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

MAR 7 1996

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)). Rhone-Poulenc Ag Co. DP Barcode D222877. CBRS 16846. MRID 43902401.

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In response to the requirements of the *Registration Standard Update* and a subsequent waiver request (S. Funk, CBRS No. 12890, DP Barcode D197077, 12/21/93), RPAC is now submitting a nature of the residue in citrus study for aldicarb. The study is entitled *A Metabolism Study with[^{14}C]-Aldicarb in Citrus (Lemon Trees)*, Project No. 478W, 01/10/96. The performing laboratory is PTRL West, Inc., Richmond, CA.

The *Aldicarb Guidance Document*, dated 3/30/84, previously determined that the qualitative nature of the residue in plants was

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adequately understood based on studies in cotton, peanuts, potatoes, and sugar beets. However, the *Aldicarb Reregistration Standard Update*, dated 8/20/90, concluded that because the acceptance criteria for plant metabolism data had become more stringent, the available data from these studies were suspect for the following general reasons: (i) data pertaining to the total recovered radioactive residues present at the time of harvest were either lacking or unclear; (ii) solvent extraction efficiencies were not reported; and (iii) although identification of radiolabeled moieties was typically by two-dimensional TLC in multiple solvent systems, confirmation by MS was either unsupported by MS scans or not attempted. Consequently, the *Update* and the ensuing Aldicarb Data Call-In Notice of 3/14/91 required new plant metabolism studies on three dissimilar crops. Alternatively, the registrant was instructed to submit and summarize the existing data in a reformatted format according to current acceptance criteria of Guideline 171-4(a) of Subdivision O.

The registrant submitted reformatted versions of previous studies, and these studies for cotton, peanuts, potatoes, and sugar beets were rejected (R. B. Perfetti, CBRS 10775, DP Barcode D183798, 04/06/93). The registrant was directed to perform three new studies, including an oil seed crop and potatoes.

At a joint meeting of SRRD, CBRS, and Rhone-Poulenc Ag Company, 11/23/93, it was agreed that one nature of the residue study, conducted on either potatoes or citrus, would suffice to fulfill the data requirements for GLN 171-4(a), provided that the study meets all Agency criteria and provided that the study confirms the apparent findings of previous nature of the residue in plants studies (P. J. Poli, SRRD Letter of 12/10/93 to Warren Davis, RPAC; S. Funk, CBRS 12890, DP Barcode D197077, 12/21/93).

Guideline	MRID	Acceptability	Additional Requirements
171-4(a)	43902401	Fully acceptable	None

Conclusions

1. The field phase of the nature of the residue in plants study is fully acceptable. Lemon trees were treated at a 1X rate with 2-methyl-2(^{14}C-methylthio)propionaldehye O-(methylcarbamoyl) oxime by the application of an acetonitrile/water solution to the soil around potted trees. Control trees and trees treated with unlabeled aldicarb (2X) were also used. The trees were maintained in a plastic-covered hoop house, and lemons were harvested 153 days after treatment. The label PHI for lemons is 90 days, and there

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are no PHI's for other citrus crops.

2a. The analytical phase of the nature of the residue in plants study is fully acceptable. The lemons treated with radiolabeled aldicarb were pooled and separated into juice, pulp, and peel, and the radiolabeled residue in each fraction was determined. The residues, as ^{14}C-aldicarb equivalents, were 0.107 ppm in juice, 0.245 ppm in pulp, 0.430 ppm in peel, and 0.234 ppm in whole lemon (calculated). The majority of the radiolabel was extracted from peel and pulp, 87.1% TRR and 88.0% TRR, respectively. The postextraction residues contained 3.8% TRR in peel and 11.4% TRR in pulp.

2b. Significant portions of the radiolabeled residues were identified by HPLC and TLC in the acetone extracts of peel and pulp and in juice. For peel, 54% TRR was identified, with sufone oxime glucoside being the largest component (22% TRR, 0.096 ppm). For pulp, 71% was identified, with sulfone acid being the largest component (20% TRR, 0.048 ppm). For juice, 88% TRR was identified, with methane sulfonic acid and other unspecified polar compounds being the major components (48% TRR, 0.052 ppm).

3. 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.

4. The currently regulated residue of aldicarb in plant commodities consists of aldicarb, aldicarb sulfone, and aldicarb sulfoxide (40 CFR §180.269). The present metabolism study and previous studies have shown none or trace aldicarb in the radiolabeled residue of rac's and variable amounts of sulfone plus sulfoxide (5% TRR - <40% TRR). The sulfone and sulfoxide are the intermediates to the identified radiolabeled metabolites of this study (see Figures 1 and 2) and of previous studies. In the absence of other cholinesterase-inhibiting metabolites, the residue of concern will remain the parent and its sulfone and 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 test crop consisted of approximately 3 year old lemon trees, variety Improved Meyer Lemon, and each tree was about 3.5 - 4.5 feet high. The trees were divided into three groups, 10 trees that were not treated (0X), 10 trees that were treated with unlabelled aldicarb, and 10 trees that were treated with ^{14}C-aldicarb. The trees were obtained at a nursery in Ivanhoe, CA and were repotted eight days before the start of the study in 4 gallon pots. The soil was loamy sand from Stanislaus County, CA. The actual test site were hoop houses (screenhouses) at Hulst Research Farm Services, Inc., Hughson, Stanislaus County, CA. The trees treated with unlabelled aldicarb and the control trees were located in a separate house from those treated with radiolabeled aldicarb. Irrigation was by a drip system after 11/22/94. The plants received 0.18 inches (10/04/94) of rainfall prior to the installation of permanent plastic covers (11/04/94).

The test substance was [^{14}C]-aldicarb, or 2-methyl-2(^{14}C-methylthio)propionaldehye O-(methylcarbamoyl) oxime. The specific activity was determined by PTRL West (HPLC, LSC) to be 239,642 dpm/μg (20.54 mCi/mmol), and the radiochemical purity (HPLC) was 97.0%. Non-labeled aldicarb was RPAC lot no. 22DEQ48, 99.8% pure.

The radiolabeled treatment solution was made by serial dilution of the acetonitrile stock solution with additional acetonitrile. From the final dilution (2.8594 mg/ml, 685,242,000 dpm/ml), exactly 9.9 ml was placed in each of ten jars. At the time of treatment, 10 ml of deionized water was added to each jar. The entire contents of each jar was applied to one of the ten trees labeled for treatment with ^{14}C-aldicarb. Each jar was rinsed with acetonitrile/water (2 X 10 ml, 1/1, v/v) and the rinses were applied to the appropriate trees. The actual amount applied to each tree, as determined by triplicate LSC measurements of 50 μl aliquots of the rinses and subtraction from the total originally in each jar, ranged from 28.302 to 28.308 mg.

The non-labeled aldicarb dosing solution was prepared in acetonitrile (5.682 mg/ml). A 10 ml aliquot was placed in each of ten jars, and 10 ml of water was added to each jar immediately before application. The jars were rinsed with acetonitrile/water (2 X 10 ml, 1/1, v/v), and the rinses were applied to the appropriate trees.

Both the labeled and unlabeled solutions were applied with Pasteur pipets in concentric circles about the trees on 06/29/94. The concentric circle pattern was followed by crisscrosses. About one hour after application, each pot was irrigated with water (100 ml/pot).

The aldicarb label for citrus (Temik 15 G, 264-417) specifies a single application per season at a maximum rate of 5 lbs. a.i./acre, with a 30 day PHI for lemons. The granules are to be spread evenly on and worked into the soil or are to be shanked at 2 to 3 inch depth with 4 - 6 shanks on a 12 inch center or with 2 shanks per furrow in irrigation furrows. It is noted that irrigation or rainfall after application will promote uptake of the aldicarb. The registrant states that a granular formation preparation was not practical with the small amount of available radiolabeled material. Each pot had a 10" diameter at the soil surface, or 75.54 in^2, or 1.25 X 10^{-5} acre. The application rate for the ^{14}C-aldicarb was 28.30 mg/1.25 x 10^{-5} acre, or 4.99 lbs. a.i./acre, or 1X. The application rate for the unlabelled aldicarb was 56.82 mg/1.25 x 10^{-5} acre, or 9.9 lbs. a.i./acre, or 2X.

The three groups of trees were harvested on 11/29/94, 153 days after treatment. The trees carried mature and immature fruit. All lemons >1 inch diameter were harvested and divided into mature and immature fruits within each treatment group. A representative sample of control group leaves, most 2X group leaves, and all ^{14}C-aldicarb group leaves were collected. Foliage was shipped on dry ice and stored frozen. Lemons were shipped on blue ice and stored at cold temperatures until prepared for analysis.

Analysis Phase

Foliage samples were homogenized with dry ice in a food processor and were stored in plastic bags at < 0° C. Whole lemons, pooled by treatment group, were halved and separated from the juice with a Pyrex juicer. Pulp and peel were next segregated with a paring knife. The juice was centrifuged, and the residue was combined with the pulp fraction. Pulp and peel were each homogenized with dry ice in a food processor. Pulp and peel were each stored in plastic bags at < 0° C. Juice samples were stored in amber bottles at < 0° C.

Lemon pulp, peel, and foliage were analyzed for total radiocarbon content by combustion and LSC. Juice was directly analyzed by LSC. The results are summarized in Table 1. Examples of the raw data from which the results were calculated were presented.

Table 1: Distribution of ^{14}C-Radiolabel in Lemons		
Matrix	Total Weight (g)	^{14}C-Aldicarb Equivalents[1] (ppm)
Juice	1840	0.107
Pulp	1373	0.245
Peel	1469	0.430
Whole Lemon	4975	0.234
Foliage	2460	17.9

[1] Based on weight of the particular matrix. Whole lemon is the sum of juice, pulp, and peel; it was not determined separately.

Pulp, peel, and foliage were each extracted sequentially with acetone, acetone/water (1/1, v/v), and 0.1 N HCL. The pulp and peel residues each were refluxed with 0.1 N HCl. Each extract or hydrolysate and the final residue were analyzed for total radiocarbon content. The acetone extracts were partitioned with methylene chloride. Lemon juice was extracted with methylene chloride/acetonitrile (1/1, v/v).

The extraction of residue from pulp, peel, and foliage is summarized in Table 2. The results of hydrolysis with 0.1 N HCl are not reported, and no further analyses of foliage extracts and pulp and peel acid extracts and acid hydrolysates were reported.

Table 2: Extraction of Radiolabeled Residue from Lemons on Trees Treated with ^{14}C-Aldicarb at 5 lbs. a.i./acre									
Matrix/TRR[1] (ppm)	Acetone Extract		Acetone/Water Extract		0.1 N HCl Extract		Postextraction Residue		Recovery[2] (% TRR)
	% TRR	PPM	% TRR	PPM	% TRR	PPM	% TRR	PPM	
Pulp/0.245	79.2	0.194	7.0	0.017	1.80	0.004[3]	11.4	0.028[3]	99.2
Peel/0.430	76.0	0.327	10.2	0.044	0.9	0.004[3]	3.8	0.016[3]	90.9
Foliage/17.9	61.6	11.0[3]	27.9	4.99[3]	1.9	0.340[3]	not determined	-	91.4

[1] TRR calculated for the particular matrix, not the whole fruit.
[2] Results of the refluxing hydrolyses with 0.1 N HCl were not reported, presumably because of low radioactivity levels in the postextraction residue.
[3] Not further characterized or analyzed.

Lemon juice fractions were separately treated with cellulase and glucosidase. The samples and controls were incubated at 35° C overnight. Additional enzyme was added, and the incubations were

continued for an additional five hours.

Pulp and peel acetone extracts were incubated with cellulase, glucosidase, and hemicellulase. Controls were run for the cellulase and glucosidase.

Extracts, hydrolysates, and enzymolysis fractions were analyzed by HPLC and TLC. For HPLC analyses, a Zorbax ODS column was utilized with uv detection (254 or 230 nm). Fractions were collected at 0.5 minute intervals and analyzed by LSC. Two solvent systems were used. In Method 1, the solvents were varied in three linear steps from 85% deionized water/5% methanol/10% trifluoroacetic acid (1%) to 55% water/35% methanol/10% trifluoroacetic acid over 35 minutes. In Method 2, the solvents were varied in three linear steps from 90% deionized water/0% methanol/1% trifluoroacetic acid (1%) to 55% water/35% methanol/10% trifluoracetic acid over 35 minutes. In Method 1, flow rates were 1 or 1.5 ml/min., and in method 2 flow rates were 1.0 ml/min.

The TLC analyses were conducted with glass silica gel F_{254} plates with one or two-dimensional development. On developed plates, cold standards were visualized with uv light (254 nm), or the plates were sprayed with typical reagents. Radiolabeled spots were detected and quantitated with a radiographic imaging system. In some cases, the appropriate sections of silica were scrapped and analyzed by LSC.

Reference standards for TLC and HPLC are given in Figure 1, a direct reproduction of the registrant's Figure 4, page 73 - 75. These standards represent potential metabolites and metabolites found in previous studies.

Semi-preparative HPLC, Method 2, was used to isolate peak regions from the acetone extract of peel and pulp. These regions were then investigated by HPLC and TLC, and comparisons were made to the HPLC chromatograms of juice. The compound identifications established are given in Table 3. Copies of relevant TLC plates and HPLC chromatograms were provided. The latter included integration data that permitted verification of the relative amounts of metabolites.

Table 3: Identification of Major Components of the Radiolabeled Residue in Lemon Fractions Resulting from the Treatment of Trees with ^{14}C-Aldicarb

Matrix	Compound	Concentration		Identification Method
		% TRR	PPM	
Peel	methane sulfonic acid + other polars	7.8	0.034	HPLC co-chromatography (juice). TLC (inconclusive). Methylation (multiple products).
	sulfone amide	3.6	0.014	TLC 2-D cochromatography. HPLC 2-D cochromatography.
	sulfone oxime glycoside sulfone acid glycoside sulfoxide acid glycoside	4.1	0.017	Cellulase. HPLC.
	sulfone oxime	9.0	0.041	HPLC.
	sulfone oxime glucoside	22	0.096	Cellulase. Glucosidase. HPLC. TLC.
	sulfone	7.4	0.030	HPLC cochromatography.
	TOTAL IDENTIFIED	53.9		
Juice	methane sulfonic acid + other polars	48.4	0.052	HPLC co-chromatography. TLC (inconclusive). Methylation (multiple products).
	sulfone amide	14.5	0.016	TLC. HPLC cochromatography.
	sulfone oxime	12.9	0.014	TLC. HPLC cochromatography.
	sulfoxide acid	1.2	0.003	HPLC cochromatography.
	sulfone acid	10.7	0.011	HPLC cochromatography.
	TOTAL IDENTIFIED	87.7		
Pulp	methane sulfonic acid + other polars	18.1	0.044	HPLC co-chromatography (juice). TLC (inconclusive). Methylation (multiple products).
	sulfone amide	4.83	0.015	TLC. HPLC cochromatography.
	sulfone acid	20.4	0.048	HPLC cochromatography.
	sulfone oxime	12.8	0.033	HPLC cochromatography.
	sulfone oxime glucoside	8.0	0.022	Cellulase. Glucosidase. HPLC. TLC.
	sulfone	4.0	0.007	HPLC cochromatography.
	sulfoxide derivatives	3.3	<0.010	HPLC.
	TOTAL IDENTIFIED	71.4		

Based on the values of Table 3 and the weights of the various fractions and whole fruit (Table 1), the identifications in whole fruit can be derived, as given in Table 4.

Table 4: Identification of the Radiolabeled Residue in Whole Lemons (0.234 ppm)		
Compound	Concentration	
	% TRR	PPM
methane sulfonic acid + other polars	18	0.042
sulfone amide	6.0	0.014
sulfoxide acid	<0.43	<0.001
sulfone oxime glycoside + sulfone acid glycoside + sulfoxide acid glycoside	2.1	0.005
sulfone oxime	11	0.025
sulfone oxime glucoside	14	0.034
sulfone	4.7	0.011
sulfone acid	8.1	0.019
TOTAL IDENTIFIED	64	-

RPAC proposes the metabolic path of Figure 2, a direct reproduction of the registrant's Figure 72, page 167. Aldicarb is oxidized to the sulfone, and the sulfone is hydrolyzed to the oxime, the amide, and the acid. Conjugates of both the oxime and acid may be formed. The sulfone and its hydrolysis products ultimately convert to methane sulfonic acid. Significantly, aldicarb per se was not detected in any lemon fraction.

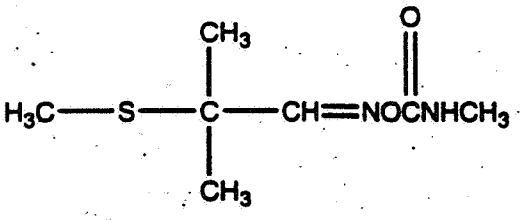
The results of this study are consistent with the findings of previous nature of the residue studies in peanuts, cotton, sugar beets, and potatoes. Although unacceptable for failures to determine the TRR of certain fractions, to report raw data, to report storage intervals of samples and extracts, to confirm identifications by a second technique, and to characterize or identify the residues in certain extracts or fractions (R. B. Perfetti, CBRS 10186, DP D180074, 04/06/93), the studies did show a general pattern of conversion of aldicarb to the sulfoxide, sulfone, and oxime, acid, nitrile, and alcohol derivatives of the sulfone and sulfoxide. The amounts of aldicarb sulfone plus aldicarb sulfoxide varied considerably with the crop: 38% of extractable residue (not TRR) for cotton forage and 15% of extractable residue for cottonseed, 18% TRR for peanut foliage and 3% TRR for nuts and 5% TRR for shells, 15% of extractable residue for potato tubers, and 11% TRR for sugarbeet tops and 24% TRR for mature sugarbeet roots.

Stability

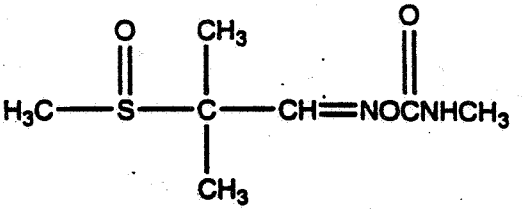
The storage stability of aldicarb in lemon juice, pulp, and peel was demonstrated by the fortification of control juice, pulp, and peel samples at 0.982 ppm with [^{14}C]-aldicarb. The spiked fractions were stored under the conditions of the study samples (<0° C) for intervals of three months and seven months. The latter represents the time span from harvest to completion of the study. Immediately after fortification (time 0) and after each storage interval, peel and pulp were extracted with acetone, and the extracts and juice were analyzed by HPLC (method 1). The juice and fractions from the HPLC were also analyzed by LSC. Copies of representative HPLC chromatograms (reconstructed radiochromatograms) were submitted. No degradation of aldicarb was found in pulp and juice over three months and in peel over seven months. Aldicarb did degrade about 20% in pulp and juice from the three to seven month storage interval. The chromatograms for peel and pulp indicate one major decomposition peak, in the retention time region of aldicarb sulfoxide and aldicarb sulfone. The chromatogram for juice at 7 months indicates that the decomposition product has a retention time slightly less than that of aldicarb and is unknown.

Pulp and peel were extracted with acetone at the start of the laboratory analysis phase and again after seven months. The initial HPLC reconstructed radiochromatograms (method 2) of peel compare favorably to those of samples stored seven months before extraction. The pulp chromatograms show significant degradation.

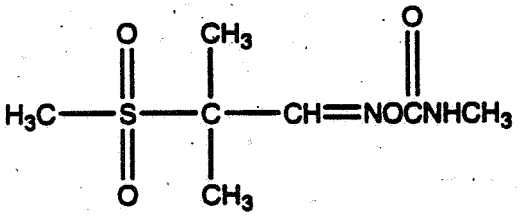
Figure 1: Structures for Aldicarb and Reference (HPLC/TLC) Standards



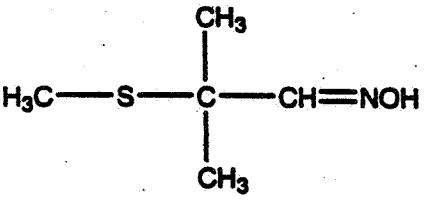
Aldicarb



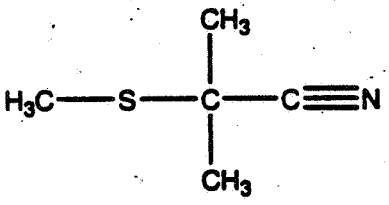
Aldicarb Sulfoxide



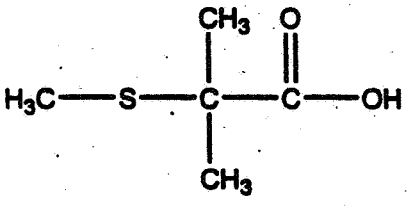
Aldicarb Sulfone



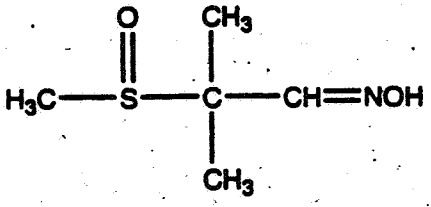
Aldicarb Oxime



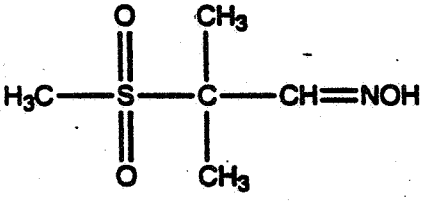
Aldicarb Nitrile



Aldicarb Acid



Aldicarb Sulfoxide Oxime



Aldicarb Sulfone Oxime

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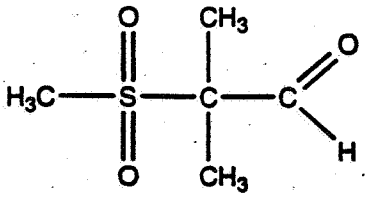
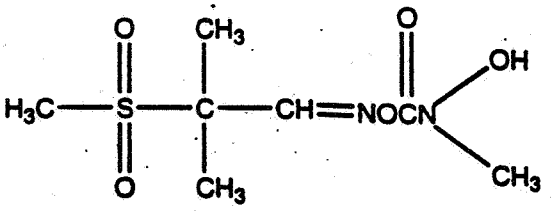
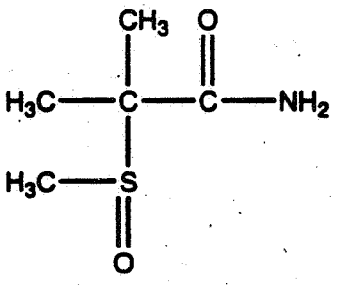
Figure 1 (continued)



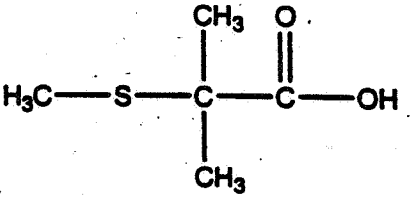
Aldicarb Sulfone Aldehyde



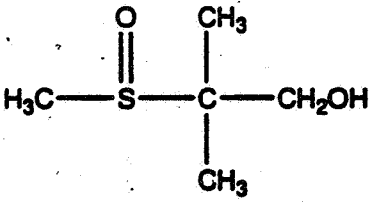
Aldicarb Sulfone Methylol



Aldicarb Sulfoxide Amide

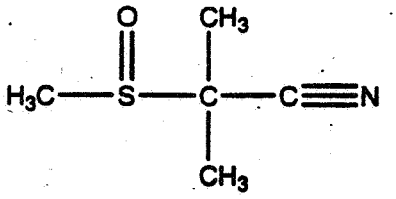


Aldicarb Sulfoxide Acid

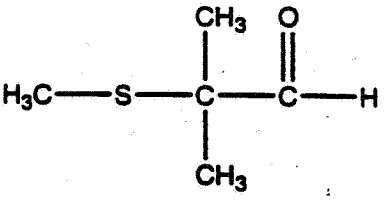


Aldicarb Sulfoxide Alcohol

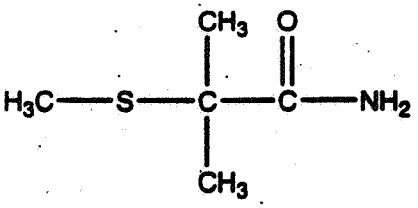
Figure 1 (continued)



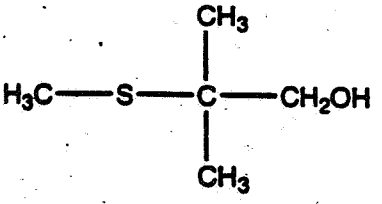
Aldicarb Sulfoxide Nitrile



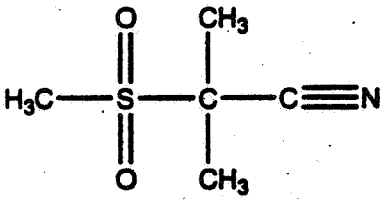
Aldicarb Aldehyde



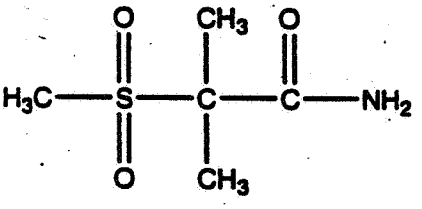
Aldicarb Amide



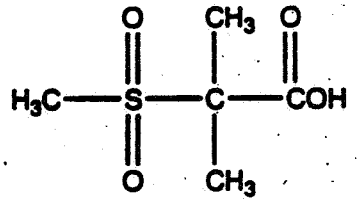
Aldicarb Alcohol



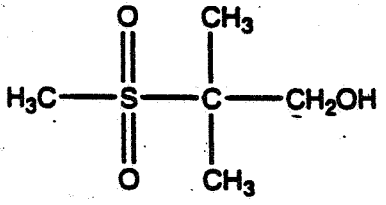
Aldicarb Sulfone Nitrile



Aldicarb Sulfone Amide

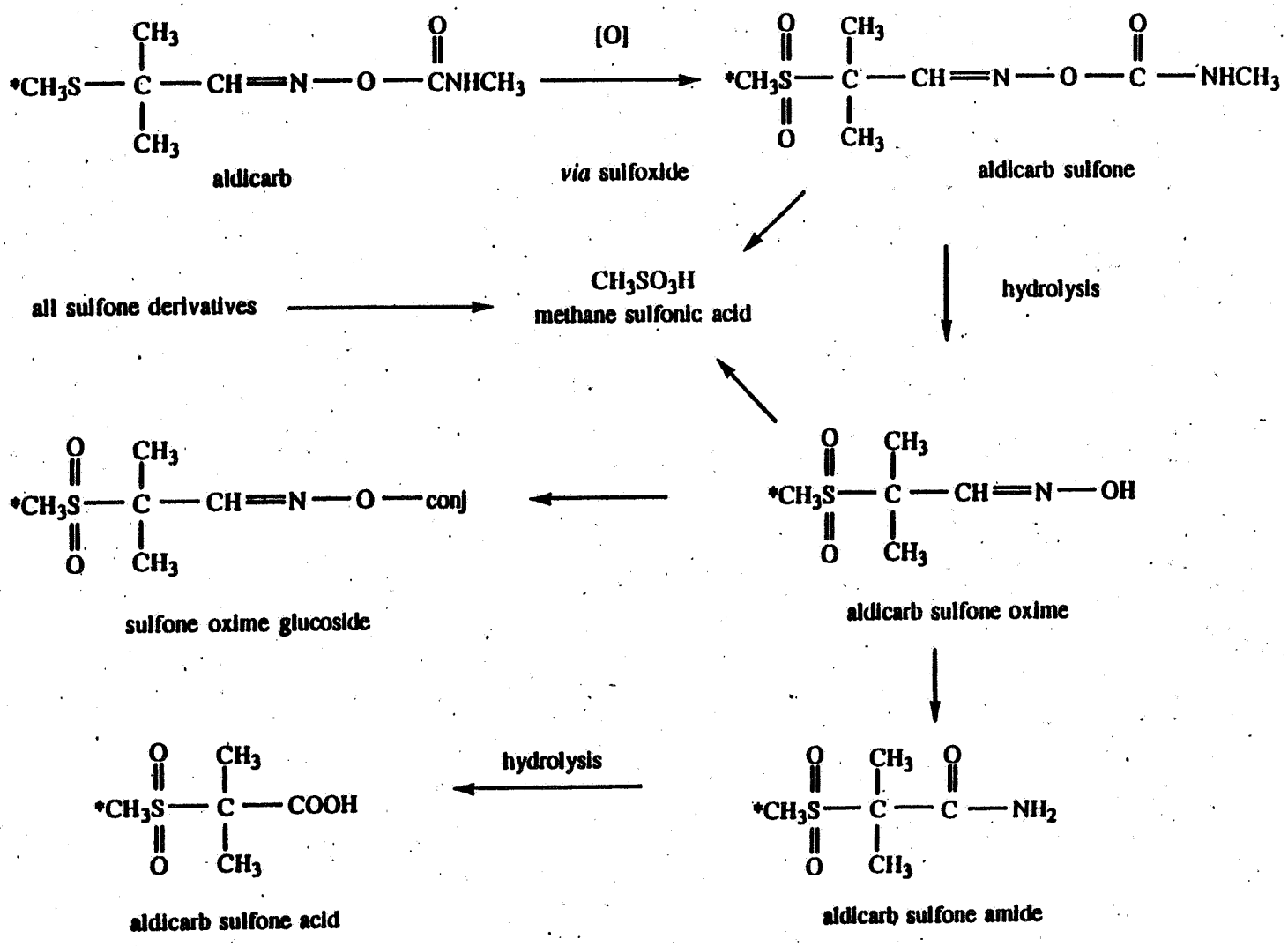


Aldicarb Sulfone Acid



Aldicarb Sulfone Alcohol

Figure 2: Proposed Metabolic Pathway of Aldicarb in Lemons



cc: S. Funk, RF, Subject File, List A, Circ.

RDI:A. Rathman:02/26/96:R. Perfetti:03/05/96:E. Zager:03/05/96:
7509C:CBRS:S.Funk:305-5430:CM#2:RM803:SF(0296.3):02/15/96.