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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Aldicarb (List A, Case 0140, Chemical 098301). Nature of the Residue in Lemons (171-4(a)). Supplemental Study. Rhone-Poulenc Ag Co. DP Barcode D225085. CBRS 17122. MRID 43970001.

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RPAC previously submitted a nature of the residue in citrus study for aldicarb. The study was found acceptable for fulfilling confirmatory data requirements for GLN 171-4(a) of the *Residue Chemistry Chapter* of the *Reregistration Eligibility Decision Document* (S. Funk, CBRS 16846, DP D222877, MRID 43902401, 03/07/96; S. Funk, CBRS 16666, DP D221975, 01/23/96; S. Funk, CBRS 16933, DP D223439, 03/14/96). The nature of the residue in/on lemons from the treatment of the soil around the tree with aldicarb is adequately understood. The aldicarb is completely oxidized to aldicarb sulfoxide (minor) and aldicarb sulfone, and these compounds form a variety of derivatives via *elimination of the methylcarbamoyl group and substitution at the C-1 carbon*, such as aldicarb sulfone oxime, aldicarb sulfone amide, aldicarb sulfone acid, and aldicarb sulfoxide acid. The oxime forms a glucoside conjugate and the acids form glycoside conjugates. The sulfone and sulfone derivatives further degrade to methane sulfonic acid.

It was concluded that in the absence of other cholinesterase-inhibiting metabolites, the residue of concern will remain the parent and its sulfone and sulfoxide.

The registrant now submits a supplement to the original study. The performing laboratory is PTRL West, Inc., Richmond. Additional characterization was conducted on foliage extracts, and LC/MS identifications were made of some metabolites.

Guideline	MRID	Acceptability	Additional Requirements
171-4(a)	43970001	Fully acceptable	None

Conclusions

1. The acetone and acetone-water extracts of lemon leaves from a previous metabolism study were analyzed by HPLC, and identifications were confirmed by LC/MS/MS. About 93% TRR was released by extraction, and about 77% TRR was identified. The major components of the extract were aldicarb sulfone amide and conjugates of aldicarb sulfone amide, aldicarb sulfone oxime, aldicarb sulfone acid, aldicarb sulfoxide amide/aldicarb sulfone aldehyde, aldicarb sulfoxide alcohol, and aldicarb sulfone alcohol. The metabolism involves elimination of the methylcarbamoyl group and substitution at the C-1 carbon. Methane sulfonic acid, a significant portion of the radiolabeled residue in whole lemons and presumably a terminal metabolism/degradation product, was not found in the present supplemental study.

2. The metabolic pathway previously identified for lemons is confirmed by the present report on lemon leaves. The metabolites of concern are the cholinesterase-inhibiting compounds aldicarb, aldicarb sulfone, and aldicarb sulfoxide.

Recommendation

CBRS recommends that no additional work be required for the nature of the aldicarb residue in/on plants. The requirements of GLN 171-4(a) have been adequately fulfilled. CBRS further recommends that the residue of concern in plant raw agricultural commodities remains as currently defined in 40 CFR §180.269.

Detailed Considerations

Field Phase

The field phase was previously reviewed (S. Funk, CBRS 16846, DP D222877, 03/07/96).

Analysis Phase

The extraction and analysis of the radiolabeled residues in peel, pulp, and foliage were previously reviewed (S. Funk, CBRS 16846, DP D222877, 03/07/96). It was originally reported that foliage contained 17.9 ppm ^{14}C-aldicarb equivalents. Sequential extraction with acetone, acetone/water, and 0.1 N HCl removed 61.6% TRR, 27.9% TRR, and 1.9% TRR, respectively. The extracts were not further characterized or analyzed. Additional analyses of foliage are now reported.

Seven extraction schemes were tested, with total percentages extracted ranging from 14% to 93%. The use of polar solvents was most effective in extracting the radiolabeled residue. The registrant now reports a foliage TRR value of 19.5 ± 1.3 ppm. Scheme VII was used for the extraction of residues for metabolite identifications. A 5 g sample was extracted sequentially with acetone/water (3/1, 50 ml, v/v) and water (50 ml). About 91% TRR and 2% TRR were removed, respectively. The extraction scheme was replicated three times. The acetone/water extracts were combined, and the water extracts were combined. The solid residue was oven-dried. A subsample was combusted, and the $^{14}CO_2$ was determined. Less than 2% TRR (0.35 ppm) remained in the postextraction solid.

The extracts were concentrated by rotary evaporation, cleaned up with C18 SPE cartridges, and analyzed by HPLC with radiochemical detector and by LC/MS/MS. Recovery from the SPE cartridge was 93 - 96%.

Foliage extracts, after concentration and clean-up, were hydrolyzed with β-glucosidase. Samples were incubated for 3 hrs at 37° C. The product mixtures tested positive with glucose testing strips (Chemstrip bG) for glucose liberation.

Aliquots of β-glucosidase-treated extracts were hydrolyzed with 1 N HCl (1.5 hr., 40 - 100° C). Aliquots of extracts that had not been treated with β-glucosidase were also hydrolyzed with 1 N HCl (1 hr., 100° C). The hyrdolysates were analyzed by HPLC and by LC/MS/MS.

The HPLC method for metabolite identification involved use of a YMC ODS-AQ column (25 cm X 4.6 mm i.d.). A series of linear gradients was used at a total flow rate of 1 ml/min. The solvent was varied from 90% water/10% acetic acid (1%) at time 0 to 88% water/10 % acetic acid (1%)/2%water-acetonitrile (50/50) at 25 minutes. From 55 minutes to 95 minutes the solvent was changed to 56% water/10% acetic acid (1%)/34%water-acetonitrile (50/50). From 95 minutes to 115 minutes, the solvent was changed to 16% water/10% acetic acid (1%)/74% water/acetonitrile (50/50). Finally, over the next five minutes the solvent was changed to 90% acetonitrile/10% acetic acid (1%). The same elution gradient was used for LC/MS/MS. Radioactivity recovery was monitored by injecting a sample of known

DPM and collecting and analyzing the fractions by LSC. Typical recovery was 104%. The sum of peak areas in a given HPLC chromatogram were assumed equal to 100% of the injected radioactivity.

Table 1 summarizes the metabolites identified in the extracts and hydrolysates of ^{14}C-aldicarb-treated lemons trees foliage. Adequate raw data and chromatograms were presented to permit verification of the reported results.

The registrant postulates the metabolic pathway of Figure 1 for aldicarb on lemon leaves. The figure is a direct reproduction of Rhone-Poulenc's Figure 9. It is in agreement with the metabolic pathway previously proposed (S. Funk, CBRS 16846, DP D222877, 03/05/96). Solvent extraction recovered about 18% TRR, the major component being identified as aldicarb sulfone amide. While aldicarb sulfone amide was only 6% TRR in whole lemons, methane sulfonic acid, a probable decomposition product of the amide, was 18% TRR. Enzyme hydrolysis of the extract released aldicarb sulfoxide alcohol (6% TRR) and aldicarb sulfone oxime (9% TRR). Thus, about 15% TRR was present as glucoside conjugates. This agrees with the 14% TRR identified as glucoside conjugates for whole lemons (S. Funk, CBRS 16846, DP D222877, 03/07/96). About 77% TRR from the acid hydrolysis of the extract was identified. This corresponds to the 64% TRR identified in whole lemons.

cc: S. Funk, RF, Subject File, List A, Circ., Jackie McQueen, CRM 63/Phillip Poli (Special Review Branch, Special Review and Reregistration Division (7508W)).

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Table 1: Metabolites in/on Lemon Leaves from the Application of ^{14}C-Aldicarb to Lemon Trees (1X)[1a, 1b]

Metabolite[2]	Acetone + Acetone/Water Extract		Enzyme Hydrolysis of Acetone + Acetone/Water Extract		Acid Hydrolysis of Enzymolysis Fraction[5]		Acid Hydrolysis of Acetone + Acetone/Water Extract[5]	
	% TRR	PPM	% TRR	PPM	% TRR	PPM	% TRR	PPM
Aldicarb Sulfoxide Acid	0		0		0.60	0.12	2.23	0.43
Aldicarb Sulfoxide Amide	0.74	0.14	0.96	0.19	[3]		[3]	
Aldicarb Sulfoxide Amide + Aldicarb Sulfone Aldehyde	-	-	-	-	32.1[3]	6.25[3]	44.9[3]	8.75
Aldicarb Sulfoxide Alcohol	0		5.65	1.10	4.98	0.97	5.92	1.15
Aldicarb Sulfone Amide	16.3	3.18	17.2	3.36	17.3	3.37	12.0	2.34
Aldicarb Sulfone Acid	0		0		2.75	0.54	4.89	0.95
Aldicarb Sulfone Alcohol	0		2.24	0.44	5.45	1.06	5.75[4]	1.12
Aldicarb Sulfoxide Nitrile	0		0		0		1.25	0.24
Aldicarb Sulfoxide Oxime	0.10	0.02	0.77	0.15	0		0	
Aldicarb Sulfone Oxime	0.46	0.09	9.02	1.76	0		0	
TOTAL	17.6	3.4	35.9	7.0	63.1	12.3	76.9	15.0

[1a] Quantitation was by HPLC. Results were confirmed qualitatively only by LC/MS/MS. A mixture of extracts from lemons treated with labeled (1X) and unlabeled (2X) aldicarb was used for the LC/MS/MS work.
[1b] Base, in part, on the registrant's tables VI - X.
[2] Aldicarb was not detected. A control matrix spiked with ^{14}C-aldicarb and processed at the same time and in the same fashion as the treated foliage showed a 35% loss of aldicarb, 22% of which was attributed to aldicarb sulfoxide.
[3] Lack of chromatographic resolution.
[4] Not confirmed by LC/MS/MS. Interference.
[5] Some metabolites are not stable under the acid hydrolysis conditions. Methane sulfonic acid, aldicarb sulfoxide acid, and aldicarb sulfoxide nitrile increase. Aldicarb sulfoxide amide, aldicarb sulfoxide alcohol, aldicarb sulfone amide, aldicarb sulfoxide oxime, aldicarb sulfone oxime, aldicarb sulfoxide, aldicarb sulfone, aldicarb oxime, and aldicarb decrease. Thus, some of the indicated metabolites may be secondary decomposition products and not the originally released conjugates.

Figure 1: Proposed Metabolic Pathway for Aldicarb in/on Lemon Leaves