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DYNAMAC CORPORATION

ARSENAL

EAB# 5253

Final Report

Task 1: Review and Evaluation of Individual Studies

Task 2: Environmental Fate and Exposure Assessment

Contract No. 68-02-4250

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Environmental Protection Agency
Arlington, VA 22202

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ARSENAL

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INTRODUCTION

This report is a scientific evaluation of environmental fate data for Arsenal submitted under Accession No. 258899. In addition to the two studies reviewed herein, several studies that were previously reviewed by EAB are in the EAB files. The contribution of studies to the fulfillment of EPA Data Requirements for Registering Pesticides is considered under Recommendations.

STUDY 1

Mallipudi, N.M., July 18, 1985. Arsenal herbicide, [AC 243,997] [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid]: Weed and soil metabolism in a field plot. American Cyanamid Company, Agricultural Research Division, Princeton, NJ. Report No. PD-M, Vol. 22-23. Acc. No. 258899, Ref: Book 2, Exhibit 4.2.

This study was submitted by American Cyanamid Company in response to an EPA request for a nonguideline study to determine the environmental fate of Arsenal in weeds and soil, specifically the contribution of pesticide residues from plants to the soil.

PROCEDURE

The small field plot (4 x 4 feet) was a Princeton sandy loam (53% sand, 39% silt, 8% clay, 0.9% organic matter, pH 4.8, CEC 7.6 meq/100 g) plot covered with two types of grasses and six types of broadleaf weeds (Table 1). Pyridine-ring-labeled [^{14}C]arsenal (11 μCi/mg, 98% pure) formulated as the isopropylamine salt, with a surfactant, was applied over the plants at 1.0 lb acid equiv/A.

Soil samples were collected in 3-inch increments from the plot on day 0, 1, 4, 8, 15, 22, 29, 104, and 231 up to a depth of 27 inches. Plant samples (shoots and leaves) were collected on day 0, 1, 4, 8, and 15 after which the plants started to die.

Soil samples were collected outside of the treated plot on day 104 and 231 to check for lateral movement of pesticide residues.

METHODOLOGY

Soil Samples: Radioactivity in most samples was determined by combustion (O_2) and radioassay (LSC).

Radioactivity in the day 8 (0-3 inch), day 104 (0-3 and 3-6 inch) and the day 231 (0-3 and 3-6 inch) was determined by extraction with sodium hydroxide (0.1 N). The basic soil slurry was centrifuged, the supernatant decanted, and the extract adjusted to pH 1 then centrifuged again to remove the humic acid. The aqueous supernatant was adjusted to pH 3.5 and centrifuged to remove additional humic acid. ^{14}C in the supernate was determined by LSC. The supernatant was saturated with sodium chloride, filtered, then passed through a C-18 SEP-PAK column.

^{14}C retained on the SEP-PAK was eluted with methanol, concentrated (rotary evaporator) and radioassayed (LSC). The humic acid and soil marc were air dried. ^{14}C in the final aqueous layer (unextractable ^{14}C) was determined by LSC and in the humic acid and the soil marc by combustion (O_2) then LSC.

Plant Samples: Plant shoots and leaves were rinsed with methanol to remove surface radioactivity. The rinses were combined, radioassayed (LSC), concentrated (0.5 ml, roto-evaporator) then analyzed by TLC. Rinsed plant samples were frozen (dry ice), ground, and refrigerated to allow for CO_2 dissipation. Subsamples were combusted (O_2) then radioassayed (LSC).

The day 8 plant samples were extracted by homogenizing the grated plant material three times with methanol:hydrochloric acid (100:0.75, v/v). The resulting slurry was centrifuged, the supernate decanted, and aliquots radioassayed (LSC). The remaining extract was concentrated (0.5 ml, roto-evaporater) then analyzed by TLC. The extracted plant material was air-dried, combusted (O_2) and evolved $^{14}CO_2$ radioassayed (LSC). TLC of the organic extract was carried out on silica gel plates using the following solvent systems:

Acetone

Ethyl acetate:methanol (1:1, v/v)

2-Butanone:pyridine:water:acetic acid
(14:3:3:4, v/v/v/v)

Dichloromethane:n-propanol:water:acetic acid
(102:150:24:24, v/v/v/v)

Dichloromethane:n-propanol:water:formic acid
(102:150:24:24, v/v/v/v)

Nonradioactive reference compounds were also chromatographed in these systems for identification of products by comparison of R_f values. Non-radioactive compounds were located by UV fluorescence quenching and radio-active materials by autoradiography. Radiolabeled materials were quantified by zonal scraping and radioassay (LSC).

RESULTS

Soil: Radioactive residues in the top 3 inches of soil inside the plot increased from an initial concentration of 0.008 ppm on day 0 to 0.234 ppm on day 231 posttreatment (Table 2). The majority of ^{14}C residues was in the top 3 inches of soil with varying amounts in the 3- to 6- and 6- to 9-inch layers. There was no detectable ^{14}C (<0.005 ppm) below 9 inches from day 29 through day 104.

Radioactive residues on days 104 and 231 (0-3 inch), three inches outside the treated field plot were 0.030 and 0.007 ppm, respectively. Below the 3-inch soil depth, ^{14}C residues were below the method detection limit (<0.005 ppm).

There was no rainfall during the first 8 days of the experiment, approx-imately 0.3 inches between 9 and 15 days, 1.0-inch between days 23 and 29, more than 3 inches between 30 and 104 days and more than 7.6 inches be-tween days 105 and 231.

Methanol extractable radioactivity for day 8, 104, and 231 soil samples ranged from 78-97% of total radioactivity in the soil (Table 3). ^{14}C resi-dues remaining in the aqueous phase, humic acid and soil marc ranged from 2.8% to 8.6% of total ^{14}C in the soil for all soil depths and sampling intervals.

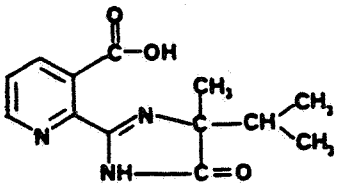
Five degradates were identified by TLC as CL 9,140, CL 60,032, CL 247, 087, CL 247,271 and CL 252,974 (Figure 1). The major compound isolated from the soil was the parent which accounted for 78-90% of extractable ^{14}C (Table 4).

The major degradate recovered from the soil extract was CL 252,974 which accounted for 6.9-13% of the extractable radioactivity. Several unidentified degradates were reported for day 104 (0-3 and 3-6 inch depth) and day 231 (3-6 inch) which were 3.2, 5.1, and 1.9% of the extractable ^{14}C. CL 247,087 was present in the day 8 (3.4%) and day 104 samples (4.2, 1.6%) but not in the day 231 soil samples. CL 247,271 was found in the day 231 (0-3 inch, 1%) sample only.

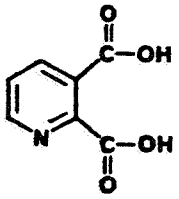
Plant Samples: The distribution of radioactivity between surface wash (external) and plant homogenate (internal) in different weeds showed that applied ^{14}C penetrated rapidly into the plants (Table 5). Less than 18% of the applied ^{14}C (except velvetleaf) could be removed by a methanol rinse one hour after application. Total ^{14}C residues declined rapidly in large crabgrass, velvetleaf and ragweed with a half-life of approximately 24 hours then remained relatively constant through day 8 then decreased again until the last sampling time of 15 days when the plants started to die. For barnyard grass, the ^{14}C half-life was approximately 4 days then the residues remained fairly constant through day 15. ^{14}C residues in horseweed remained relatively constant from day 0 through day 15 posttreatment.

External ^{14}C in all samples was identified by TLC as Arsenal. Internal ^{14}C in the day 8 large crabgrass and ragweed samples was readily extractable with only about 4-5% remaining in the plant marc after extraction.

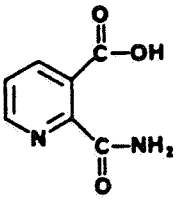
Four degradates present in plant extracts were identified as CL 9,140, CL 60,032, CL 247,087 and CL 252,974. The major compound isolated was the parent which comprised ~78% of the extractable radioactivity (Table 6). Several unknowns totaling <10% of the extractable ^{14}C were present in both ragweed and crabgrass extracts. No attempt was made to identify these degradates. CL 247,087 was 6.5% of extractable ^{14}C for ragweed and levels of all other degradates in both extracts were less than 4%.



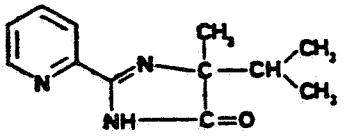
2-(4'-Isopropyl-4'-methyl-5'-oxo-2'-imidazolinyl) nicotinic acid
(AC 243,997)



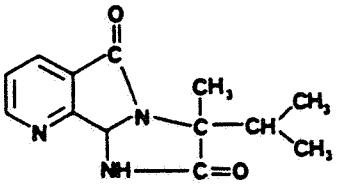
2,3-Pyridedicarboxylic acid
(CL 9,140)



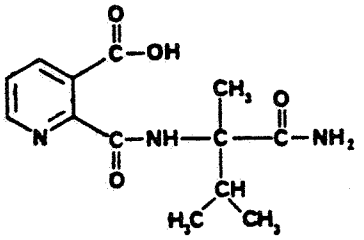
2-Carbamaylnicotinic acid
(CL 60,032)



2,(2'-Pyridyl)-4-methyl-4-isopropyl-5-oxo-imidazole
(CL 247,271)



[2,3b]-(2-Methyl-2-isopropyl-3-oxo)-imidazolidino-
[2,3b]-pyrido-1-oxo-pyrrole
(CL 247,087)



2-[2'-Carbamyl-N-2',3'-dimethylbutamido]-nicotinic acid
(CL 252,974)

Figure 1. Structures of Arsenal and degradates.

Table 1. Weed species present in the test plot in Princeton, New Jersey and treated with [^{14}C]arsenal.

Grasses	
Digitaria sanquinalis	Large Crabgrass
Echinochloa crus-galli	Barnyardgrass
Broadleaves	
Abutilon theophrasti	Velvetleaf
Ambrosia artemissifolia	Common Ragweed
Conyza canadensis	Horseweed
Daucus carota	Wild Carrot
Plantago lanceolata	Plantain
Xanthium pensylvanicum	Common Cocklebur

Table 2. ^{14}C Distribution (ppm) at different soil depths after application of [^{14}C]-arsenal at 1.0 lb ai/A over weeds in a sandy loam soil plot.[a]

Sampling depth (inches)	Sampling interval (days)								
	0	1	4	8	15	22	29	104	231
0-3	0.008	0.012	0.030	0.029	0.094	0.148	0.179	0.223	0.234
3-6	-	0.006	0.010	0.011	0.008	0.026	0.008	0.074	0.073
6-9	-	-	0.008	<0.005	0.009	0.009	0.006	0.017	0.016
9-12	-	-	-	b	b	b	b	b	0.015
12-15	-	-	-	-	b	b	b	b	b
15-18	-	-	-	-	-	-	b	b	b
18-21	-	-	-	-	-	-	b	b	b
21-24	-	-	-	-	-	-	-	b	b
24-27	-	-	-	-	-	-	-	-	b

[a] Ppm calculated as arsenal equivalents.

[b] Nondetectable levels (<0.005).

Table 3. Distribution of ^{14}C residues following the aging of [^{14}C]arsenal in a field plot with weeds at 1.0 lb acid equiv/A.

Sampling interval (days)	% ^{14}C extracted							
	Organic phase[a]		Aqueous phase		Humic acid		Soil marc	
	0-3 in	3-6 in	0-3 in	3-6 in	0-3 in	3-6 in	0-3 in	3-6 in
8	97	-	2.8		4.8		4.2	
104	89	94	4.9	3.8	5.5	3.7	6.8	4.4
231	78	85	7.4	6.2	7.9	8.6	4.3	5.5

a Methanol extract of soil treated with 0.1 N NaOH.

Table 4. Distribution of ^{14}C residues (% of extractable) in the organic extract[a] of the soil following treatment with [^{14}C]arsenal in a field plot with weeds at 1.0 lb acid equiv/A.

Sampling interval (days)	Arsenal		CL 9,140		CL 60,032		CL 247,087		CL 247,271		CL 252,924		Unidentified	
	0-3 in	3-6 in	0-3 in	3-6 in	0-3 in	3-6 in	0-3 in	3-6 in	0-3 in	3-6 in	0-3 in	3-6 in	0-3 in	3-6 in
8	81 (0.023)		0.2 (b)		3.0 (b)		3.4 (b)		-		13 (b)		-	-
104	78 (0.153)	76 (0.053)	2.2 (0.005)	1.0 (b)	1.6 (b)	2.9 (b)	4.2 (0.008)	1.6 (b)	-	-	12 (0.023)	13 (b)	3.2 (2)[c]	5.1 (3)[c]
231	90 (0.163)	87 (0.054)	1.0 (b)	0.5 (b)	2.4 (b)	2.7 (b)	-	-	1.0 (b)	-	6.9 (0.013)	7.9 (0.005)	-	1.9 (2)[c]

[a] Methanol extract of soil following treatment with 0.1 N NaOH.

[b] Below detection limit (<0.005 ppm).

[c] Number of unidentified compounds.

Table 5. Distribution of [^{14}C]arsenal residues (ppm)[a] in plants after application at 1.0 lb ai/A.

Time (days)	Crabgrass		Barnyard grass		Velvetleaf		Ragweed		Horseweed	
	Ext[b]	Int[c]	Ext	Int	Ext	Int	Ext	Int	Ext	Int
0	4.2	67	8.3	50	46	142	17	118	6.6	60
1	1.8	20	5.4	53	5.4	54	3.5	72	2.7	44
4	1.3	26	2.0	26	1.9	28	2.2	74	1.3	57
8	0.8	27	0.9	31	2.0	25	1.2	65	0.7	53
15	0.4	25	0.3	24	0.2	14	0.6	30	1.2	73

[a] Ppm calculated as arsenal equivalents.

[b] Surface wash (external).

[c] Plant homogenate (internal).

Table 6. Distribution of plant degradation products (% of recovered) in extracts from day 8 weed samples.

Compound	Large crabgrass	Ragweed
Arsenal	78 (21)[a]	77 (53)
CL 9,140	3.5 (0.95)	1.3 (0.89)
CL 60,032	3.0 (0.81)	2.5 (1.7)
CL 247,087	2.8 (0.76)	6.5 (4.4)
CL 252,974	3.2 (0.87)	2.9 (2.0)
Others[b]	9.8 (3)[c]	9.7 (6)[c]

[a] Number in parentheses represents amount in ppm calculated as arsenal equivalents.

[b] Unidentified ^{14}C degradates.

[c] Number in parentheses represents number of different unknown compounds.

CONCLUSIONS

[^{14}C]Arsenal was readily absorbed by five weeds at 1.0 lb acid equiv/A. External rinses less than one day post-application recovered <18% of applied ^{14}C. Internal ^{14}C residues decreased rapidly within 24-48 hours then leveled off through day 15 when the weeds started to die.

The decrease in ^{14}C residues in plants coincided with an increase in soil ^{14}C residues. The majority of ^{14}C was in the top 3 inches of soil where total residues increased from 8 ppb on day 0 to 29 ppb on day 8. Since there was no rain during the first eight days after application, increased soil ^{14}C residues can be attributed to translocation from plant shoots to roots and exudation into the soil. ^{14}C residues in soil continued to increase and peaked on day 104. There was little change between days 104 and 231 due to colder temperatures. Trace amounts of ^{14}C was only found in the top 3 inches of soil at a distance of 3 inches outside the plot indicating a low potential for lateral movement of arsenal.

The major compound isolated from both soil and plants was the parent which comprised ~80% of the extractable radioactivity. Degradates CL 9,140, CL 60,032, CL 247,087 and CL 252,974 were identified both in soil and plants. Trace amounts of degradate CL 247,271 were identified from soil samples only. The major soil degradate was CL 252,974 which was present at a concentration four times greater than in the plant.

The study does not meet the data requirement. However, the study was an EPA nonguideline request for determination of soil pesticide residues from treated (crop) plants.

STUDY 2

McAllister, W.A., B. Bunch, and J. Burnett. July, 1985. Bioconcentration and depuration of ^{14}C-AC 243,997 by bluegill sunfish (Lepomis macrochirus). American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey. Report No. ABC 32819, Acc. No. 258899. Ref. Book 2, Exhibit 4.3.

PROCEDURE

Bluegill sunfish (mean weight of 4.5 g and mean length of 53 mm) were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, was maintained. During the holding, acclimation and test periods, the fish received a standard commercial fish food daily in an amount equivalent to 3% of body weight.

Flow-through aquatic exposure systems were prepared using two 100-l aquaria equipped with continuous-flow proportional dilution apparatus as described by Mount and Brungs (1967. Water Res. 1:21). Aerated well water (pH 7.8-8.3, total hardness ($CaCO_3$) 255-275 ppm, 9.2-10.2 ppm dissolved O_2) was delivered to the aquaria at a rate sufficient for 6.0 volume changes in 24 hours. Test aquaria were immersed in a water bath and held at 22 ± 1 C. [14C]Arsenal, (4.48 x 109 DPM), in dimethylformamide, was supplied to the

test aquarium at 1.0 mg/l. The control aquarium received dimethylformamide only.
The test solution was allowed to flow through the test aquaria for a 24-hour equilibration period and the test concentration confirmed by radioanalysis.

The uptake phase was initiated by transferring groups of 120 fish each to the control and test aquaria and observing them initially then every 24 hours during the exposure period for mortality and adverse behavior. Water and fish were sampled throughout the uptake period on day 1, 3, 7, 14, 21 and 28.

On day 28, addition of radiolabeled material to the test aquarium was stopped and the water siphoned from both test and control aquaria to a level 3-inches above the bottom. The aquaria were filled with approximately 70 liters of uncontaminated well water then siphoned again to 3 inches. This procedure was repeated, then the fish were exposed to flowing uncontaminated well water for 14 days. During the depuration period, water and fish were sampled and radioassayed on day 1, 3, 7, 10 and 14.

METHODOLOGY

All water and tissue samples were radioassayed (LSC). Fish from the control and treated aquariums were dissected into filet and viscera in triplicate at each sample interval. Three fish from each aquarium were collected at each interval for whole fish analysis. Fish samples were stored frozen until radioassay when individual samples were homogenized with dry ice in a grinder, allowed to sublime, weighed and combusted (O_2).

Mean recovery data for [^{14}C]arsenal in tissue sample oxidations was 98% for all tissue types.

RESULTS

Radioassay of fillet, whole fish and viscera after 28 days of exposure to 1.0 mg/l [^{14}C]arsenal and 14 days of depuration showed that all mean ^{14}C tissue concentrations were less than the radioassay minimum quantifiable limits (MQL) of 0.54, 0.55 and 0.57 mg/kg, respectively. These data suggests that there was no active or passive transport of the parent across the gill membrane. There was no adverse behavior in fish in the control or treated chambers and no mortality was observed in fish in the test chambers during this study.

CONCLUSION

[^{14}C]Arsenal, at 1.0 mg/l, does not accumulate in bluegill sunfish exposed in a flow-through system. The study meets the data requirements for registration.

EXECUTIVE SUMMARY

[^{14}C]Arsenal was readily adsorbed by five weeds in <1 day postapplication. Internal ^{14}C residues decreased rapidly within 24-48 hours then leveled off through day 15 when the weeds started to die.

The decrease in ^{14}C residues in plants coincided with an increase in soil 14C residues. The majority of 14C was in the top 3 inches of soil. Trace amounts of 14C were found only in the top 3 inches of soil at a distance of

3 inches outside the plot, indicating a low potential for lateral movement of arsenal.

The major compound isolated from both soil and plants was the parent, which comprised ~80% of the extractable radioactivity. Degradates CL 9,140, CL 60,032, CL 247,087, and CL 252,974 were identified both in soil and plants. Trace amounts of degradate CL 247,271 were identified from soil samples only. The major soil degradate was CL 252,974 which was present at a concentration four times greater than in the plant.

There was no bioaccumulation of [^{14}C]arsenal from a water concentration of 1.0 mg/l by bluegill sunfish during 28 days of exposure.

RECOMMENDATIONS

Available data are insufficient to fully assess the environmental fate of arsenal. The submission of data to fulfill registration requirements (Subdivision N) is summarized below:

Hydrolysis studies: Based on previous EAB reviews, data requirements have been fulfilled.

Photodegradation studies in water: Based on previous EAB reviews, data requirements have been fulfilled.

Aerobic soil metabolism studies: Based on previous EAB reviews, a study is needed using ^{14}C-labeling in another portion of the molecule (other than carboxyl-label) to better define and identify degradates.

Leaching and adsorption/desorption studies: Based on previous EAB reviews, data requirements have been fulfilled.

Terrestrial field dissipation studies: One study was reviewed (Mallipudi, 1985, Acc. No. 258899) that does not satisfy data requirements. However, the study was an EPA nonguideline request for determination of soil pesticide residues from treated plants.

Laboratory studies of pesticide accumulation in fish: One study was reviewed (McAllister et al., 1985, Acc. No. 258899) that is scientifically valid and fulfills data requirements by showing that [^{14}C]arsenal does not bioaccumulate in bluegill sunfish.

REFERENCES

Mallipudi, N.M., July 18, 1985. Arsenal herbicide, AC 243,997 [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid]: Weed and soil metabolism in a field plot. American Cyanamid Company, Agricultural Research Division, Princeton, NJ. Report No. PD-M, Vol. 22-23. Acc. No. 258899, Ref: Book 2, Exhibit 4.2.

McAllister, W.A., B. Bunch, and J. Burnett. July, 1985. Bioconcentration and depuration of ^{14}C-AC 243,997 by bluegill sunfish (Lepomis macrochirus). American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey. Report No. ABC 32819, Acc. No. 258899. Ref. Book 2, Exhibit 4.3.