlormid may be extensively metabolized in goats, as dichlormid parent was found in only a few samples (32 hour milk and liver PES supernatants), where it represented a lower % TRR (0.2-8.5%) than other components. Its metabolic pathway was not well-defined since only a small fraction (0.6-22.7%) of the TRR was identified in each matrix. Dichlormid metabolism is proposed to involve N-dealkylation and dechlorination followed by oxidation, since the metabolites N-allyl-2,2-dichloro-acetamide and N,N-diallyl glycolamide were found in almost all matrices (the latter was not found in muscle, which had TRR of only 0.056 ppm). It is unknown if the metabolic pathway was the same in all tissues because >77% of the TRR was not identified.

This goat metabolism study (MRID 46015802) did not provide sufficient information to establish the residues of concern for dichlormid in goat milk and tissues. This is concluded because only a small fraction (0.6-22.7%) of the TRR was identified in each matrix, and these identities were not confirmed by a second analytical method (as required by OPPTS 860.1300 guidelines). Total residue levels in the evaluated tissues and milk were low (<0.001 - 0.064 ppm), which likely contributed to the inability to identify some unknowns, although the parent and metabolites R 326590 and R 305588 were identified at similarly low levels (<0.001- 0.040 ppm).

The livestock metabolism data are classified as scientifically **Unacceptable/Guideline** for a metabolism study in ruminants (OPPTS 860.1300) because it did not adequately define the nature of the residues or the residue(s) of concern for dichlormid, and 84.4% is an inadequate animal mass balance accounting. It may be upgraded upon further identification of residues

representing $\geq 10\%$ TRR and/or 0.05 ppm, and an adequate explanation of the poor mass balance accounting. The study is acceptable for the purpose of extending time-limited tolerances.

Laying Hen:

In a hen metabolism study, the herbicide safener dichlormid (N,N-diallyl-2,2-dichloroacetamide, 99.8% a.i., [^{14}C]-labeled at the carbonyl carbon)(1.75 mg/day; 10 ppm) was given to 5 laying hens by gavage once/day for 14 days. Within 24 hours of administration, the majority of each dose was excreted and a steady state was achieved for excreted residues. Residues accumulated somewhat in egg whites and yolks, which attained steady state residue levels after 3 and 8 days, respectively. After 14 days, 96.91% of the TRR was accounted for, being found in the excreta (94.38% TRR), cage washes (1.14% TRR), egg yolks and whites, liver, breast and thigh muscle, fat, and skin with (latter each $<1\%$ of the administered radioactivity). Limited identification of residues was achieved for each matrix using two HPLC methods, varying from 0% TRR (fat) to 15.4% TRR (day 13 egg whites). This precluded a complete characterization of the nature of the residues, or residues of concern, of dichlormid in hen eggs and tissues. The residue identities were not confirmed by a second analytical method, as suggested by OPPTS 860.1300 guidelines, although an unsuccessful attempt was made to use LC-MS with several tissues. The parent dichlormid and/or metabolites R326590 and R305588 were identified at low levels (<0.001 - 0.077 ppm) in all matrices except fat, and could possibly be used to regulate dichlormid residue levels for tolerance purposes.

The finding of very little parent dichlormid in tissues ($\leq 1\%$ tissue TRR), at levels lower than of other identified and/or unknown components, indicates that it is extensively metabolized. The presence of metabolite R326590 (N-allyl-2,2-dichloro-acetamide) and R305588 (N,N-diallyl glycolamide) in most matrices, and of R336075 (N,N-diallyloxamic acid) and R327940 (N,N-diallylglyoxylamide) in egg yolks and thigh muscle, respectively, indicates that dichlormid metabolism involves N-dealkylation and dechlorination coupled with various degrees of oxidation. Because only a small fraction ($\leq 15.4\%$) of the TRR was identified in each matrix, however, the dichlormid metabolic pathway and similarities among tissues cannot be defined with certainty.

This metabolism study is classified **Unacceptable/Guideline** for a metabolism study in laying hens (OPPTS 860.1300) because it did not adequately define the nature of the residues or the residue(s) of concern for dichlormid (0-15.4% TRR was identified in each matrix). It is upgradeable upon further identification of residues representing $\geq 10\%$ TRR and/or 0.05 ppm. It may be possible to use levels of dichlormid, R326590, and/or R305588 to regulate residue levels in hen tissues for tolerance purposes, since one or more of these was found in all examined matrices except fat. The study is acceptable for the purpose of extending time-limited tolerances.

860.1340 Residue Analytical Methods

PP#:6F3344, DP Barcode: D248305, S. Chun, 09/21/99.

An enforcement method has been submitted for the determination of residues of dichlormid in field corn, grain, fodder, and forage. A petition method validation (PMV) was successfully completed with minor revisions recommended by the Analytical Chemistry Branch (ACB) (PP#: 6F03344, DP Barcode: D199320, G. Kramer, 08/29/94). The registrant was requested to submit standards of dichlormid to the EPA repository and submit a revised version

of the proposed analytical enforcement method. The Agency received a pure active ingredient (PAI) standard (dichlormid) for the EPA Repository from the registrant in August of 2003. **Until the receipt of the revised method, the requirements for analytical enforcement methodology will remain unfulfilled.** However, for the purposes of extending the time-limited tolerance, the method is adequate.

860.1360 Multiresidue Methods

PP#:6F3344, DP Barcode: D248305, S. Chun, 09/21/99.

A report on Multiresidue testing of dichlormid was received and forwarded to FDA (PP#: 6F03344, DP Barcode: D191195, G. Kramer, 9/16/93). Dichlormid was evaluated using multiresidue method Protocols C, D and E. Protocol C demonstrated dichlormid to be amenable to detection by electron capture, nitrogen/phosphorous and electrolytic conductivity detectors. The recovery from lettuce samples fortified at 0.1 ppm was 79.2% with Protocol D and 41.4% with Protocol E. The recovery from soybean samples fortified at 0.1 ppm was 38.3% with Protocol E.

860.1380 Storage Stability

PP#:6F3344, DP Barcode: D248305, S. Chun, 09/21/99.

Storage stability data were submitted for dichlormid in field corn ears (Accession# 005802). Twenty-five gram samples were fortified with 0.10 ppm of dichlormid. Samples were kept frozen at approximately $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ for up to three years. Periodic analyses of the samples were completed to determine if dichlormid deteriorated with time during frozen storage. Samples were analyzed at day 0 and after storage for 3, 8, 12, 24, and 36 months. At the 24 and 36 month intervals, newly fortified control samples were also analyzed to verify the accuracy of the analytical procedure. At each interval, 2 fortified samples and 1 unfortified sample were analyzed.

The samples were analyzed for dichlormid using analytical method RRC-83-64, "Determination of Residues of Cycloate, R29148, and R25788 in Corn Fodder and Corn Grain by Gas Chromatography". The results of this study are presented in Table 4.

Time Interval (days)	Corrected % Recovery^a
0	93
96	101
240	86
360	99
751	95
1095	73

^a Each value is the average of 2 individual determinations.

No dichlormid residues, < 0.05 ppm (LOQ), were detected in the control samples.

The study does not specify what different corn RACs were analyzed and uses the term "corn ears." The study does include storage stability data of dichlormid in wheat grain and straw. These data can be translated to corn RACs. Wheat samples were stored at intervals of 270, 818, and 1240 days. The wheat data are presented in Table 5.

RAC	Time Interval (days)	Corrected % Recovery ^a
Wheat Grain	0	93
	270	88
	818	96
	1240	100
Wheat Straw	0	93
	270	96
	818	96
	1240	87

^a Each value is the average of 2 individual determinations

The storage stability data are acceptable. The data shows dichlormid to be stable in corn and wheat for up to 3 years when stored frozen. HED concluded that storage stability had been demonstrated for the purposes of time-limited tolerances for dichlormid. If other residues are found to be of regulatory interest, storage stability studies for those residues will be required, as well.

860.1400 Water, Fish, and Irrigated Crops

Dichlormid is presently not registered, nor is the registrant seeking registration for direct use on water and aquatic food and feed crops; therefore, no residue chemistry data are required under these guideline topics.

860.1460 Food Handling

Dichlormid is presently not registered, nor is the registrant seeking registration for use in food-handling establishments; therefore, no residue chemistry data are required under these guideline topics.

860.1480 Meat, Milk, Poultry, and Eggs

MRID 46015802, D. Rate, 09/13/04.

PP# 3E6676, DP Barcode: D294741, D. Rate, 09/14/04

Currently, there are no registered direct animal treatments of dichlormid to livestock. However, dichlormid has time-limited tolerances for use on field corn, popcorn, silage corn and production seed corn, with a request for the addition of sweet corn, which contains animal feedstuffs. The tentative maximum theoretical dietary burdens (MTDB) of dichlormid to

livestock from the treatment of sweet corn are presented in Table 6. Based on the submitted study on lactating goats, the dichlormid residues found in animal tissues were between <0.001 - 0.023 ppm when treated at a level ~200X the proposed tolerance level. TRB does not expect quantifiable residues in animal commodities when fed corn treated by the proposed use of dichlormid, therefore dichlormid tolerances are not required on animal commodities.

Feedstuff (Sweet Corn)	Estimated Tolerance (ppm)	% Dry Matter	Beef Cattle		Dairy Cattle	
			% Diet ¹	Dietary Contribution (ppm) ²	% Diet ¹	Dietary Contribution (ppm) ²
forage	0.05	48	40	0.042	50	0.052
stover	0.05	83	25	0.015	15	0.009
cannery waste	0.05	30	35	0.058	20	0.033
Total Burden			100 ³	0.115	85 ³	0.094

¹ %Diet is the maximum % of a diet on a dry weight basis for finishing beef and lactating dairy cattle from OPPTS 860.1000 Table I.

² The amount of residues received by the animal from each source of feedstuff. ((Tolerance / %DM) X %Dietary Contribution).

³ The remainder of the diet will be composed of feedstuffs derived from crops that do not have dichlormid uses.

860.1500 Crop Field Trials

PP#:6F3344, DP Barcode: D248305, S. Chun, 09/21/99.

MRID 46353807, D. Rate, 08/25/05.

Corn field trial data were previously submitted and reviewed in support of the post-emergent use (PP#: 5F4505, DP Barcode: D214735, G. Herndon, 06/25/96) of acetochlor. A formulation, designated Acetochlor EC Herbicide, was used in the field trials. Eight field trials were conducted during the 1993 growing season in IA (Region 5), IL (Region 5), IN (Region 5), MN (Region 5), NE (Region 5), OH (Region 5), TX (Region 8), and WI (Region 5), 1 trial per state. Each treated plot received one post-emergence application of emulsifiable concentrate (EC) formulation when the corn plants had reached a height of 5-9" at an application rate of 3.0 lbs. acetochlor/A. The application rate of dichlormid was 0.5 lb. dichlormid/A. Table 7.1 summarizes this data. All field trials had residues below the LOQ (0.01 ppm).

TABLE 7.1. Summary of Residue Data From Previous Crop Field Trials with Dichlormid.

Crop Matrix	Total Applic. [Target] Rate ¹ (lb a.i./A)	PHI (days)	Residue Levels (ppm)						
			n ²	Min.	Max.	HAFT [*]	Median (STMdR)	Mean (STMdR) Std. Dev.	Std.Dev.
Dichlormid									
Forage	0.5	12-31	8	<0.01	<0.01	<0.01	<0.01	<0.01	0
Grain	0.5	104-131	8	<0.01	<0.01	<0.01	<0.01	<0.01	0
Stover	0.5	104-131	19	<0.01	<0.01	<0.01	<0.01	<0.01	0

¹This is the rate of application of dichlormid itself.

²Includes duplicate analysis of some samples.

*HAFT= Highest Average Field Trial.

The reviewed field trials were submitted prior to the current OPPTS Test Guidelines, Series 860. HED concluded that assuming residues are less than the LOQ, a total of 15 field trials should be conducted on field corn and analyzed for dichlormid in accordance with OPPTS Test Guideline 860.1500.

In response to the previous HED review, Pyxant Labs Inc. has submitted field trial data for dichlormid on field corn. Eight additional trials were conducted encompassing EPA Regions 1 (1, PA), 2 (1, VA), 5 (5, IA, IL, IN, OH, WI), and 6 (1, TX) during the 2002 growing season. The number and locations of field trials were chosen in order to satisfy an EPA request for an additional eight magnitude of residue trials to be conducted in EPA regions 1, 2, 5 and 6.

At each test location, treatment consisted of a single foliar application to field corn when it was 9-12 inches tall of dichlormid at the target rate of 0.48 lb a.i./A (0.54 kg a.i./ha). This mixture resulted from dissolving the GF-670 Capsule Suspension formulation of TopNotch Herbicide in water. An adjuvant was not added to the spray mixture in any applications. Forage, grain, and stover were harvested at pre-harvest intervals (PHIs) of 62-77, 102-135, and 102-135 days, respectively. Table 7.2 summarizes this data.

TABLE 7.2. Summary of Residue Data From Crop Field Trials with Dichlormid

Crop Matrix	Total Applic. [Target] Rate ¹ (lb a.i./A)	PHI (days)	Residue Levels (ppm)						
			n ²	Min.	Max.	HAFT [*]	Median (STMdR)	Mean (STMdR) Std. Dev.	Std.Dev.
Dichlormid									
Forage	0.48	62-77	19	<0.003	0.0046	<0.003	<0.003	<0.003	0
Grain	0.48	102-135	20	<0.003	0.0033	<0.003	<0.003	<0.003	0
Stover	0.48	102-135	19	<0.003	0.003	<0.003	<0.003	<0.003	0

¹This is the rate of application of dichlormid itself.

²Includes duplicate analysis of some samples.

*HAFT= Highest Average Field Trial.

Residues of dichlormid were quantified using Zeneca Agrochemical's analytical method RAM-244/02, which uses gas chromatography with nitrogen phosphorous detection. Satisfactory method performance in detecting residues was demonstrated by concurrent recoveries. The

results from these trials show that maximum residues in forage, grain, and stover never exceeded the method's LOQ, which is 0.01 ppm. The petitioner stated that a freezer storage study is in progress showing that dichlormid is stable for up to four months in all three matrices. Storage stability of dichlormid is adequate as shown in previously submitted data (PP#:6F3344, DP Barcode: D248305, S. Chun, 09/21/99). There was no residue decline study.

Though the submitted field trial data report residue levels <0.01 ppm, the enforcement method's LOQ is 0.05 ppm. Therefore, the appropriate tolerance level is 0.05 ppm for all corn RACs. **If other residues are found to be of regulatory interest, additional field trials will be required.**

The submitted studies reflect the use patterns for dichlormid, and the storage stability studies support the residue data. The enforcement methods are adequate for detecting the parent compound, dichlormid. However, because the metabolism studies were not adequate to determine residues present at levels >10% TRR, residues of concern in addition to the parent compound have not been identified. Based on the previously submitted data on corn, TRB can only recommend for an extension to the current time-limited tolerances of 0.05 ppm for the use of dichlormid on field corn (forage, grain, stover), sweet corn (K+CWHR, forage and stover) and pop corn (grain, stover).

860.1520 Processed Food and Feed

No processing studies are required for field corn.

860.1850 Confined Accumulation in Rotational Crops

MRID 46353807; D. Rate 08/25/05.

In a confined rotational crop study, sandy loam soil was sprayed with the herbicide safener dichlormid (N,N-diallyl-2,2-dichloroacetamide, 99.8% a.i., [¹⁴C]-labeled at the carbonyl carbon) prepared as an emulsifiable concentrate with the herbicide acetochlor. The single application was at the maximum seasonal application rate (1X) of 0.56 kg a.i./ha (0.5 lb/acre). At 30, 120, and 365 days after application (DAA), spring wheat, carrot, and soybean seeds were planted in the treated soil.

Samples were initially extracted with acetonitrile (ACN), ACN:water, and water, and soybean grain also with hexane, and the extracts characterized by HPLC. The polar extracts were subjected to solid phase extraction (SPE) and ACN extracts to acid hydrolysis at 95°C, followed by HPLC. Post-extraction solids (PES) were extracted with acid, some were partitioned with dichloromethane, and supernatants analyzed by HPLC. The remaining insolubles were resuspended and the radioactive residues shown to be incorporated into plant cell wall polysaccharides, starch, monosaccharides, proteins, and lignin by driselase, pullulanase and amyloglucosidase digestion, trichloroacetic acid precipitation, and base hydrolysis, respectively. The storage stability study of wheat hay and early forage, and soybean early forage (55-63 weeks at -20°C) indicated that dichlormid and its metabolites are stable frozen at -20°C for a year. Residues in soil were not evaluated, although a natural water sample from an environmental fate study with [¹⁴C]-dichlormid was analyzed by HPLC.

Total radioactive residues (TRR) at the 30, 120, and 365 DAA wheat samples were: 0.005-0.169 ppm in early forage, 0.017-0.639 ppm in hay, 0.014-0.629 ppm in straw, and 0.017-

Dichlormid

Summary of Analytical Chemistry and Residue Data

Barcode: D318075

0.295 ppm in grain. TRR in carrot shoots were 0.005-0.115 ppm and in 30 DAA roots were 0.038 ppm. TRR in soybean samples were: 0.005-0.122 ppm in early forage, 0.014-0.331 ppm in hay, 0.010-0.139 ppm in straw, and 0.019-0.039 ppm in grain. In every matrix, maximum residues occurred at 30 DAA, and TRR levels decreased as the DAA increased. TRR recoveries of extracts were acceptable for all matrices. Radioactive residues were also found in control matrices, which was likely due to incorporation of $^{14}\text{CO}_2$ released from dichlormid in the soil.

Dichlormid was extensively metabolized, as the parent was found in only wheat early forage and hay at low levels (0.01 ppm). Based on their partitioning behavior, most of the known and unknown metabolites from all three crops were polar. Wheat forage, hay, and/or straw metabolites included N,N-diallyl-2-hydroxyacetamide, N,N-di-2-propenylacetamide, N,N-diallyl glyoxylamide, 2-chloro-N,N-di-2-propenylacetamide, N-allyl-2,2-dichloroacetamide, N-allyl-2,2-glyoxylamide, and dichloroacetic acid (each 0.001-0.024 ppm, 0.3-3.7% TRR). Residues were also present in wheat hay and straw cell wall polysaccharides and lignin, and in starch and cell walls in grain. The 120 DAA carrot shoots contained N,N-di-2-propenylacetamide (0.001 ppm, 6.3% TRR) and radiolabeled glucose (0.001 ppm, 2.0% TRR) was detected in 30 DAA roots. Identified metabolites in 30 and/or 120 DAA soybean early forage, hay, and/or straw included N,N-diallyl glyoxylamide, N,N-diallyl-2-hydroxyacetamide, N-allyl-2,2-dichloroacetamide, and 2-chloro-N,N-di-2-propenylacetamide (each 0.001-0.013 ppm, 0.9-3.9% TRR). No dichlormid-related metabolites were identified in soybean grain. The environmental fate study water sample contained N,N-di-2-propenylacetamide, 2-chloro-N,N-di-2-propenylacetamide, and N,N-diallyl-2-hydroxyacetamide (59.7, 33.0 and 7.2% of the applied sample radioactivity, respectively).

Dichlormid metabolism in all rotational crops is proposed to involve two routes. In one, dichlormid is first de-chlorinated to form 2-chloro-N,N-di-2-propenylacetamide and N,N-di-2-propenylacetamide, and in the other, dichlormid first loses an allyl group to form N-allyl-2,2-dichloroacetamide. The final product of both routes is CO_2 , which can be re-assimilated into endogenous plant cell components. This study did not establish the residues of concern for dichlormid because 12.8% of the TRR was identified in each matrix, although the low levels of metabolites and the similarities in proposed metabolic pathway with a primary crop (corn, MRID 46015801) suggest that no new metabolites will be present at levels of concern in the rotational crops.

Based on this confined rotational crop study, the label crop rotation restriction interval for all crops is one year, because residues > 0.01 ppm were found at 30 DAA and 120 DAA in all three rotational crops.

860.1900 Field Accumulation in Rotational Crops

No studies in field accumulation in rotational crops have been submitted. **The registrant should submit a field accumulation in rotational crop study in accordance with OPPTS Guideline, 860.1900.**

860.1550 Proposed Tolerances

Tolerance expressions are set in terms of the parent compound only. Because no other data exists on the associated metabolites, the recommended time-limited tolerance is based only on the parent compound. Once additional data is submitted and reviewed, a complete tolerance expression can be established for the herbicide safener, dichlormid.

Currently there are no international harmonization issues associated with the use of dichlormid on corn.

Dichlormid

Summary of Analytical Chemistry and Residue Data

Barcode: D318075

Commodity	Established/Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments (correct commodity definition)
Corn, field, forage	0.05		Expires 12/31/05
Corn, field, grain	0.05		Expires 12/31/05
Corn, field, stover	0.05		Expires 12/31/05
Corn, pop, grain	0.05		Expires 12/31/05
Corn, pop, stover	0.05		Expires 12/31/05
Corn, sweet, forage	0.05		Expires 12/31/05
Corn, sweet, grain	0.05		Expires 12/31/05
Corn, sweet, stover	0.05		Expires 12/31/05

INTERNATIONAL RESIDUE LIMIT STATUS			
Chemical Name: 2,2'-dichloro-N,N'-di-2-propenylacetamide	Common Name: Dichlormid	<input checked="" type="checkbox"/> Proposed tolerance <input type="checkbox"/> Reevaluated tolerance <input type="checkbox"/> Other	Date: 08/10/05
Codex Status (Maximum Residue Limits)		U. S. Tolerances	
<input checked="" type="checkbox"/> No Codex proposal step 6 or above <input type="checkbox"/> No Codex proposal step 6 or above for the crops requested		Petition Number: 4F6950 DP Barcode: D318075 Other Identifier:	
Residue definition (step 8/CXL) N/A		Reviewer/Branch: Rate/TRB	
		Residue definition: Dichlormid	
Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm)
		corn, field	0.05
		corn, pop	0.05
		corn, sweet	0.05
Limits for Canada		Limits for Mexico	
<input checked="" type="checkbox"/> No Limits <input type="checkbox"/> No Limits for the crops requested		<input checked="" type="checkbox"/> No Limits <input type="checkbox"/> No Limits for the crops requested	
Residue definition:N/A		Residue definition:N/A	
Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)

Dichlorimid

Summary of Analytical Chemistry and Residue Data

Barcode: D318075

Notes/Special Instructions: S. Funk, 08/23/2005.			

Template Version November 2003

APPENDIX A:

Dichlormid

Summary of Analytical Chemistry and Residue Data

Barcode: D318075

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Dichlormid/900497/Dow AgroSciences/62719
 DACO 7.4.3/OPPTS 860.1850/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
 Confined Accumulation in Rotational Crops - Spring Wheat, Carrot, and Soybean

Primary Evaluator	Oak Ridge National Laboratory, Oak Ridge, TN	Date: 26/APR/2005
Peer Reviewer	Debra Rate, Biologist, TRB / RD <i>Debra M. Rate</i>	Date: 08/AUG/2005

This DER was originally prepared by Toxicology and Hazard Assessment Group, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831. The DER has been reviewed by TRB and revised to reflect current OPP policies.

STUDY REPORTS:

MRID No. 46353808. Chapleo, S., J. Gray, and K. Paterson (2003). The uptake and metabolism of [¹⁴C]-dichlormid in confined rotational crops. Inveresk Research, Tranent, Scotland. Laboratory Study ID Inveresk Number 802290, November 27, 2003 (original signature date) and December 9, 2003 (report amendment signature date), 536 pages.

EXECUTIVE SUMMARY:

In a confined rotational crop study, sandy loam soil was sprayed with the herbicide safener dichlormid (N,N-diallyl-2,2-dichloroacetamide, 99.8% a.i., [¹⁴C]-labeled at the carbonyl carbon) prepared as an emulsifiable concentrate with the herbicide acetochlor. The single application was at the maximum seasonal application rate (1X) of 0.56 kg ai/ha (0.5 lb/acre). At 30, 120, and 365 days after application (DAA), spring wheat, carrot, and soybean seeds were planted in the treated soil.

Samples were initially extracted with acetonitrile (ACN), ACN:water, and water, and soybean grain also with hexane, and the extracts characterized by high performance liquid chromatography (HPLC). The polar extracts were subjected to solid phase extraction (SPE) and ACN extracts to acid hydrolysis at 95°C, followed by HPLC. Post-extraction solids (PES) were extracted with acid, some were partitioned with dichloromethane, and supernatants analyzed by HPLC. The remaining insolubles were resuspended and the radioactive residues shown to be incorporated into plant cell wall polysaccharides, starch, monosaccharides, proteins, and lignin by driselase, pullulanase and amyloglucosidase digestion, trichloroacetic acid precipitation, and base hydrolysis, respectively. The storage stability study of wheat hay and early forage, and soybean early forage (55-63 weeks at -20°C) indicated that dichlormid and its metabolites are stable frozen at -20°C for a year. Residues in soil were not evaluated, although a natural water sample from an environmental fate study with [¹⁴C]-dichlormid was analyzed by HPLC.

Total radioactive residues (TRR) at the 30, 120, and 365 DAA wheat samples were: 0.005-0.169 ppm in early forage, 0.017-0.639 ppm in hay, 0.014-0.629 ppm in straw, and 0.017-0.295 ppm in grain. TRR in carrot shoots were 0.005-0.115 ppm and in 30 DAA roots were 0.038 ppm. TRR in soybean samples were: 0.005-0.122 ppm in early forage, 0.014-0.331 ppm in hay, 0.010-0.139 ppm in straw, and 0.019-0.039 ppm in grain. In every matrix, maximum residues occurred at 30 DAA, and TRR levels decreased as the DAA increased. TRR recoveries of extracts were acceptable for all matrices. Radioactive residues were also found in control matrices, which was likely due to incorporation of ¹⁴CO₂ released from dichlormid in the soil.

Dichlormid was extensively metabolized, as the parent was found in only wheat early forage and hay at low levels (≤0.01 ppm). Based on their partitioning behavior, most of the known and unknown metabolites from all three crops were polar. Wheat forage, hay, and/or straw



Dichlormid/90497/Dow AgroSciences/62719

DACO 7.4.3/OPPTS 860.1850/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6

Confined Accumulation in Rotational Crops - Spring Wheat, Carrot, and Soybean

metabolites included N,N-diallyl-2-hydroxyacetamide, N,N-di-2-propenylacetamide, N,N-diallyl glyoxylamide, 2-chloro-N,N-di-2-propenylacetamide, N-allyl-2,2-dichloroacetamide, N-allyl-2,2-glyoxylamide, and dichloroacetic acid (each 0.001-0.024 ppm, 0.3-3.7% TRR). Residues were also present in wheat hay and straw cell wall polysaccharides and lignin, and in starch and cell walls in grain. The 120 DAA carrot shoots contained N,N-di-2-propenylacetamide (0.001 ppm, 6.3% TRR) and radio labeled glucose (0.001 ppm, 2.0% TRR) was detected in 30 DAA roots. Identified metabolites in 30 and/or 120 DAA soybean early forage, hay, and/or straw included N,N-diallyl glyoxylamide, N,N-diallyl-2-hydroxyacetamide, N-allyl-2,2-dichloroacetamide, and 2-chloro-N,N-di-2-propenylacetamide (each 0.001-0.013 ppm, 0.9-3.9% TRR). No dichlormid-related metabolites were identified in soybean grain. The environmental fate study water sample contained N,N-di-2-propenylacetamide, 2-chloro-N,N-di-2-propenylacetamide, and N,N-diallyl-2-hydroxyacetamide (59.7, 33.0 and 7.2% of the applied sample radioactivity, respectively).

Dichlormid metabolism in all rotational crops is proposed to involve two routes. In one, dichlormid is first de-chlorinated to form 2-chloro-N,N-di-2-propenylacetamide and N,N-di-2-propenylacetamide, and in the other, dichlormid first loses an allyl group to form N-allyl-2,2-dichloroacetamide. The final product of both routes is CO₂, which can be re-assimilated into endogenous plant cell components.

STUDY ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the confined rotational crop residue data are classified as scientifically acceptable and satisfies OPPTS 860.1850 guideline requirements for a confined rotational crop study. No major deficiencies were identified that would invalidate the study results. Several typographic errors were found in naming metabolites. One concern is the finding of radioactive CO₂ in controls, although the description of the test system explains the reason this occurred. It would have been helpful if dichlormid metabolites were subjected to a confirmatory analytical method (i.e., in addition to HPLC).

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D318075 and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.



Dichlormid/900497/Dow AgroSciences/62719
 DACO 7.4.3/OPPTS 860.1850/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
 Confined Accumulation in Rotational Crops - Spring Wheat, Carrot, and Soybean

A. BACKGROUND INFORMATION:

The subject compound dichlormid (N, N-diallyl-2, and 2-dichloroacetamide) is a safener present as a safener in various herbicide formulations containing the active ingredient acetochlor produced by Dow AgroSciences. As a safener, it functions by protecting the crop from cytotoxicity caused by the a.i. End-use products containing dichlormid and acetochlor are listed in Table A.1.

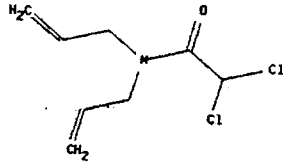
TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure 
Common name	Dichlormid
Company experimental name	Dichlormid, CGA-185072
IUPAC name	N,N-diallyl-2,2-dichloroacetamide
CAS name	2,2-dichloro-N,N-di-2-propenylacetamide
CAS #	37764-25-3
End-use product/(EP)	'Surpass' (+ acetochlor); 'Trophy' (+ acetochlor); 'Topnotch' (+ acetochlor); 'Trophée' (+ acetochlor) (all from Dow AgroSciences)

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound		
Parameter	Value	Reference
Melting point/range	5.0-6.5 °C (95% pure technical)	http://www.agrochemchina.com/dichlormid.htm
pH	6.9	MRID No. 42773501
Density (Specific gravity)	1.202 (20 °C)	http://www.agrochemchina.com/dichlormid.htm
Water solubility (20°C)	5 g/L	http://www.agrochemchina.com/dichlormid.htm
Solvent solubility (mg/L at not stated °C)	15 g/L in kerosene; miscible with acetone, ethanol, and xylene	http://www.agrochemchina.com/dichlormid.htm
Vapour pressure at 25°C	800 mPa	http://www.agrochemchina.com/dichlormid.htm
Dissociation constant (pK _a)	not found	
Octanol/water partition coefficient Log(K _{ow})	1.84 ± 0.7 (25 °C)	http://www.agrochemchina.com/dichlormid.htm
UV/visible absorption spectrum	not found	

B. EXPERIMENTAL DESIGN:

B.1. Test Site and Crop Information:

TABLE B.1.1. Test Site Information



Dichlormid/90497/Dow AgroSciences/62719
 DACO 7.4.3/OPPTS 860.1850/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
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Testing Environment and location	Soil characteristics						
	Type	% Sand	% Silt	% Clay	%OM	pH	CEC
Greenhouse, Inveresk Research, Scotland	sandy loam/loamy sand	78.16	14.42	7.42	3	7.3	13.9 mequiv/100 g

TABLE B.1.2. Test Site Information

Testing Environment and location	Temperature		Relative Humidity (%)	
	Min.	Max.	Min.	Max.
Greenhouse, Inveresk Research, Scotland	8 °C	40 °C	33 %	> 96%

TABLE B.1.3. Crop Information

Crop/Crop Group	Variety	Plant-back Intervals (days after soil treatment)	Growth stage at harvest	Harvested RAC	Harvesting Procedure
Spring Wheat	<i>Triticum aestivum</i> , cv. <i>Paragon</i>	30, 120, 365	5-7 leaves, 6-8" high, one node	Early Forage	Harvested just above soil surface (cutting method not specified); hay was dried for 2-8 days at ambient conditions to 19.5-19.7% moisture content; grain was separated from ears with an ear thrasher and the chaff combined with the straw.
			30-50% flowering or milky ripe	Hay	
			maturity (caryopsis hard)	Straw, grain	
Carrot	<i>Daucus carota</i> , cv. Bangor F1	30, 120, 365	Maturity	Shoots, roots	Separated into shoots and roots at the soil surface (cutting method not specified); soil removed from roots with paper tissues.
Soybean	<i>Glycine max</i> , cv Northern Conquest	30, 120, 365	6 nodes, 6-8" high	Early forage	Harvested just above the soil surface (cutting method not specified); pods were separated from grain by hand and combined with straw; hay was dried for 2-5 days to 10.4-19.6% moisture content.
			30-50% flowering	Hay	
			Maturity (fully ripe)	Straw, grain	

*Bq = disintegrations per second

B.2. Test Materials:

TABLE B.2.1. Test Material Characteristics



Dichlormid/900497/Dow AgroSciences/62719
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 Confined Accumulation in Rotational Crops - Spring Wheat, Carrot, and Soybean

Chemical structure	
Radiolabel position	carbonyl carbon
Batch number:	INV1624
Purity	94.1% by normal-phase TLC, 100.0% by reversed-phase TLC, and 97.8% by HPLC
Specific activity (Bq)*	5.88 MBq/mg (353,109 dpm/μg; 33.1 mCi/mmmole)

*Bq = disintegrations per second

B.3. Study Use Pattern:

Chemical name	Dichlormid
Application method	Applied onto soil in 30 ml with a hand-held manual sprayer. During application, the test area was surrounded by polythene sheeting to avoid contaminating other crates. After application, the upper 3-4 cm of the soil surface was gently raked to incorporate the test material into the soil.
Application rate	0.56 kg ai/ha (0.5 lb/A), the 1X maximum seasonal use rate
Number of applications	one
Timing of applications	Soil was treated 30, 120, or 365 days before planting crops
Time (days) from soil treatment to harvest of RACs for 30, 120, and 365 DAA, respectively	Wheat early forage: 90, 169, 423 Wheat hay: 111, 187, 441 Wheat straw, grain (maturity): 163, 220, 500 Carrot shoots, roots (maturity): 161, 217, 504 Soybean early forage: 149, 177, 429 Soybean hay: 170, 199, 441 Soybean straw, grain (maturity): 231, 239, 510

B.4. Identification/Characterization of Residues:

B.4.1. Sample Handling and Preparation:

The samples were weighed, chopped into shorter pieces if needed, and frozen at -20°C on the day of sampling. Samples were homogenized with dry ice using a Hobart or Waring blender, or a Glen Creston knife mill. The 30 DAA soybean grain sample was not homogenized due to insufficient sample.

Homogenized tissue samples that contained >0.01 mg/kg residues were further characterized. Samples were initially extracted twice with acetonitrile (ACN), followed by ACN:water (1:1 v/v), and water. Soybean grain was also first extracted with hexane, but the extracts contained <0.01 ppm and were not further characterized. Equal volumes of ACN and ACN:water extracts were combined and subjected to HPLC. Water extracts were concentrated and analyzed by HPLC System 2. Water extracts of spring wheat early forage and hay sown 30 DAA were passed under vacuum through hydrophobic solid phase extraction (SPE) cartridges (Isolute ENV+®) pre-equilibrated with ACN and



Dichlormid/90497/Dow AgroSciences/62719

DACO 7.4.3/OPPTS 860.1850/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6

Confined Accumulation in Rotational Crops - Spring Wheat, Carrot, and Soybean

water. The column was eluted with ACN:water (1:1 v/v), ACN:water (8:2 v/v), ACN, and finally methanol. Aliquots of spring wheat 30 DAA hay water extracts were also acidified to pH 5.0 with 50 mM acetic acid and passed under vacuum through Isolute ENV+[®] and anion exchange SPE cartridges pre-equilibrated with methanol followed by 50 mM acetic acid: sodium acetate buffer. The columns (acidic residues) were eluted with 10% sulfuric acid in methanol. A portion of the spring wheat 30 DAA concentrated ACN + ACN:water straw extracts and hay and straw water extracts were subjected to acid hydrolysis with 1 M HCl in a 95°C shaking water bath for ~4 hours. Following centrifugation to remove particulates, the hydrolysate was subjected to HPLC System 2. The extraction methods were validated by spiking control wheat and soybean early forage, carrot roots, and wheat grain with [¹⁴C]-dichlormid, and incubating in acetone for 1 hour at ambient temperature. Each sample was extracted with ACN, ACN:water, and water as for the treated samples, and analyzed by HPLC System 2.

More severe conditions were used to release radioactive residues from the post-extraction solids (PES). PES from 30 DAA wheat early forage and hay were extracted twice with 100 mM HCl for 1 hour, followed by water extraction and analysis of supernatants by HPLC System 2. PES from 30 DAA wheat and soybean early forage and carrot shoots, and from 30 and 120 DAA soybean hay were refluxed for 2 hours with acid detergent (2% v/v hexadecyltrimethyl ammonium bromide in 1 M H₂SO₄). The extracts, along with water and acetone rinses of the reflux flask, were centrifuged to separate the supernatant (containing hemicellulose and soluble plant proteins) and solid residue (containing cellulose and lignin). An unsuccessful attempt was made to remove detergent from the supernatants in wheat 30 DAA early forage samples by partitioning 3X with dichloromethane and SPE extraction (Varian SCX). A similar acid (1 M HCl) reflux extraction, but without detergent, was conducted on 30 and 120 DAA spring wheat hay and straw PES. The extract, with 1 M HCl rinsate of the reflux flask, were centrifuged to separate the supernatant and solids. The 30 DAA hay was also partitioned against dichloromethane. The hay and straw final supernatants were rotor-evaporated, resuspended in water, and analyzed by HPLC System 2.

Plant cell wall polysaccharides in PES from 30 and/or 120 DAA wheat hay, straw, and grain, as well as carrot roots and soybean grain were solubilized by incubation in 2% driselase in 50 mM acetic acid: sodium acetate pH 4.5 buffer for 24-26 hours at 37°C. Following centrifugation, the solid residue was washed with water and the driselase and water supernatants combined. The wheat hay and straw contained insufficient radioactivity for further characterization, but the wheat grain (30 and 120 DAA) samples and the carrot root 30 DAA samples were rotor-evaporated and resuspended in methanol: water (2:1 v/v). Both wheat grain samples were subjected to HPLC by System 3, and the 30 DAA sample also by thin layer chromatography (TLC) System 5. The carrot root sample was too viscous for injection into HPLC System 3 and, since it contained only 0.011 ppm, was not analyzed further.

The presence of radioactivity in starch was evaluated in 30, 120, and 365 DAA wheat grain and in 30 DAA carrot roots by incubating samples with dimethyl sulfoxide (DMSO): water (9:1 v/v) for ~4 hours with periodic stirring. After refrigeration for 19 hours the insolubles were removed by centrifugation and ethanol added to the extracts for ≥30 minutes (24 hours for 365 DAA grain sample) to precipitate the starch. The insoluble, starch-enriched fraction was collected by centrifugation. Radioactivity was



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counted in the soluble and insoluble fraction in grain, but only in the soluble fraction in carrot due to low levels of starch therein. The starch-enriched (insoluble) wheat grain (30 and 120 DA) and carrot root was hydrolyzed with pullulanase for 24 hours at 25°C in 50 mM acetic acid: sodium acetate pH 4.7 buffer, followed by addition of amyloglucosidase and additional incubation for 24 hours at 55°C. The supernatant following centrifugation was tested for the presence of glucose and other monosaccharides by HPLC System 3.

The incorporation of radioactivity into protein was determined in PES from 120 DAA soybean grain was extracted with pH 7.5 phosphate buffer in a top-drive homogenizer, shaken for 2 hours, residue removed by centrifugation, and trichloroacetic acid (TCA):water (40 g : 40 mL) added to precipitate the proteins. After shaking, the insoluble protein-rich precipitate was collected by centrifugation (3300 rpm, 45 minutes) and the presence of protein was confirmed by TLC. An aliquot was placed on a TLC plate (Whatman KC₁₈F₂₅₄) pre-soaked with 12.5% TCA, sprayed with Coomassie blue in water: methanol: acetic acid (87.5:7.5:5 v/v/v) and the color developed for 30 minutes.

The incorporation of radioactivity into lignin in PES from 30 DAA wheat hay, 120 DAA straw, and 120 DAA soybean grain was determined by extraction of samples for 24 hours with 5 M NaOH under reflux. Following removal of unextractable residues by centrifugation, the hydrolysates were acidified with HCl to pH ≤ 1.0, and refrigerated for 3.5 hours to precipitate the lignin. The precipitate was centrifuged, dried, and radioactivity counted.

B.4.2. Analytical Methodology:

Total radioactive residues (TRR) were determined in 6 aliquots from each homogenized matrix by oxidative combustion, except the 30 DAA soybean grain sample was combusted without homogenization (insufficient sample). For combustion analysis, samples were weighed into Combustocones[®], combusted using a Model 307 Tricarb automatic sample oxidizer, and the evolved ¹⁴CO₂ was trapped in Carbo-Sorb[®] and mixed with Permafluor[®] E scintillant (all materials from Packard Bioscience). The resulting samples were radioanalyzed by liquid scintillation counting (LSC). The oxidative combustion efficiencies were ≥96.6%. Radioactivity of liquid samples was determined directly LSC using a Packard 2100 Tricarb liquid scintillation analyzer with automatic quench correction. Radioactivity of highly colored liquid samples and of homogenized solid samples was determined by oxidative combustion followed by LSC. Prior to LSC, the liquid samples were mixed with Quickszint 1[®] (Zinsser Analytic) or Ultrima-Gold XR (Packard BioScience) scintillation fluid; the latter was used for HPLC eluates. Samples that were too large were evaporated at room temperature and resuspended in water and scintillant prior to LSC. Samples were stabilized to heat and light prior to being counted for 5 minutes.

Three HPLC systems were used. System 1 determined the radiochemical purity of [¹⁴C]-dichlormid as supplied and in the treatment formulation, as well of the metabolite reference standards. The system used a Hewlett-Packard (HP) model 1050 instrument with UV detection at 254 nm, a Phenomenex Luna C₁₈ analytical column (5 μm, 250 x 4.6 mm i.d.), and solvents (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. System 2 was used to analyze plant extracts for dichlormid and its potential metabolites with a HP model 1100 instrument. UV detection was at 220 nm, the analytical column was Hypersil Hypercarb (5 μm, 100 x 4.6 mm i.d.), and the solvents were (A) water and (B) acetonitrile with 0.1% formic acid. System 2 was the



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main chromatographic system used in the study. System 3 was used to detect monosaccharides in the plant extracts with a HP model 1100 instrument at 80°C and the Waters model 2414 refractive index detector. The analytical column was Rezex RPM-Monosaccharide (Pb²⁺ form, 8 µm, 300 x 7.8 mm i.d.), and the solvent was water. Reference standards included unlabeled arabinose, fructose, galactose, glucose, xylose, and [¹⁴C]-glucose.

Five TLC systems were used. The first three measured the radiochemical purity of [¹⁴C]-dichlormid, and the fourth and fifth measured dichloroacetic acid and monosaccharides, respectively, in plant extracts. The System 1, 2, 4, and 5 solid phases was Merck Kieselgel 60F₂₅₄, 0.25 mm thick. The corresponding System liquid phases were (1) hexane: ethyl acetate, 3:1 v/v, (2) dichloromethane: diethyl ether, 9:1 v/v, (4) acetone: butanol: 1.5 M ammonium hydroxide: water, 65:20:10:5 v/v/v/v, and (5) ethyl acetate: pyridine: water: acetic acid, 60:30:15:5 v/v/v/v. System 1 was also run in two dimensions to evaluate if radioactivity bound to the solid phase. The System 3 solid phase was Merck KC18F, 0.20 mm thick, and the solvent was acetonitrile: water (7:3 v/v). Radioactivity was determined on the plates with a phosphor imager, and non-radioactive reference standards were visualized at 254 nm. Dichloroacetic acid and reducing sugars were visualized by spraying the TLC plates with 0.1% aqueous bromocresol purple (15 minutes at 60°C) and aniline-diphenyl amine (10 minutes at 85°C), respectively. Reference standards of dichlormid and metabolites were run alongside plant extracts in System 4, and glucose was the reference standard for System 5.

The limits of detection (LOD) and quantitation (LOQ) were calculated using equations developed by Currie (1968). The LOD (ppm) was defined as the LOD (dpm) divided by the [sample weight (g) x specific activity (dpm/µg)]. In the equation, the LOD (dpm) is defined as $[2.71 + (4.65 \times \text{square root of (background dpm} \times \text{count time)})]$, which is divided by the count time. The LODs for the various tissues ranged from 0.00063 - 0.00189 ppm. The LOQ (ppm) was defined as the LOQ (dpm) divided by the [sample weight (g) x specific activity (dpm/µg)]. In the equation, the LOQ (dpm) is defined as 50 times the sum of $\{1 + \text{the square root of } [1 + (\text{background dpm} \times 1/12.5 \text{ count time})]\}$, all divided by the count time. The LOQs for the various tissues ranged from 0.00237 - 0.00726 ppm.

C. RESULTS AND DISCUSSION:

C.1. Storage Stability:

Recovery of radioactivity was adequate (95.1-113.8% of combustion vales) in the wheat hay, wheat early forage, and soybean early forage extracts following storage for 55-63 weeks at -20°C. Residue levels and distribution in the three extracts were generally similar to that obtained with the pre-storage extractions, as can be seen by comparing Table C.1.2. with Tables C.2.3. (A) and C.2.3. (C). The HPLC profiles of the combined, concentrated ACN + ACN:water extracts for wheat forage and hay contained dichlormid and most of the previously identified (and unidentified) metabolites, albeit at somewhat lower levels. For wheat forage, several metabolites not found originally in any matrix were identified (N,N-diallyloxamic acid and 2-chloro-N,N-di-2-propenylacetamide¹), and

¹This compound appears to be erroneously called 2-chloro N,N-di-2-phenylacetamide on page 96 of MRID 46353808.



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the amount of radioactivity eluting at the solvent front increased from 0.021 ppm to 0.053 ppm. The HPLC profile of the re-extracted soybean early forage differed more substantially from that obtained initially, as one of the two earlier identified metabolites was missing, and three new metabolites were found. The greater discrepancy in the soybean forage pre- and post-storage extracts may be in part due to the lower residue levels in this matrix, as many of the metabolites were near their LOD. The data overall suggest that dichlormid and its metabolites are stable in frozen storage for a year, the results for hay being the most consistent (and hay had the greatest residue levels). The results for the characterization and identification of post-storage residues in wheat and soybean samples are shown in Table C.1.2.

OPPTS 860.1300 guidelines require storage stability studies when matrices are frozen more than 4-6 months before analysis, which was not the case for any of the initially extracted matrices except for starch in wheat grain. Therefore, the ambiguities of the storage stability study results are largely irrelevant to the interpretation of the confined rotational crop study.

Matrix (RAC)	Plant-back interval (days after soil treatment)	Storage temp. (°C)	Actual storage duration (weeks)	Interval of demonstrated storage stability (weeks)
Wheat early forage	30	-20	63	63
Wheat hay	30	-20	60	60
Soybean early forage	30	-20	55	55



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RAC (30 DAA)	Wheat early forage	Wheat hay	Soybean early forage
TRR (ppm) by combustion	0.169 (100.0)	0.639 (100.0)	0.122 (100.0)
ACN	0.047 (28.1)	0.024 (3.7)	0.038 (31.1)
ACN:water	0.037 (21.7)	0.113 (17.7)	0.018 (14.7)
<u>ANC + ACN:water, concentrated</u>	<u>0.077 (45.3)</u>	<u>0.141 (22.0)</u>	<u>0.054 (44.2)</u>
N, N-diallyl-2-hydroxyacetamide	0.005 (2.8)	0.008 (1.3)	ND
N-allyl-2,2-dichloroacetamide	ND	ND	0.005 (4.0)
N,N-diallyl glyoxylamide	ND	ND	0.002 (1.3)
2-chloro-N,N-di-2-propenylacetamide	0.002 (1.0)	0.014 (2.1)	0.008 (6.9)
N,N-diallyloxamic acid	0.002 (1.1)	ND	ND
N,N-di-2-propenylcetamide	ND	0.018 (2.9)	0.004 (3.5)
Dichlormid	0.001 (0.6)	0.006 (0.9)	ND
Unknown components	Four: <0.001-0.053 (0.3-30.9)	Five: 0.001-0.030 (0.2-4.8)	Four: 0.004-0.019 (3.6-15.9)
Water (not further characterized)	0.013 (7.8)	0.105 (16.4)	0.015 (11.9)
PES (not further characterized)	0.078 (46.0)	0.366 (57.3)	0.068 (56.1)
TOTAL residue recovery, as % of combustion TRR	103.6	95.1	113.8

Date from pp. 160-162 of MRID 46353808.

ACN = acetonitrile; DAA = days after application; ND = not detected; PES = post-extraction solids

C.2. Identification, Characterization, and Distribution of Residues:

C.2.1. Total radioactive residues (TRR):

TRR levels in RACs of spring wheat, carrot, and soybean grown in [¹⁴C]-dichlormid-treated and untreated (control) soil are shown in Table C.2.1. Radioactive residues in all three crops declined as time after dichlormid application (DAA) increased, with one exception. The 120 DAA soybean grain had slightly higher residue levels than the 30 DAA grain, which may be a reflection of the soybean plants' stunted and necrotic growth and low grain yield. Radioactive residues were also found in control samples, which were grown in greenhouses adjacent to those containing treated soil. The two chambers were separated by unsealed glass panes and volatile radioactivity appeared to have transferred between them. Residue levels in RACs grown in untreated soil paralleled those from treated soil, decreasing as the DAA increased, except for the soybean grain samples.



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TABLE C.2.1. Total Reactive Residues (TRR, ppm) Obtained by Combustion Analysis for Rotational Crops Spring Wheat, Carrot, and Soybean RACS Planted in Soil Treated with Dichlormid and in Control (Untreated) Soil.

RAC	Spring wheat			Carrot			Soybean		
	30	120	365	30	120	365	30	120	365
<u>Treated soil</u>									
Early forage	0.169	0.061	0.005	-	-	-	0.122	0.040	0.005
Hay	0.639	0.155	0.017	-	-	-	0.331	0.113	0.014
Straw	0.629	0.260	0.014	-	-	-	0.139	0.061	0.010
Grain	0.295	0.080	0.017	-	-	-	0.034	0.039	0.019
Shoots	-	-	-	0.115	0.021	0.005	-	-	-
Roots	-	-	-	0.038	ND	ND	-	-	-
<u>Control soil</u>									
Early forage	0.012	ND	ND	-	-	-	0.008	NQ	ND
Hay	0.040	0.007	ND	-	-	-	0.021	0.006	ND
Straw	0.028	0.012	ND	-	-	-	0.006	NQ	NQ
Grain	0.022	0.007	NQ	-	-	-	NQ	0.004	ND
Shoots	-	-	-	0.006	ND	ND	-	-	-
Roots	-	-	-	NQ	ND	ND	-	-	-

ND = not detectable; NQ = not quantifiable (between LOD and LOQ); DAA = days after application

"-" = RAC not applicable

Data from pp. 117-122 of MRID 46353808.

C.2.2. Distribution and characterization of ¹⁴C-residues:

(A) Spring wheat:

Total residue levels were greatest in hay and straw, followed by grain and early forage, and for any given RAC, residue levels decreased as the DAA increased. Residues were characterized from the 30, 120, and 365 DAA samples of all wheat RACs except the 365 DAA early forage, which had TRR below 0.01 ppm. The majority (62-67.1% TRR) of the 30 and 120 DAA early forage radioactive residues were recovered in the initial extractions (ACN, ACN:water, and water), with 32.9-38.0% TRR being in the PES. A slightly higher fraction of the TRR (38.3-44.9%) was in the PES of the 30 and 120 DAA hay and straw initial extracts, whereas the 365 DAA hay and straw PES contained 51.2-68% of the TRR. Grain residues were released the least by the initial extractions, as 76.1-83.1% of the TRR was in the PES.

Acid hydrolysis of early forage, hay, and straw PES containing >0.05 ppm released 10.6-22.2% TRR per matrix. Further treatment of the 30 DAA hay and 120 DAA straw unextracted acid hydrolysis residues with driselase and base hydrolysis (for residues incorporated into cell wall polysaccharides and lignin, respectively), released an additional 5.2-8.9% and 11.4-16.3% TRR, respectively. Grain PES was subjected to DMSO extraction, and starch was ethanol-precipitated from the resulting supernatant and subsequently digested with driselase. The majority of the 30 and 120 DAA grain



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radiolabel was in the starch fraction (365 DAA grain PES not evaluated), and was digestible by driselase, indicating that the starch was incorporated into cell wall polysaccharides.

The overall recoveries of the 30, 120, and 365 DAA spring wheat RACs were adequate, ranging from 87.4-114.9% of the initially applied TRR. For all RACs except straw and grain, the fraction of unextractable radioactivity increased with the DAA. The distribution and characterization of ¹⁴C-residues in the spring wheat RACs are shown in Table C.2.2.(A).

TABLE C.2.2.(A) Rotational Crop – Spring Wheat – Planted in Dichlormid-treated Soil: RAC Characterization and Recoveries Following Extraction and Hydrolysis.												
RAC	Early forage			Hay			Straw			Grain		
DAA (days)	30	120	365	30	120	365	30	120	365	30	120	365
TRR: ppm (%TRR)	0.169 (100.0)	0.061 (100.0)	0.005 (100.0)	0.639 (100.0)	0.155 (100.0)	0.017 (100.0)	0.629 (100.0)	0.260 (100.0)	0.014 (100.0)	0.295 (100.0)	0.080 (100.0)	0.017 (100.0)
Initial extracts												
Acetonitrile	0.067 (39.8)	0.018 (30.0)	NC	0.045 (7.0)	0.017 (11.0)	0.001 (4.1)	0.061 (9.7)	0.026 (10.0)	0.003 (24.6)	0.003 (0.9)	0.001 (1.2)	NC
Aqueous acetonitrile	0.042 (25.0)	0.015 (24.7)	NC	0.116 (18.1)	0.036 (23.2)	0.003 (18.4)	0.104 (16.6)	0.054 (20.9)	0.003 (23.0)	0.009 (3.1)	0.003 (4.1)	NC
Water	0.013 (7.6)	0.006 (9.4)	NC	0.131 (20.5)	0.026 (16.7)	0.002 (11.5)	0.086 (13.7)	0.041 (15.7)	0.002 (16.1)	0.019 (6.3)	0.002 (2.3)	NC
100 mM HCl	0.008 (5.0)	NC	NC	0.061 (9.6)	NC	NC	NC	NC	NC	NC	NC	NC
PES	0.056 (32.9)	0.023 (38.0)	NC	0.245 (38.3)	0.070 (44.9)	0.012 (68.0)	0.298 (47.4)	0.098 (37.8)	0.007 (51.2)	0.245 (83.1)	0.061 (76.1)	NC
Characterization of PES (post-extraction solids)												
1M acid hydrolysate	0.038 (22.2)	NC	NC	0.088 (13.8)	0.025 (16.0)	NC	0.067 ² (10.6)	0.055 (21.0)	NC	NC	NC	NC
Driselase digest ³	NC	NC	NC	0.033 (5.2)	NC	NC	NC	0.023 (8.9)	NC	0.099 (33.7)	0.021 (26.2)	NC
5M NaOH hydrolysate ³	NC	NC	NC	0.073 (11.4)	NC	NC	NC	0.042 (16.3)	NC	NC	NC	NC
DMSO extr. aq. fraction	NC	NC	NC	NC	NC	NC	NC	NC	NC	0.007 (2.3)	0.005 (6.2)	0.004 (21.8)
DMSO extr. starch ppt.	NC	NC	NC	NC	NC	NC	NC	NC	NC	0.092 (31.3)	0.031 (38.9)	0.005 (26.9)
Residue recoveries												
Extractable	0.168 (99.6)	0.039 (64.1)	NC	0.547 (85.6)	0.104 (66.9)	0.006 (34.0)	0.318 (50.6)	0.241 (92.8)	0.008 (63.7)	0.229 (77.6)	0.063 (78.9)	0.009 (48.7)



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Unextracted	0.018 (10.7)	0.023 (38.0)	NC	0.050 (7.9)	0.035 (22.3)	0.012 (68.0)	0.131 (20.8)	0.023 (8.7)	0.007 (51.2)	0.051 (17.4)	0.008 (9.9)	0.009 (50.5)
TOTAL	110.3	102.1	NC	93.5	89.2	102	87.4 ²	101.5	114.9	95	88.8	99.2

Data from pp. 24, 28, and 124-130 of MRID 46353808.

NC = not conducted; DAA = days after application; PES = post-extraction solids

¹Acid was 1M H₂SO₄ in 2% detergent for 30 DAA forage, and was 1M HCl for all other samples.

²Some residue (0.101 ppm; 16.0% TRR) was lost upon acid hydrolysis; this amount is included in the total.

³Unextracted 1M hydrolysis residues were digested with driselase, and the latter residues hydrolyzed with base.

(B) Carrot:

Residues were characterized from the 30 and 120 DAA shoot samples and the 30 DAA root samples, as other samples had TRR below 0.01 ppm. Residue levels were low for all three tissues, being greatest in the 30 DAA shoots. Approximately half of the recovered TRR for each matrix was in the initial extractions and the rest in the PES. Acid detergent hydrolysis of the 30 DAA shoot PES yielded 45.7% TRR, which was not characterized further due to low radioactivity and interference from the detergent (0.053 ppm). The 30 DAA root PES was extracted with DMSO and the starch fraction precipitated with ethanol, hydrolyzed with pullulanase and amyloglucosidase, and driselase. A small fraction of the TRR was in the ethanol supernatant and precipitate, whereas driselase digestion extracted the majority of the TRR in the PES. Total recoveries of applied radioactivity for the three characterized matrices were reasonable (80.9-112.3% TRR), considering the sample low residue levels. As was the case for spring wheat, extractability decreased as DAA increased. The distribution and characterization of ¹⁴C-residues in carrot RACs are shown in Table C.2.2.(B).



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TABLE C.2.2. (B) Rotational Crop – Carrot – Planted in Dichlormid-treated Soil: RAC Characterization and Recoveries Following Extraction and Hydrolysis.						
RAC	Shoots			Roots		
DAA (days)	30	120	365	30	120	365
TRR: ppm (%TRR)	0.115 (100.0)	0.021 (100.0)	0.005 (100.0)	0.038 (100.0)	Not detected	Not detected
Initial extracts						
Acetonitrile	0.025 (21.9)	0.006 (26.6)	NC	0.004 (10.4)	NC	NC
Aqueous acetonitrile	0.017 (15.1)	0.005 (23.4)	NC	0.006 (15.8)	NC	NC
Water	0.007 (5.9)	0.002 (8.7)	NC	0.004 (9.3)	NC	NC
PES	0.055 (47.5)	0.011 (53.6)	NC	0.015 (39.2)	NC	NC
Characterization of PES (post-extraction solids)						
Acid detergent hydrolysis	0.053 (45.7)	NC	NC	NC	NC	NC
DMSO extraction						
--aqueous fraction	NC	NC	NC	0.002 (5.1)	NC	NC
--starch ethanol ppt. ¹	NC	NC	NC	0.001 (2.0)	NC	NC
Driselase digestion	NC	NC	NC	0.011 (28.4)	NC	NC
Residue recoveries						
Extractable	0.102 (88.6)	0.013 (58.7)	NC	0.028 (71.0)	NC	NC
Unextracted	0.002 (1.8)	0.011 (53.6)	NC	0.004 (9.9)	NC	NC
TOTAL	90.4	112.3	NC	80.9	NC	NC

NC = not conducted; DAA = days after application; PES = post-extraction solids

Data from pp. 25, 30, and 131-134 of MRID 46353808.

¹Quantified by HPLC after hydrolysis with pullulanase and amyloglucosidase.

(C) Soybean:

Residues were characterized from all matrix samples except the 365 DAA early forage and the 30 DAA grain, due to insufficient sample radioactivity. Residue recoveries from the initial extractions decreased with increasing DAA and growth stage: ~40-60% TRR was recovered from the 30 and 120 DAA early forage, hay, and straw and the 365 DAA straw, whereas ~20-40% TRR was recovered from the 365 DAA hay and 120 and 365 DAA grain initial extracts. Straw PES was not further characterized because it is not a RAC. The PES from early forage (30 DAA) and hay (30 and 120 DAA) were subjected to acid detergent hydrolysis, which yielded 38.6–49.9% TRR (0.047-0.165 ppm), but were not further characterized due to detergent interference. The 120 DAA grain PES was extracted with aqueous phosphate: NaCl buffer and the released protein precipitated with TCA, which yielded 5.3% TRR (0.002 ppm) protein and 3.0% TRR (0.001 ppm) in the supernatant. The unextracted 52.8% TRR (0.021 ppm) was digested with driselase, which yielded no radioactivity, hydrolyzed with base (59.0% TRR released; 7.0% unextractable) and acidified to precipitate lignin (11.0% TRR; 27.9% remained aqueous). Total recoveries of applied radioactivity for the four characterized matrices were reasonable (83.7-120.3% TRR), considering the low residue levels of the samples. The



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distribution and characterization of ^{14}C -residues in the soybean matrices are shown in Table C.2.2.(C).

TABLE C.2.2.(C) Rotational Crop – Soybean Planted in Dichlormid-treated Soil: Characterization and Recoveries of Four Matrices Following Extraction and Hydrolysis.												
RAC	Early forage			Hay			Straw			Grain		
DAA (days)	30	120	365	30	120	365	30	120	365	301	120	365
TRR: ppm (%TRR)	0.122 (100.0)	0.040 (100.0)	0.005 (100.0)	0.331 (100.0)	0.113 (100.0)	0.014 (100.0)	0.139 (100.0)	0.061 (100.0)	0.010 (100.0)	0.034 (100.0)	0.039 (100.0)	0.019 (100.0)
Initial extracts												
Hexane	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	0.002 (5.0)	0.002 (8.5)
Acetonitrile	0.037 (30.0)	0.018 (45.1)	NC	0.013 (3.8)	0.018 (15.7)	0.001 (3.7)	0.020 (14.3)	0.016 (26.6)	0.001 (11.4)	NC	0.002 (5.4)	<0.001 (1.6)
Aqueous acetonitrile	0.020 (16.7)	0.005 (12.2)	NC	0.071 (21.3)	0.022 (19.9)	0.001 (9.4)	0.037 (26.4)	0.013 (21.0)	0.001 (13.2)	NC	0.005 (13.6)	0.001 (4.7)
Water	0.010 (7.8)	0.002 (5.4)	NC	0.042 (12.7)	0.020 (17.6)	0.002 (11.4)	0.014 (10.0)	0.006 (9.2)	0.001 (7.5)	NC	0.009 (22.0)	0.002 (10.5)
PES	0.061 (49.7)	0.016 (38.9)	NC	0.171 (51.8)	0.058 (51.0)	0.011 (78.3)	0.055 (39.3)	0.022 (35.4)	0.005 (51.6)	NC	0.027 (69.4)	0.014 (72.7)
Characterization of PES (post-extraction solids)												
Acid hydrolysis	0.047 (38.6)	NC	NC	0.165 (49.9)	0.047 (41.8)	NC	NC	NC	NC	NC	NC	NC
Base hydrolysis	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	0.023 ² (59.0)	NC
Residue recoveries (% of TRR obtained by combustion analysis)												
Extractable	0.114 (93.1)	0.025 (62.7)	NC	0.291 (87.7)	0.107 (95.0)	0.004 (24.5)	0.071 (50.7)	0.035 (56.8)	0.003 (32.1)	NC	0.044 ³ (113.3)	0.005 (25.3)
Unextracted	0.014 (11.1)	0.016 (38.9)	NC	0.006 (1.9)	0.010 (9.2)	0.011 (78.3)	0.055 (39.3)	0.022 (35.4)	0.005 (51.6)	NC	0.003 (7.0)	0.014 (72.7)
TOTAL	104.2	101.6	NC	89.6	104.2	102.8	90	92.2	83.7	NC	120.3	98

NC = not conducted; PES = post-extraction solids

Data from pp. 25, 32, and 135-139 of MRID 46353808.

¹There was insufficient sample for homogenization or further analysis of this sample.

²Residue after PES extraction with phosphate:NaCl buffer to release protein (0.001 ppm soluble + 0.002 ppm protein fraction), digestion with driselase (no radioactivity), and base hydrolysis.

³Includes 0.018 ppm from initial extracts, 0.003 ppm from protein release, and 0.023 ppm from base hydrolysis.

C.2.3. Identification of metabolites:

The parent dichlormid and its metabolites were identified chromatographically by HPLC, and in one case TLC, by comparison to R_f values of reference standards.

(A) Spring wheat:

The parent dichlormid was found in only 30 DAA early forage and hay (ACN:water and



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water extracts). Six dichlormid metabolites were identified by HPLC System 2 and one by TLC System 5 in spring wheat 30 DAA and/or 120 DAA RACs. Based on their partitioning behavior, the majority of the known and unknown metabolites were polar, and were not bound by the SPE hydrophobic matrix. For each matrix sample, the greatest radioactive HPLC component was the (polar) solvent front. The identified metabolites ranged from 0.001-0.024 ppm, and for a given metabolite, were generally greater at 30 DAA than at 120 DAA.

N,N-diallyl-2-hydroxyacetamide (ACN:water and water extracts) and N,N-di-2-propenylacetamide (ACN:water, water, and HCl extracts) were found in forage, hay, and straw. N,N-diallyl glyoxylamide (ACN:water extracts), 2-chloro-N,N-di-2-propenylacetamide (ACN:water, water, and HCl extracts), and N-allyl-2,2-dichloroacetamide (ACN:water and water extracts) were found in hay and straw. N-allyl-2,2-glyoxylamide was detected only in hay (water extracts), and dichloroacetic acid was identified by TLC in straw (water extracts). As discussed in Section C.2.2.(A) and shown in Table C.2.2.(A), driselase digestion followed by base hydrolysis indicated that some residues were incorporated into cell wall polysaccharides and lignin, respectively, in hay and straw.

In wheat grain, dichlormid was degraded and incorporated into starch as glucose, and into the cell wall as unidentified components, based on results of the results of the pullulanase + amyloglucosidase digestion, and driselase digestion, respectively. Attempts were made to identify all components of wheat RACs representing ≥ 0.05 ppm of each matrix, as well as some lower-abundance components. The characterization and identification of residues in spring wheat RACs is summarized in Table C.2.3.(A).

TABLE C.2.3.(A) Rotational Crop – Spring wheat: Characterization and Identification of Residues from Plants Grown in Dichlormid-treated Soil.		
RAC: Early Forage	30 DAA	120 DAA
Initial sample: ppm (%TRR)	0.169 (100.0)	0.061 (100.0)
<u>Combined, concentrated ACN + ACN:water</u>	0.112 (66.3)	0.025 (40.6)
N,N-diallyl-2-hydroxyacetamide	0.016 (9.7)	0.002 (3.9)
N,N-di-2-propenylacetamide	ND	0.001 (1.3)
Dichlormid	0.005 (3.1)	ND
Unknown component(s) ¹	Seven: 0.002 – 0.022 (1.4 – 13.1)	Four: 0.002 – 0.016 (2.5 – 25.6)
<u>Concentrated water extract</u>	0.012 (6.9)	NC
Unknown component(s)	Three: 0.001 – 0.010 (0.7 – 5.8)	
SPE aqueous eluates	0.012 (7.2)	NC
SPE organic eluates	Four: each ND	
RAC: HAY		
Initial Sample: ppm (%TRR)	0.639 (100.0)	0.155 (100.0)



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TABLE C.2.3.(A) Rotational Crop – Spring wheat: Characterization and Identification of Residues from Plants Grown in Dichlormid-treated Soil.		
<u>Combined, concentrated ACN + ACN:water</u>	0.178 (27.9)	0.052 (33.3)
N,N-diallyl glyoxylamide	0.012 (1.9)	ND
N,N-diallyl-2-hydroxyacetamide	0.020 (3.2)	ND
2-chloro-N,N-di-2-propenylacetamide	0.011 (1.7)	ND
Dichlormid	0.010 (1.6)	ND
Unknown component(s)	Six: 0.004 – 0.042 (0.6 – 6.6)	One: 0.052 (33.3)
<u>Concentrated water extract</u>	0.133 (20.8)	0.025 (15.9)
N-allyl-2,2-dichloroacetamide	ND	0.001 (0.6)
N,N-diallyl-2-hydroxyacetamide	ND	0.002 (1.5)
2-chloro-N,N-di-2-propenylacetamide	ND	0.001 (0.3)
N,N-diallyl glyoxylamide		
Dichlormid	0.010 (1.5)	ND
Unknown component(s)	One: 0.123 (19.3)	Six: 0.001 – 0.009 (0.6 – 5.7)
SPE ENV [®] aqueous eluates	0.127 (19.8)	NC
SPE ENV [®] organic eluates (four solvents)	ND	
SPE ENV [®] acidified aqueous eluates	0.095 (14.9)	NC
SPE ENV [®] acidified organic eluates (two solvents)	0.20 (3.1), ND	
SPE SAX acidified aqueous eluates	0.077 (12.0)	NC
SPE SAX acidified organic eluates	Two: 0.050 (7.9), ND	
Acid (1N HCl, 95°C, 4 hr) hydrolysate	0.107 (16.7)	NC
Unknown component(s)	Three: 0.029 – 0.048 (4.6 – 7.5)	
Acid hydrolysate residue	0.013 (2.1)	
<u>Concentrated 100 mM HCl extract</u>	0.053 (8.3)	NC
N,N-di-2-propenylacetamide	0.004 (0.7)	
2-chloro-N,N-di-2-propylacetamide	0.002 (0.3)	
Unknown component(s)	Two: 0.002, 0.045 (0.3, 7.0)	
<u>Concentrated 1N acid hydrolysate: one unknown</u>	0.075 (11.7)	NC
RAC: Straw		
Initial sample: ppm (%TRR)	0.629 (100.0)	0.260 (100.0)
<u>Combined, concentrated ACN + ACN:water</u>	0.188 (29.9)	0.082 (31.5)
N-allyl-2,2-dichloroacetamide	0.008 (1.3)	0.004 (1.4)
N,N-diallyl glyoxylamide	ND	0.006 (2.1)
N,N-diallyl-2-hydroxyacetamide	0.024 (3.7)	2
N,N-di-2-propenylacetamide	0.21 (3.4)	ND
2-chloro-N,N-di-2-propenylacetamide	0.003 (0.4)	ND
Unknown component(s)	Nine: 0.003 – 0.053 (0.4 – 8.4)	Three: <0.001–0.070 (0.2 – 26.7)
Acid (1N HCl, 95°C, 4 hr) hydrolysate	0.157 (25.0)	NC
Unknown component(s)	Three: 0.032 – 0.084 (5.2 – 13.3)	
Acid hydrolysate residue	0.028 (4.4)	
TLC System 4 analysis: unresolved areas	Two: 0.033, 0.096 (5.3, 15.2)	NC



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<u>Concentrated water extract</u>	0.087 (13.9)	0.041 (15.6)
N-allyl-2,2-dichloroacetamide	0.005 (0.8)	ND
N,N-diallyl-2-hydroxyacetamide	0.004 (0.7)	ND
N,N-di-2-propenylacetamide	0.002 (0.3)	ND
Unknown component(s)	Six: 0.001 – 0.055 (0.1 – 8.8)	One: 0.041 (15.6)
Acid (1N HCl, 95°C, 4 hr) hydrolysate	0.072 m(11.5)	NC
Unknown component(s)	Two: 0.019, 0.053 (3.0, 8.5)	
Acid hydrolysate residue	0.012 (1.9)	
TLC System 4 analysis: unknowns	Four: 0.006 – 0.011 (0.9 – 1.7)	NC
Dichloroacetic acid	0.009 (1.5)	
N,N-diallyl-2-hydroxyacetamide	0.008 (1.2)	
Concentrated 1N HCl hydrolysate: one unknown	NC	0.056 (21.4)
RAC: Grain		
Initial sample: ppm (%TRR)	0.295 (100.0)	0.080 (100.0)
<u>Starch digest (pullulanase + amyloglucosidase)</u>	0.92 (31.3)	0.31 (38.9)
Glucose	0.066 (22.3)	0.23 (29.3)
Unknown component(s)	Three: 0.001 – 0.003 (0.4 – 0.9)	ND
<u>Driselase digest</u>	0.099 (33.7)	0.021 (26.2)
HPLC System 3: arabinose, fructose, galactose, glucose, or xylose	ND	ND
TLC System 5: poorly resolved from origin	0.80 (27.0)	NC

Data from pp. 142-144, 149-153, 155-157, and 211-281 of MRID 46353808.

¹In each case the solvent front was the largest unknown (and very polar) component.

²This compound eluted together with N,N-diallyl glyoxylamide.

ACN = acetonitrile; DAA = days after application; NC = not characterized;

ND=not detectable; SPE = solid phase extraction

(B) Carrot:

Parent dichlormid was not found in any samples. The majority of radioactivity in the ACN:water + water extracts of shoots and roots was unidentified but polar, eluting at the solvent front. In the combined and concentrated ACN:water + water extracts, the metabolite N,N-di-2-propenylacetamide was identified in the 120 DAA shoots (0.001 ppm; 6.3% TRR). Digestion of 30 DAA roots with pullulanase and amyloglucosidase followed by HPLC analysis showed the presence of glucose (0.001 ppm; 2.0% TRR), but subsequent digestion with driselase produced a sample that was too viscous for HPLC analysis. The characterization and identification of residues in carrot roots and shoots is summarized in Table C.2.3.(B).



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RAC: Shoot	30 DAA	120 DAA
Initial sample: ppm (%TRR)	0.115 (100.0)	0.021 (100.0)
<u>Combined, concentrated ACN + ACN:water extract</u>	0.039 (34.1)	0.008 (39.1)
N,N-di-2-propenylacetamide	ND	0.001 (6.3)
Unknown component(s)	Six: 0.002-0.019 (2.1-16.4)	One: 0.008 (32.8)
RAC: Root		
Initial sample: ppm (%TRR)	0.038 (100.0)	ND
<u>Combined, concentrated ACN + ACN:water extract</u>	0.014 (38.1)	NC
Unknown component(s)	One: 0.014 (38.1)	
<u>Starch digest (pullulanase + amyloglucosidase)</u>	0.001 (2.0)	NC
Glucose	0.001 (2.0)	
<u>Driselase digest, concentrated</u>	0.008 (21.1)	NC
No results – sample too viscous		

Data from pp. 145, 155, and 282-293 of MRID 46353808.

ACN = acetonitrile; DAA = days after application; NC = not characterized; ND = not detectable

(C) Soybean:

The parent dichlormid was not found in any samples. Four dichlormid metabolites were identified by HPLC System 2 in soybean 30 DAA and/or 120 DAA RACs. Based on their partitioning behavior, the majority of the known and unknown metabolites were polar. For all but the 120 DAA early forage ACN + ACN:water extract, the greatest radioactive HPLC component was the (polar) solvent front. Levels of most metabolites were greater at 30 DAA than at 120 DAA. The identified metabolites ranged from 0.001-0.013 ppm. N,N-diallyl glyoxylamide and N,N-diallyl-2-hydroxyacetamide were found in early forage, hay, and straw ACN:water and/or water extracts. N-allyl-2,2-dichloroacetamide and 2-chloro-N,N-di-2-propenylacetamide were found in hay and straw ACN:water and/or water extracts. The identification of only 4 metabolites, compared to 6 in wheat, may be in part due to the lower residue levels in the soybean matrices. The characterization of soybean grain was discussed in Section C.2.2.(C). The characterization and identification of residues in soybean RACs, as well as straw, is summarized in Table C.2.3.(C); no individual grain metabolites were identified.



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TABLE C.2.3.(C) Rotational Crop – Soybean – Characterization and Identification of Residues of Plants Grown in Dichlormid-treated Soil.		
RAC: Early forage	30 DAA	120 DAA
Initial sample: ppm (%TRR)	0.122 (100.0)	0.040 (100.0)
<u>Combined, concentrated ACN + ACN:water extract</u>	0.053 (43.3)	0.023 (56.6)
N,N-diallyl glyoxylamide	ND	0.002 (5.4)
N,N-diallyl-2-hydroxyacetamide	0.004 (3.3)	0.002 (5.1)
Unknown component(s)	Three: 0.006-0.022 (4.7-18.1)	Six: <0.001-0.006 (0.8-14.0)
<u>Concentrated water extract</u>	0.009 (7.3)	NC
Unknown component(s)	One: 0.009 (7.3)	
RAC: Hay		
Initial sample: ppm (%TRR)	0.331 (100.0)	0.113 (100.0)
<u>Combined, concentrated ACN + ACN:water extract</u>	0.096 (29.0)	0.036 (31.7)
N-allyl-2,2-dichloroacetamide	0.003 (0.9)	²
N,N-diallyl glyoxylamide	ND	0.005 (4.3)
N,N-diallyl-2-hydroxyacetamide	0.013 (3.9)	0.004 (3.2)
2-chloro-N,N-di-2-propenylacetamide	0.010 (3.2)	ND
Unknown component(s)	Four: 0.007-0.043 (2.0-12.8)	Five: <0.001-0.012 (0.4-10.3)
<u>Concentrated water extract</u>	0.041 (12.5)	0.019 (16.5)
N-allyl-2,2-dichloroacetamide	0.004 (1.1)	ND
2-chloro-N,N-di-2-propenylacetamide	0.002 (0.7)	ND
N,N-diallyl-2-hydroxyacetamide	ND	0.001 (0.9)
Unknown component(s)	Two: 0.005, 0.029 (1.7, 9.0)	Two: 0.001, 0.017 (1.1, 14.5)
non-RAC: Straw		
Initial sample: ppm (%TRR)	0.139 (100.0)	0.061 (100.0)
<u>Combined, concentrated ACN + ACN:water extract</u>	0.065 (46.5)	0.029 (47.1)
N-allyl-2,2-dichloroacetamide	0.008 (5.7)	0.002 (3.9)
N,N-diallyl glyoxylamide	0.007 (4.7)	ND
N,N-diallyl-2-hydroxyacetamide	²	0.006 (10.4)
2-chloro-N,N-di-2-propenylacetamide	ND	0.002 (3.9)
Unknown component(s)	One: 0.050 (36.0)	Five: 0.001-0.011 (1.2-17.9)

Data from pp. 146-147 and 294-329 of MRID 46353808.

¹For all samples, except the 120 DAA early forage ACN + ACN:water extract, the solvent front was the largest unknown (and very polar) component.²This compound eluted together with N,N-diallyl glyoxylamide.

ACN = acetonitrile; DAA = days after application; NC = not characterized; ND = not detectable

C.3. Proposed Metabolic Profile:

Dichlormid (N,N-diallyl-2,2-dichloroacetamide) metabolism in confined rotational crops is proposed to involve two routes. In one, dichlormid is de-chlorinated to form 2-chloro-N,N-di-2-propenylacetamide and N,N-di-2-propenylacetamide, and is then transformed to N,N-diallyl glyoxylamide and N,N-diallyl hydroxyacetamide. The latter is oxidized to form N,N-diallyl oxamic acid. In the second route, dichlormid loses an allyl group to form N-allyl-2,2-dichloroacetamide, which may be oxidized to dichloroacetic acid. The

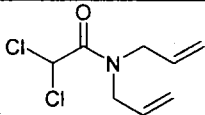
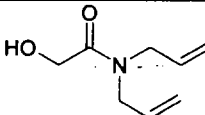
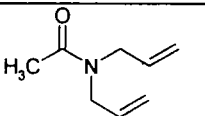
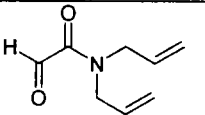
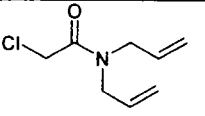
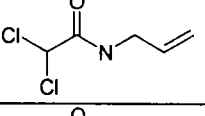
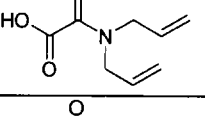
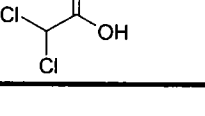


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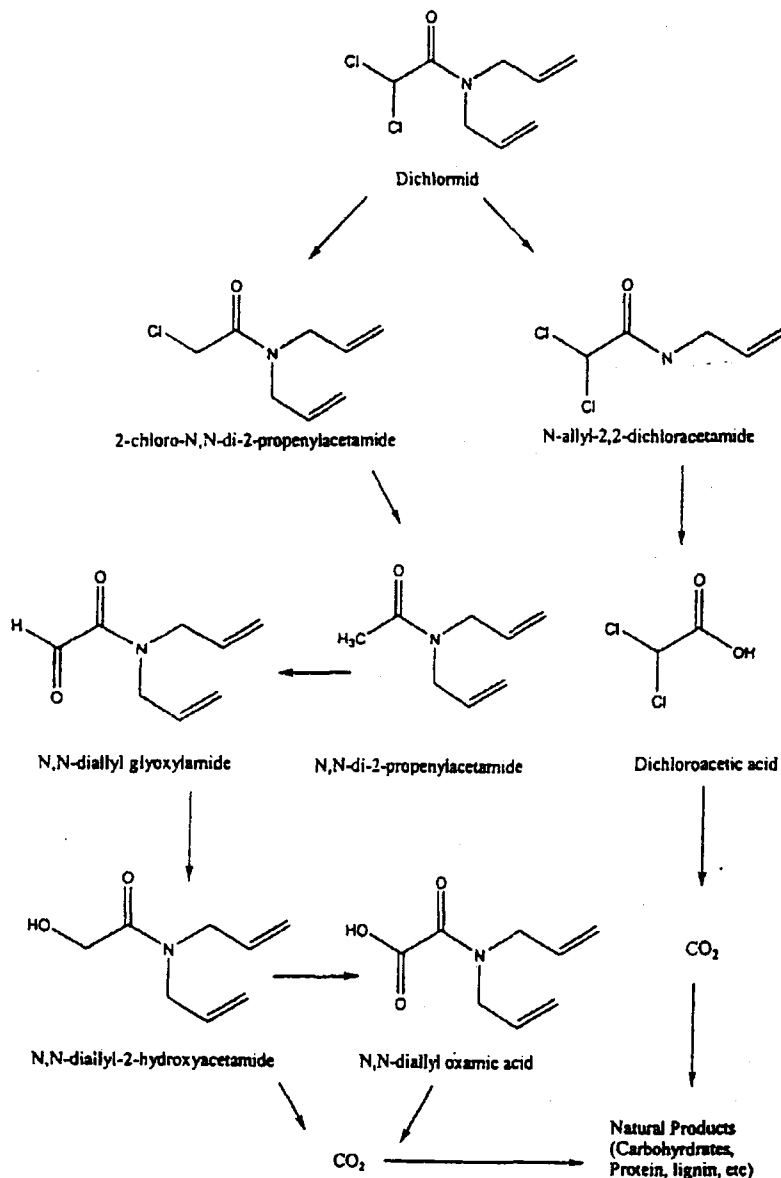
final product of both routes is CO₂, which can then be re-assimilated into endogenous plant cell components including sugars, starches, lignin, protein, and starch. This pathway is consistent with that proposed for the primary crop corn (MRID 46015801). Figure C.3.1. shows schematically the proposed metabolic pathways of dichlormid in rotational crops.

TABLE C.3.1. Identification of Compounds from the Confined Rotational Crop Study	
Dichlormid (parent)	
N,N-diallyl-2-hydroxyacetamide (N,N-diallyl glycolamide)	
N,N-di-2-propenylacetamide	
N,N-diallyl glyoxylamide	
2-chloro-N,N-di-2-propenylacetamide	
N-allyl-2,2-dichloroacetamide	
N,N-diallyl oxamic acid	
Dichloroacetic acid	



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FIGURE C.3.1. Proposed Metabolic Profile of Dichlormid in Rotational Wheat, Carrots, and Soybeans



D. CONCLUSION:

In this confined rotational crop study, wheat, corn and soybean were shown to metabolize dichlormid and incorporate it into cellular components. In every matrix, TRR levels decreased as the DAA increased, as is expected since dichlormid volatilizes from soil over time. Radioactive residues were also found in control matrices, which was likely due to incorporation of ¹⁴CO₂ released from physically proximate dichlormid-treated crops. The majority of the radioactivity was released from the plant matrices upon initial extraction, except for wheat and soybean grain



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and 365 DAA hay. PES were further characterized by acid and base hydrolysis, enzyme digestion, and DMSO extraction to release residues, although further dichlormid-related metabolites were not identified. Dichlormid was extensively metabolized, as the parent was found in only wheat early forage and hay at low levels (at ≤ 0.01 ppm). Based on their partitioning behavior, most of the known and unknown metabolites from all three crops were polar. Dichlormid metabolism in all rotational crops is proposed to involve two routes, involving an initial de-chlorination or loss of an allyl group.

E. REFERENCES:

Currie, L.A. 1968. Limits for qualitative detection and quantitative determination - application to radiochemistry. Analytical Chemistry 40: 586-593.

F. DOCUMENT TRACKING:

RDI: D. Rate (08/25/05); W.Cutchin (08/25/05)
Petition Number(s): 4F6950
DP Barcode(s): D318075
PC Code: 900497

Template Version September 2003



Dichlormid/900497/Pyxant Labs Inc./62719
 DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
 Crop Field Trial - Field Corn

Primary Evaluator Oak Ridge National Laboratory,
 Oak Ridge, TN

Date: 26/APR/2005

Peer Reviewer Debra Rate, Biologist,
 TRB / RD

Date: 25/AUG/2005

This DER was originally prepared by Toxicology and Hazard Assessment Group, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831. The DER has been reviewed by TRB and revised to reflect current OPP policies.

STUDY REPORTS:

MRID No. 46353807. McCormick, R.W. and S.C. Dolder (October 23, 2003.) Magnitude of residue of dichlormid in field corn: Dow AgroSciences Study 020035. Unpublished study prepared by Regulatory Laboratories—Indianapolis Lab, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268-1054, 114 pages.

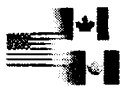
EXECUTIVE SUMMARY: Pyxant Labs Inc. has submitted field trial data for dichlormid on field corn. Eight trials were conducted encompassing EPA Regions 1 (1, PA), 2 (1, VA), 5 (5, IA, IL, IN, OH, WI), and 6 (1, TX) during the 2002 growing season. The number and locations of field trials were chosen in order to satisfy an EPA request for an additional eight magnitude of residue trials to be conducted in EPA regions 1, 2, 5 and 6.

Dichlormid is a safener that is applied with the herbicide acetochlor. At each test location, treatment consisted of a single foliar application to field corn when it was 9-12 inches tall of a mixture of acetochlor at the target rate of 3.0 lb a.i./A (3.4 kg a.i./ha) and dichlormid at the target rate of 0.48 lb a.i./A (0.54 kg a.i./ha). This mixture resulted from dissolving the GF-670 Capsule Suspension formulation of TopNotch Herbicide in water. An adjuvant was not added to the spray mixture in any applications. Forage, grain, and stover were harvested at pre-harvest intervals (PHIs) of 62-77, 102-135, and 102-135 days, respectively.

Residues of dichlormid were quantified using Zeneca Agrochemical's analytical method RAM-244/02, which uses gas chromatography with nitrogen phosphorous detection. Satisfactory method performance in detecting residues was demonstrated by concurrent recoveries. Residues were not shown to be stable in any matrices for the duration of storage during this study. A freezer storage study in progress showed that dichlormid is stable for up to four months in all three matrices. The results from these trials show that maximum residues in forage, grain, and stover never exceeded the method's Limit of Quantitation (LOQ), which is 0.01 ppm. There was no residue decline study.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are classified



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DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3

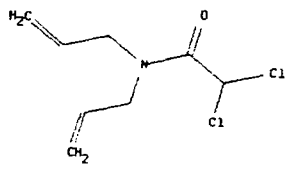
Crop Field Trial - Field Corn

as scientifically acceptable. Although storage stability data has not provided here, adequate storage stability data has been previously submitted for field corn ears, wheat grain and wheat straw (Accession# 005802) for storage duration up to 3 years for the parent compound, dichlormid. **If other residues are found to be of regulatory interest, storage stability studies for those residues will be required.**

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D318075 and in Canada's Regulatory Decision Document.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

A. BACKGROUND INFORMATION: Dichlormid is needed as a safener in the formulation of the GF-670 capsule suspension formulation (TopNotch Herbicide) to prevent damage to the corn due to phytotoxic effects of acetochlor.

TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure 
Common name	Dichlormid
Company experimental name	R-25788
IUPAC name	N,N-diallyl-2,2-dichloroacetamide
CAS name	2,2-dichloro-N,N-di-2-propenylacetamide
CAS #	37764-25-3
End-use product/(EP)	Safener in TopNotch Herbicide



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Parameter	Value	Reference
Melting point/range	5.0-6.5 °C (for 95% pure Technical)	www.agrochemchina.com/dichlormid Said to be 5.5 °C in experimental database for EPIWIN v3.12
pH	6.9	MRID No. 42773501
Density	1.202 g/cm ³ at 20 °C	www.agrochemchina.com/dichlormid
Water solubility (20 °C)	5000 mg/L	Shiu, W.Y et al. (1990) according to EPIWIN v3.12
Solvent solubility (mg/L at __°C)	NA	
Vapour pressure at 25°C	800 mPa	www.agrochemchina.com/dichlormid and also from experimental database for EPIWIN v3.12; converted from 6.00E-3 mm Hg
Dissociation constant (pK _a)	NA	
Octanol/water partition coefficient Log(K _{ow})	1.84	Tomlin, C. (1994) according to EPIWIN v3.12
UV/visible absorption spectrum	NA	

B. EXPERIMENTAL DESIGN:

B.1. Study Site Information:

Trial Identification (City, State/Year)	Soil characteristics	Meteorological data	
	Type	Overall monthly rainfall range (inches)	Overall T°C range
Germansville, PA /2002	Loam	1.1-7.4 also irrigated	13.4-33.2
Goochland, VA /2002	Clay loam	0.0-3.8 also irrigated	2.2-33.6
Carlyle, IL /2002	Silt loam	1.6-5.8	7.4-32.8
Fowler, IN /2002	Loam	0.8-4.6	4.1-30.4
Webster City, IA /2002	Loam	2.0-10.2	1.3-29.3
New Holland, OH /2002	Silt loam	1.9-6.3	7.9-30.9
Arkansaw, WI /2002	Sandy loam	5.3-12.1 also irrigated	1.2-29.4
Uvalde, TX /2002	Clay loam	0.4-15.7 also irrigated	20.4-36.1

The actual temperature recordings were within average historical values for the residue study period. The actual rainfall average was within the historical rainfall average at all sites except for Uvalde, TX, and Arkansaw, WI. The 15.7 inches of rain at the Texas site in July was far above the historical average of 1.7 inches for that month. The 12.1 inches of rain at the Wisconsin site in June was also well above the historical average of 5.9 inches for that month. Irrigation was used to supplement rainfall as needed at the four sites indicated in Table B.1.1.



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TABLE B.1.2. Study Use Pattern.

Location (City, State/Year)	EP ¹	Application					Tank Mix Adjuvants
		Method/Timing	Vol (GPA ²)	Rate ⁴ , (lb a.i./A) (g a.i./ha)	RTI, ³ days	Total Rate ⁴ , (lb a.i./A) (kg a.i./ha)	
Germansville. PA 2002	DA	1. Sprayed when corn was stage BBCH 13-14	31.1 291	0.49 0.55	None	0.49 0.55	None
Goochland. VA 2002	DA	1. Sprayed when corn was stage BBCH 19	19.2 180	0.46 0.52	None	0.46 0.52	None
Carlyle. IL 2002	DA	1. Sprayed when corn was stage BBCH 13-14	25.9 242	0.49 0.55	None	0.49 0.55	None
Fowler. IN 2002	DA	1. Sprayed when corn was stage BBCH 15	19.9 186	0.47 0.53	None	0.47 0.53	None
Webster City. IA 2002	DA	1. Sprayed when corn was stage BBCH 14	20.0 187	0.49 0.55	None	0.49 0.55	None
New Holland. OH 2002	DA	1. Sprayed when corn was stage BBCH 14-15	16.0 150	0.45 0.50	None	0.45 0.50	None
Arkansaw. WI 2002	DA	1. Sprayed when corn was stage BBCH 15	20.1 188	0.48 0.54	None	0.48 0.54	None
Uvalde. TX 2002	DA	1. Sprayed when corn was stage BBCH 17	21.6 202	0.48 0.54	None	0.48 0.54	None

¹ EP = End-use Product = DA = dichlormid and acetochlor in the GF-670 Capsule Suspension formulation of TopNotch Herbicide
² Gallons per acre. L/ha
³ Retreatment Interval
⁴ This is the rate of application of dichlormid itself.



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TABLE B.1.3. Trial Numbers and Geographical Locations		
NAFTA Growing Region	Field Corn	
	Submitted	Requested
		US
1	1	1
1A		
2	1	1
3		
4		
5	4	17
5A	1	
5B		
6	1	1
7		
7A		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
Total	8	20



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Crop Field Trial - Field Corn

B.2. Sample Handling and Preparation:

At each site, a single sample was taken from the control plot, and duplicate composite samples were collected independently from the treated plot. Samples were frozen within four hours of sampling and remained frozen during shipment by ACDS freezer truck to Dow AgroSciences in Indianapolis, Indiana, where they were stored in temperature-monitored freezers at approximately -20 °C. Before extraction, they were frozen with liquid nitrogen and ground using an Agvise Model 2001 Hammermill, which had a 1/8-inch screen.

B.3. Analytical Methodology:

The analytical method used in this study, Zeneca Agrochemicals analytical method RAM 244/02 (Crook, 1994), utilizes gas chromatography with nitrogen phosphorous detection to analyze all residues of dichlormid. Each sample was extracted with methanol and filtered through two Whatman filter papers into vacuum flasks. The extracts were rotary evaporated to 10 -20 ml in a cold water bath. The extracts were brought back to volume with NaCl solution (40 ml) and toluene (5 ml). The sample solution was then cleaned-up using a C¹⁸ solid phase extraction (SPE) column that was prewetted with toluene. The SPE clean-up was performed and the samples analyzed by gas chromatography with nitrogen phosphorus detection. The method's LOD was 0.003 ppm, and its LOQ was 0.01 ppm.

C. RESULTS AND DISCUSSION: Dichlormid levels in samples of corn RACs collected in the eight field trials are shown in Tables C.3 and C.4. Residue data were not corrected for concurrent recoveries. There was a corresponding control sample for each group of experimental samples, and the control residue levels of dichlormid were always below the LOD. (Table 10 on page 28 of the MRID indicated that the level of residue in the untreated control sample of stover in TX was 0.0011 ppm; however, because this is far below the LOD of 0.003, it seems reasonable to conclude that residues were not detected in untreated controls.) All of the RACs had at least one sample that exceeded the LOD, with there being four such samples out of 48 total samples (and 58 readings gathered, including duplicates) overall. However, none of the samples exceeded the LOQ.

The storage stability data submitted with this MRID do not support the storage durations or conditions of samples in the crop field trials, which were kept in frozen storage for intervals ranging up to 270 days after harvest. However, adequate storage stability data (up to 3 years) has been previously submitted for the following RACs: field corn ears, wheat grain and wheat straw (Accession# 005802). Tables C2.2 and C2.3 summarize the storage stability data previously reviewed. It was stated in the storage stability section on page 40 of the MRID that a storage stability study of dichlormid residues in corn grain, forage and stover was in progress at Pyxant Labs (Cervantes, in progress), with early results showing stability through at least 4 months in all three matrices (Table C2.1).

The results in Table C.1 show that the concurrent recoveries were almost all within the



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OPPTS 860.1340 guideline acceptable range of 70-120%. Recoveries overall ranged from 64-113%, with a mean and SD of $83.7 \pm 11.7\%$ ($n = 30$), as calculated by the reviewer. The two values outside the acceptable range were 64% and 67%. These results show that the analytical method effectively measured residues of dichlormid in field corn RACs. The numerous chromatograms presented show that the control samples of various crop matrices are free from interferences.

There was no decline study, which is reasonable considering that none of the RACs had dichlormid residues that exceeded the LOQ. The rather wide range of PHIs used for grain and stover provide no reason to think that dichlormid residues increase with longer PHIs on these commodities.

It seems unlikely that the farming practices used or the reported environmental conditions adversely affected the study. The number of trials and their geographic representation was adequate. The minimum distribution that is recommended (see Table B.1.3) does not apply to this study because it was done to satisfy an EPA request for an additional eight trials to be conducted in EPA regions 1, 2, 5 and 6 (Memo. S.Chun, 21/SEP/1999). The eight trials represent geographically diverse areas to complement the previously submitted field trial data (MRID No. 43616401) for post-emergent treatment of field corn using this pesticide formulation. These studies provide strong support for the view that levels of residue of dichlormid are extremely low.

TABLE C.1. Summary of Concurrent Recoveries of Dichlormid from Field Corn Forage, Grain and Stover.

Matrix	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean \pm std. dev.
Dichlormid				
Forage	0.10	3	73-75	74.3 \pm 1.2
Forage	0.01	6	73-95	86.3 \pm 8.0
Grain	0.10	4	64-87	76.0 \pm 12.2
Grain	0.01	8	72-110	87.1 \pm 12.2
Stover	0.10	3	78-82	79.3 \pm 2.3
Stover	0.01	6	70-113	88.5 \pm 16.3

TABLE C.2.1 Summary of Storage Conditions

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
Forage	approximately -20 °C	162- 270	was being tested
Grain	approximately -20 °C	87-211	was being tested
Stover	approximately -20 °C	115-228	was being tested



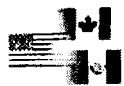
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Time Interval (days)	Corrected % Recovery ^a
0	93
96	101
240	86
360	99
751	95
1095	73

^a Each value is the average of 2 individual determinations.

RAC	Time Interval (days)	Corrected % Recovery ^a
Wheat Grain	0	93
	240	88
	818	96
	1240	100
Wheat Straw	0	93
	240	96
	818	96
	1240	87

^a Each value is the average of 2 individual determinations



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TABLE C.3. Residue Data from Crop Field Trials with Dichlormid.						
Trial ID (City, State/Year)	Region	Crop/ Variety	Commodity or Matrix	Total Rate ¹ , (lb a.i./A) (kg a.i./ha)	PHI (days)	Residues of Dichlormid ² (ppm)
Germansville, PA 2002	1	Doebler's 609XRR	Forage	0.49 0.55	70	<LOD: <LOD. <LOD
Goochland, VA 2002	2	Dekalb 687 RR AR	Forage	0.46 0.52	77	<LOD: <LOD
Carlyle, IL 2002	5	Burrus 442	Forage	0.49 0.55	66	<LOD: <LOD: <LOD
Fowler, IN 2002	5	Pioneer 34A55	Forage	0.47 0.53	71	<LOD: <LOD
Webster City, IA 2002	5	N2555BT	Forage	0.49 0.55	64	<LOD: <LOD
New Holland, OH 2002	5	SC11R12	Forage	0.45 0.50	68	(0.0046) ³ : <LOD
Arkansaw, WI 2002	5A	Pioneer 3751	Forage	0.48 0.54	70	<LOD: <LOD. <LOD
Uvalde, TX 2002	6	Pioneer 32A59	Forage	0.48 0.54	62	<LOD: (0.0031) ³
Germansville, PA 2002	1	Doebler's 609XRR	Grain	0.49 0.55	109	<LOD: <LOD: <LOD
Goochland, VA 2002	2	Dekalb 687 RR AR	Grain	0.46 0.52	120	<LOD: (0.0033) ³
Carlyle, IL 2002	5	Burrus 442	Grain	0.49 0.55	135	<LOD: <LOD. <LOD
Fowler, IN 2002	5	Pioneer 34A55	Grain	0.47 0.53	115	<LOD: <LOD. <LOD
Webster City, IA 2002	5	N2555BT	Grain	0.49 0.55	131	<LOD: <LOD
New Holland, OH 2002	5	SC11R12	Grain	0.45 0.50	102	<LOD: <LOD
Arkansaw, WI 2002	5A	Pioneer 3751	Grain	0.48 0.54	121	<LOD: <LOD. <LOD
Uvalde, TX 2002	6	Pioneer 32A59	Grain	0.48 0.54	104	<LOD: <LOD

¹This is the rate of application of dichlormid itself.

²Semicolons separate the two samples; results of duplicate analyses of a single sample are separated by commas.

³A result shown in parentheses is below the LOQ but at or above the detection level.



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TABLE C.4. Summary of Residue Data From Crop Field Trials with Dichlormid									
Commodity	Total Applic. [Target] Rate ¹ (lb a.i./A) (kg a.i./ha)	PHI (days)	Residue Levels (ppm)						
			n ²	Min.	Max.	HAFT ²	Median (STMdR)	Mean (STMdR) Std. Dev.	Std.Dev.
Dichlormid									
Forage	0.48 0.54	62-77	19	<0.003	0.0046	<0.003	<0.003	<0.003	0
Grain	0.48 0.54	102-135	20	<0.003	0.0033	<0.003	<0.003	<0.003	0
Stover	0.48 0.54	102-135	19	<0.003	0.003	<0.003	<0.003	<0.003	0

HAFT = Highest Average Field Trial.
¹This is the rate of application of dichlormid itself.
²Includes duplicate analyses of a some samples.

D. CONCLUSION:

The field trial residue data are classified as scientifically acceptable contingent upon the analytical method measuring all of the residues of concern for this safener. At this time, only the parent compound is required for storage stability studies. The study crop field trials were well conducted and appear to indicate that the level of dichlormid residues in field corn RACs is extremely low. There was no decline study, but none may be required in view of the extremely low residue levels. This study may be adequate to make decisions regarding the use of dichlormid in combination with acetochlor.

E. REFERENCES:

Crook, S.J. (1994) The Determination of Acetochlor and R-25788 (Dichlormid) in Maize Grain, Forage and Fodder; Soybean Seed and Hay. RAM-244-02, unpublished method of Zeneca Agrochemical.

Cervantes, T.J. (in progress) Pyxant Labs Inc Study Number Dow-1424, "A Frozen Storage Stability Study for Dichlormid Residues on Corn Grain, Corn Forage, and Corn Stover", a study in progress at the Pyxant Labs Inc. in 2002.

PP#: 6F3344, DP Barcode: D248305, S. Chun, 09/21/99.

F. DOCUMENT TRACKING:

RDI: D. Rate (08/25/05); W. Cutchin (08/25/05)
 Petition Number(s): 4F6950
 DP Barcode(s): 318075
 PC Code: 900497



13544

R115319

Chemical: Acetamide, 2,2-dichloro-N,N-di-2-propeny

PC Code: 900497
HED File Code 11500 Petition Files Chemistry
Memo Date: 09/14/2005
File ID: DPD318075
Accession Number: 412-06-0006

HED Records Reference Center
10/06/2005