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

MAR 24 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Partial Response (November 10, 1987) by Centre International d'Etudes du Lindane (CIEL) to Data Gap §171-4 (Nature of the Residue in Plants as Identified in the Residue Chemistry Chapter of the September 30, 1985 Lindane Registration Standard - (RCB No. 3267) MRID Nos. 404312-01, 404312-04, and 404109-02

FROM: Gary F. Otakie
Tolerance Petition Section II
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

Gary F. Otakie

THRU: Charles L. Trichilo, Ph.D., Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

TO: Amy Rispin, Chief
Science Integration Staff
Hazard Evaluation Division (TS-769C)

and

George LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

and

Toxicology Branch (Attention: Edwin Budd)
Hazard Evaluation Division (TS-769C)

The law firm of McKenna, Conner, and Cuneo has submitted a partial response to Residue Chemistry (§158.125) data gaps cited in the Lindane Registration Standard (September 30, 1985) on behalf of its client, CIEL, the Centre International d'Etudes du Lindane [Rhone-Poulenc, Inc.; Celamerck GmbH & Co., KG and its U.S. affiliate, E.M. Industries, Inc.; and Inquinosa (Quimocos de

Noroeste SA Industries)]. The submission consists of a cover letter from C.A. O'Conner (McKenna, Conner, and Cuneo) and three radiolabeled plant metabolism studies in spinach, cucumbers, and apples.

Pertinent data gaps cited in the Registration Standard will be restated below, followed by CIEL's response and RCB's Comments/ Conclusions.

Summary of Deficiencies That Need Resolution, Data Gap re: Plant Metabolism (171-4)

- o Outstanding issues relating to the spinach, cucumber, and apple metabolism studies need to be resolved (see the RCB's Comments/Conclusion section that follows in this review). Plant metabolism is still a data gap for lindane.

- o Additional metabolism studies may be required.

Recommendations

RCB recommends that the registrant address all of the issues raised in the RCB's Comments/Conclusion section that follows in this review. It is of upmost importance that these issues be resolved as soon as possible so that the required field residue data may be generated. A deference to TB on those residues that are of toxicological significance will be made.

RCB's Comments/Conclusions

Spinach Metabolism

1. Legible copies of all spinach spray test calibration data and calculations for extrapolation of the data to application rates (i.e., lb/A) are required.

2. Legible and appropriately identified copies of the HPLC chromatographs are required.

3. Data on the total radioactivity in the spinach samples before extraction are required. The registrant should provide an accounting for the parent compound and its metabolites when present.

4. A complete sample storage history including time and conditions of frozen storage (if any)

as well as time and conditions before frozen storage, extraction, clean up and analysis is required.

5. Tabulated numerical radioactivity data (e.g., dpm/gm and ppm) for the samples before extraction (if available), of the extracts, and of the remaining solid residue subjected to combustion analysis with extraction efficiencies and accountabilities, presented in an accountable manner are required.

6. The registrant also mentioned in his study that samples were concentrated to dryness and then taken up in another solvent before analyses. Is it necessary to take the samples to <u>dryness</u> since some of the more volatile lindane metabolites may escape?

7. A detailed description of the daily watering procedure utilized is required.

8. RCB will defer a decision on the adequacy of the spinach metabolism study until the above issues are resolved.

<u>Cucumber Metabolism Study</u>

1. Legible copies of all cucumber spray test calibration data and calculations for extrapolation of the data to application rates are required.

2. Legible and appropriately identified copies of the HPLC chromatograms and GC-MS results are required.

3. Data on the total radioactivity in the cucumber samples before extraction are required.

4. A complete sample storage history including time and conditions of frozen storage (if any) as well as time and conditions before frozen storage, extraction, clean up, and analysis is required.

5. Tabulated numerical radioactivity data (e.g., dpm/gm and ppm) for the samples before extraction

(if available), of the extracts, (including acid hydrolysis) and of the remaining solid residue subjected to combustion analysis with extraction efficiencies and accountabilities, presented in an intelligible manner are required.

6. Since the current use, for lindane on cucumbers, includes a 1-day PHI; data on radioactivity levels in the cucumbers before 39 days after treatment, preferably in intervals from day 1, are required.

7. The registrant also mentioned in his study that in the acid hydrolysis of leaf residues, the hydrolysate was concentrated to dryness. Is it necessary to take the samples to dryness since some of the more volatile lindane metabolites may escape?

8. A detailes description of the daily watering procedure utilized is required.

9. RCB will defer a decision on the adequacy of the cucumber metabolism study until the above issues are resolved.

Apple Metabolism

1. A complete sample storage history including time and conditions of frozen storage as well as time and conditions before frozen storage, extraction, clean up and analysis is required.

2. An explanation of the significant variation in the total ^{14}C-residues found in mature fruit from oxidative combustion/LSC (.04 ppm) compared to extraction/LSC (.08 ppm) is required.

3. Are GLC confirmation data available for lindane in the mature fruit, or for any of the metabolites identified by TLC in any samples? Confirmation of the claimed metabolites characterized by TLC, by another method (e.g., GLC/MS) is required (particularly for mature fruit).

4. The nature of the residue in apples is not adequately understood. Data on the characterization of the metabolites by TLC appears

marginal (especially for mature fruit) due to inadequate resolution from other potential metabolites. How were the metabolites detected by TLC chosen and/or what is the proposed metabolic pathway? RCB notes a similarity in the metabolites detected by TLC and a metabolic pathway suggested for lindane in Metabolism of Pesticides An Update By Calvin M. Menzie (1974) and included as Attachment 6 to this review.

5. Tabulated numerical radioactivity data (e.g., dpm/gm and ppm) for the samples before extraction (if available), of the extracts and of the remaining solid residue subjected to combustion analysis with extraction efficiencies and accountabilities, are required. Some of this data was presented but appears incomplete and does not include mature fruit or foliage.

6. Appendix 1 of the report, the protocol for determining the metabolic fate of radiolabeled lindane (reference 3.5.4) provided conjugated metabolites to be hydrolyzed with acids, bases and/or enzymes, yet only data on acid hydrolysis of the aqueous fractions remaining after the ethyl acetate extraction were discussed. Were additional efforts made to release conjugated residues and if not, why?

7. The registrant has mentioned in his study that samples were concentrated to dryness and then taken up in another solvent before analyses. Is it necessary to take the samples to dryness since some of the more volatile metabolites may escape.

8. A new radiolabeled apple metabolism study conducted at exaggerated rates and including acidic basic and enzymatic hydrolysis to release conjugated residues, may be necessary to provide sufficient residue to allow adequate characterization of metabolites. If a new apple metabolism study is necessary, the registrant may submit a protocol for this study.

9. RCB will defer a decision on the adequacy of the apple metabolism study until the above issues are resolved.

Note: An updated section of the relevant portion of Table A from the Lindane Registration Standard is Attachment 7 of this review.

§158.125 Residue Chemistry

171-4: Nature of the Residue (Metabolism)

- The data gaps relative to the metabolism in plants (spinach, cucumber, and apple) metabolism have not been fulfilled.

Plants

The following conclusions were made in the Residue Chemistry Chapter of the September 30, 1985 Lindane Registration Standard:

- None of the available metabolism studies has provided an adequate accounting of the terminal plant residues, which may include polar and nonpolar metabolites. Metabolism studies of radiolabeled lindane are required for representative crops from three crop groupings (cucurbits, leafy vegetables, and a pome or stone tree fruit). If metabolism data differ significantly among these crops, then metabolism data will be required for a representative crop from each crop grouping for which there is a registered tolerance.

CIEL's Response

The petitioner has submitted plant metabolism studies in spinach, cucumbers, and apples.

Spinach Metabolism Study (MRID No. 404312-01)

A metabolism study was conducted using two varieties of spinach plants (i.e., Viroflay and Perpetual), following post-emergence application at the two leaf stage of radiolabeled lindane (gamma isomer) at a rate equivalent to 0.9 kg ai/ha (.81 lb/acre) and 1.5 kg ai/ha (1.35 lb/acre), respectively. Plants of each variety were sampled at 0, 1, 3, 7, and 14 days after treatment for radioactivity. Only plants of the Perpetual variety were kept to maturity and also sampled at 28, 60, and 92 days since the Viroflay variety was found to be producing flowers on day 28.

Lindane ^{14}C was prepared by Pathfinder Laboratories Inc., and had a specific activity of 25.26 mCi/mmol at a purity of 99 percent and was mixed with nonradiolabeled lindane and solvent, resulting in an EC formulation with a specific activity of 2.06 mCi/mmol, which was diluted in water for application. Seeds were planted in 5-inch pots containing clay-loam soil (28% clay) and maintained in a covered enclosure. Plants were watered daily and fed using a liquid plant food, once each week.

A material balance system was set up to determine the extent of lindane distribution throughout the test system. Originally, six plants were set up in a sealed polyethylene enclosure and the system dismantled and assayed for radioactivity for both varieties of spinach; the system was redesigned using glass equipment. A single plant was placed in a glass enclosure and air pulled through the enclosure and traps designed to trap volatile organic compounds and carbon dioxide to test the system.

The diluted EC formulation was applied to the leaves using a Hamilton syringe. At each sampling time the plants were separated into leaves and roots, cut into pieces and soaked overnight in acetone. The acetone was then decanted and duplicate samples (1 ml) taken for liquid scintillation counting. Extracts containing sufficient radioactivity were than analyzed by HPLC. All samples were shown to contain a single compound which coeluted with lindane. The solid residue was allowed to dry prior to assay for radioactivity by combustion followed by liquid scintillation counting. Samples at 0, 1, 3, and 7 days after application were taken for autoradiography and placed between sheets of card and subjected to freeze-drying over a 3-day period and placed in direct contact with x-ray film for 15 days and developed according to manufacturer's recommendations.

The following table summarizes the activity found in the leave and root extracts by LSC analysis, for both plant varieties.

Distribution of Total Radioactive Residue
In Spinach (as ug Lindane)

	Viroflay		Perpetual	
Day	Leaves (ug)	Roots (ug)	Leaves (ug)	Roots (ug)
0	7.570		13.10	
1	0.418	< 0.001	1.462	0.012
3	0.201	< 0.001	0.746	0.002
7	0.042	0.006	0.104	0.025
14	0.015	< 0.001	0.077	0.012
28			0.088	0.047
60			0.001	0.001
92			0.012	0.055

The table indicates the total radiolabeled residue associated with plant extracts declined markedly by day 7 to less than 1 percent of the dosed radioactivity. A greater portion of the radiolabeled residue was found in leaves than in the roots. The extractable component (total residue from all sources) declined rapidly to less than 0.25 ug residue per plant by 7 days after treatment for both varieties of spinach. The following table depicts the significant growth dilution of the residue:

Growth Dilution of the Residue In Spinach
(As ppm Lindane)

Day	Leaf Residue	Root Residue
7	0.4142	0.2494
14	0.1098	0.0587
28	0.0119	0.0339
60	< 0.0001	0.004
92	0.0004	0.0033

The results of the autoradiography showed an immediate translocation of radioactivity throughout the plant. After 1 day the radioactivity was confined to the leaves and by 7 days the radioactivity had disappeared. The results of the material balance experiment for plants in the plastic enclosures showed recoveries of radioactivity of 6 and 12% for Viroflay and Perpetual plants, respectively; while the plants in glass enclosures provided satisfactory recoveries of 70 and 109 percent for Viroflay and Perpetual plants, respectively. The distribution of radioactivity indicated that the radioactivity migrates from the point of application throughout the experimental system.

No summary tables depicting numerical extraction efficiencies or total radioactivity before extraction were provided. However, graphs were provided (see Attachment 1) which plotted the total radioactivity in the extract versus the activity remaining in the residue after extraction (i.e., combustion/LSC) for each variety of spinach. It appears that a majority of the total radioactivity was extracted with the acetone. Appendix VII of the study does include some raw data on the radioactivity extracted from the leaves and roots, but the data is illegible and is not presented in a clear manner. Sample HPLC chromatograms are provided in Appendix VI of the report; but they are not clearly labeled and are thus not understandable.

In summary the petitioner indicates spinach plants were assayed for radioactivity at 0, 1, 3, 7, 14, 28, 60, and 92 days following treatment, at the two leaf stage, with radiolabeled lindane (gamma isomer). The results showed that 7 days following treatment approximately 99 percent of the applied radioactivity was lost to the air which was confirmed by the mass balance study. The results showed that the extractable radiolabeled components declined rapidly during the early stages and the nonextractable component remained approximately constant from 1 day following treatment until 28 days and then declined to nearly undetectable levels by day 60. HPLC analysis where practical showed only lindane in the extractable fraction from the spinach plants.

Cucumber Metabolism Study (MRID No. 404312-04)

A metabolism study was conducted with cucumber plants, following three postemergence applications of radiolabeled lindane (gamma isomer) at a rate equivalent to .71 kg/ha or .64 lb/acre (Table 3 of the study summarizes the volumes and concentrations used in the applications). The first application was applied at the two leaf stage with the second and third applications applied one and two weeks, respectively, after the first application. Plants were sampled at 0, 1, 4, 7, 14, 28, and 61 days and fruits

at 39, 42, 46, 53, 61, 64, 70, and 83 days after the third application for radioactivity.

Lindane ^{14}C was prepared by Pathfinder Laboratories, Inc., and had a specific activity of 25.26 mCi/mmol at a purity of 99 percent and was mixed with nonradiolabeled lindane and solvent, resulting in an EC formulation with a specific activity of 2.06 mCi/mmol, which was diluted in water for application. Seedlings were propagated in 2-inch pots of peat based compost and at the two leaf stage were placed in 10" pots containing sandy loam soil (84% sand). The plants were watered daily and fed using a liquid plant food once a week.

A material balance system was set up to determine how the lindane applied was distributed during the study. Three plants were placed in each of two enclosures made from a wire frame and large plastic bag, which was sealed and a single plant was placed in a sealed glass enclosure. Air was pulled through the enclosure and traps designed to trap volatile organic compounds and carbon dioxide.

The test plants were sampled at 0, 1, 4, 7, 14, 28, and 61 days after the third application for radioactivity and at 0, 1, 3, and 7 days for autoradiography. Plants in the plastic enclosure were found to be adversely affected by the environmental at 20 days after application. Four of the six plants were taken for analysis, with the two remaining plants allowed to grow outside the plastic enclosures, with the test plants. The plant in the glass enclosure was dismantled and assayed for radioactivity, 7 days after application.

The leaves, stems, and roots were soaked in acetone overnight. After discontinuing the acetone the residue was homogenized with methanol. The mixture was then centrifuged and the supernatant measured and duplicate samples taken for liquid scintillation counting. Extracts containing sufficient radioactivity were then analyzed by HPLC. The solid was allowed to dry prior to radioanalysis by combustion followed by liquid scintillation counting. The cucumber fruits were sliced homogenized with acetone, filtered, the volume measured and taken for liquid scintillation counting. The solid was allowed to dry prior to radioanalysis by combustion followed by liquid scintillation counting (LSC).

HPLC analysis was performed on all extracts containing sufficient radioactivity. All samples analyzed were shown to contain a compound which coeluted with lindane. Putative metabolites, chlorinated benzenes, cyclohexanes, phenols, etc., were shown not to interfere with these analyses. Chromatographs

obtained from extracts of plants taken after four days following application, showed a small peak at the origin which corresponded to a larger peek in the LVN channel of the detector. Thus, it was concluded that the peak observed at the origin was due to chemiluminescence and not of interest.

Residual material obtained from solvent extraction of the leaves from the glass enclosure plant was subjected to acid hydrolysis by mixing with methanol/2 N hydrochloric (1:1 V/V) acid and refluxed for 2 hours. The resulting mixture was centrifuged for 20 minutes, the supernatant decanted and the remaining residue washed further with the same mixture. After centrifugation the supernatants were pooled and assayed by LSC. The hydrolysate was concentrated to dryness and dissolved in acetonitrile/water/glacial acetic acid (70:30:01 by volume) and subjected to HPLC. The chromatograph of the hydrolysate showed a peak at the origin using a isocratic HPLC system. A chromatograph of the the same sample, using the gradient system, showed no single identifiable peak, which suggested the hydrolysate contained a multitude of polar components.

Following preliminary analysis, the acetone leaf extract from the glass enclosure was reacted with diazomethane and the ethered solution concentrated and taken up in acetone for analysis by gas chromatography-mass spectroscopy. The leaf extracts (both methylated and not methylated) were shown to contain lindane with no other lindane-related material detected.

The following three tables summarize the results of the data obtained from the test plants extracted with acetone:

Distribution of Total Radiolabeled Residue (as ug Lindane)

	Leaves		Stem		Roots	
Day	Extracted	Bound	Extracted	Bound	Extracted	Bound
0.125	191.21*	4.63*	----	----	----	----
1	113.14	6.65	6.45	0.10	0.38	0.03
4	24.71	10.83	0.98	0.31	0.05	0.08
7	15.81	10.72	0.09	0.25	0.56	0.14
14	11.92	10.70	7.12	0.17	0.84	1.04
28	3.12	9.21	0.50	0.42	0.21	0.30
61	4.56	10.88	0.39	0.47	< 0.01	0.26

*Combined leaves and stem.

Growth Dilution of the Residue (as ppm Lindane)

Day	Assay	Leaves	Stem	Roots
7	Extraction	1.581	0.094	0.022
	Combustion	1.072	0.041	0.035
61	Extraction	0.025	0.002	< 0.001
	Combustion	0.060	0.002	0.014

Cucumber Fruits: Total Radiolabeled Residue (as ppb Lindane)

Day						
39	0.14[a]	0.12	0.11			
42	0.09					
46	0.37					
53	0.61	0.42	0.26[a]	0.12[a]	0.11	0.07[a]
61	0.33	0.22	1.73	1.03[a]		
64	1.18					
70	3.16	1.93	1.78	1.62	1.38	
83	1.47	0.35	1.08			

[a]Results produced from two cucumbers harvested on the same day from the same plant.

In summary, following the third application, plants were assayed for radioactivity at 0, 1, 4, 7, 14, 28, and 61 days. The results indicated that 4 days following the final application 90 percent of the radioactivity in the leaves was lost. This was confirmed by the material balance experiment which showed that the radioactivity migrated from the point of application throughout the whole experimental system, due to the volatility of lindane and/or its metabolites. Cucumber fruits taken when ripe from 39 to 83 days after the final application were found to contain low radioactivity levels; the highest sample contained 3.2 ppb. Chromatographic analysis where practical showed only lindane in the extractable residue. Hydrolysis of the bound residue produced polar material which after chromatographic examination was found to contain many components which were not present in large enough quantities to permit characterization.

RCB cautions the registrant on concentrating his samples to dryness since may of the more volative lindane metabolites may be lost.

Apple Metabolism Study (MRID No. 404109-02)

A metabolism study was conducted on a mature bearing red delicious apple tree which was isolated before application, from the orchard, with an enclosure of lumber, chicken wire and plastic which measured 20' x 20' x 13' high. An application of 1.008 gm (equivalent to 3.6 lb/acre) or 88 mCi ^{14}C-lindane (>96% purity) was made to the apple tree with a stainless steel sprayer. The sprayer was hand pumped with air and the entire tree sprayed. Foliage (leaves and small branches) were sampled at the following intervals: 0, 1, 8, 14, 21, 28, 57, 84, 117, and 131 days and immature fruit was sampled at 28, 57, 84, and 117 days, and mature fruit sampled at 131 days. All samples were returned to the laboratory and immediately frozen.

The foliage and/or fruit samples were taken and ground to a fine consistency with dry ice using a Waring blender. Samples were blended for two minutes with 10% water/acetonitrile, vacuum filtered and the extract radioassayed by LSC to determine total extractable (TER). The remaining filter cakes were then further extracted by refluxing with 2% HCl in methanol, radioassayed, and the extract residue added as part of the TER; while the remaining filter cake subjected to oxidative combustion analysis in order to determine bound residues (TBR). Attachment 2 summarizes these data.

The water/acetonitrile extract was evaporated to aqueous, partitioned with part ether which was evaporated to dryness and the residue redissolved in acetone; and the aqueous fraction subjected to aqueous hydrolysis with concentrated HCl for 1 hour and partitioned with ethyl acetate. The aqueous fraction from the filter cake extraction was also partitioned with ethyl acetate, evaporated to dryness and the residue redissolved in Acetone. The aqueous fractions were subjected to LSC and metabolites were characterized by thin layer chromatography (TLC) of the organic fractions. Attachment 3 summarizes the extraction procedures utilized and Attachment 4 provides the efficiency of the fractionation steps.

Total ^{14}C-residues (TR) in the plant tissues were also determined by oxidative combustion. However, these TR values were not always consistent with the TR values (Reference Attachment 2) from the extracts (TER). TR values obtained by oxidative combustion for foliage at day 0, 28, 84, and 131 were 123.5, 1.8, 0.95, and 0.81 ppm, respectively; while for fruit at

28, 84 and 131 days, TR values were 0.38, 0.07, and 0.04 ppm, respectively. The largest variation was the 131-day fruit where the TR from the extracts and combustion of the filter cake was 0.08 ppm and the TR from combustion only, was 0.04 ppm.

The characterization of metabolites by TLC analyses of the initial and filter cake extracts of the apple leaves and fruit are summarized in Attachment 5. The major product identified for all the foliage and immature fruit samples was lindane. However, the major metabolite found in mature fruit (131 days) was pentachlorphenol. The lindane residue in foliage represented 77 percent of the total activity at day 0, and dissipated to 3 percent at harvest (131 days); while the lindane residue in fruit represented 20.2 percent of the total activity at day 28 and dissipated to 10.9 percent of the total activity at harvest. The pentachlorophenol residue in foliage was 0.2 and 1.8 percent of the total activity at day 21 and 131, respectively; while in fruit the residue was 0.9 and 13.6 percent of the total activity at day 28 and 131, respectively. Other metabolites identified in the foliage or fruit in minor amounts were 2,3-dichlorophenol; 2,4-dichlorophenol; 2,3,5-trichlorphenol; 2,4,5-trichlorophenol; 2,4,6-trichlorophenol; 2,3,4-5-tetrachlorophenol; and 2,3,5,6-tetrachlorophenol.

Only the presence of lindane in the foliage and fruit pet-ether fraction, was confirmed by GLC analysis using and election capture detector (i.e. 28 day foliage and fruit and 131 day foliage and fruit had 0.07 and 0.12, and 0.05 and 0.015 ppm lindane, respectively).

In summary, the total ^{14}C-residues from extract/LSC data in foliage dissipated from 129 ppm at day 0 to 0.72 ppm at 131 days (harvest); with the half-life calculated to be approximately 4.8 days. The total ^{14}C-residues in fruit ranged from 0.49 ppm at 28 days (immature fruit) to 0.08 ppm at harvest (131 days). Extractability of the ^{14}C-residues in in foliage ranged from 99 percent at day 0 to 61 percent at 131 days; and for fruit ranged from 69 percent at 28 days to 75 percent at 131 days, from the acetonitrile/water extraction; further partitioning of the acetonitrile/water extract (75% of activity) from the mature fruit (131 days) resulted in the following radioactivity distribution: pet ether, 25%; ethyl acetate, 12.5%; and the aqueous phase, 37.5%. RCB is concerned that the concentration of samples to dryness may have resulted in the dissipation of lindane and/or its metabolites due to their volatility.

Attachments:

1. Figure 4 - Perpetual Residue Extracted and Bouund
2. Accountability of ^{14}C Residue from Fruit and Foliage
3. Figure 2 - Flow Diagram
4. Table 5 - Efficiency of Plant Tissue Fractionation Steps
5. Table 6 - Metabolite Distribution in Apple Foliage and Fruit Samples
6. BHC Metabolites - Suggested Relationships
7. Table A - Generic Data Reguirements for Lindane

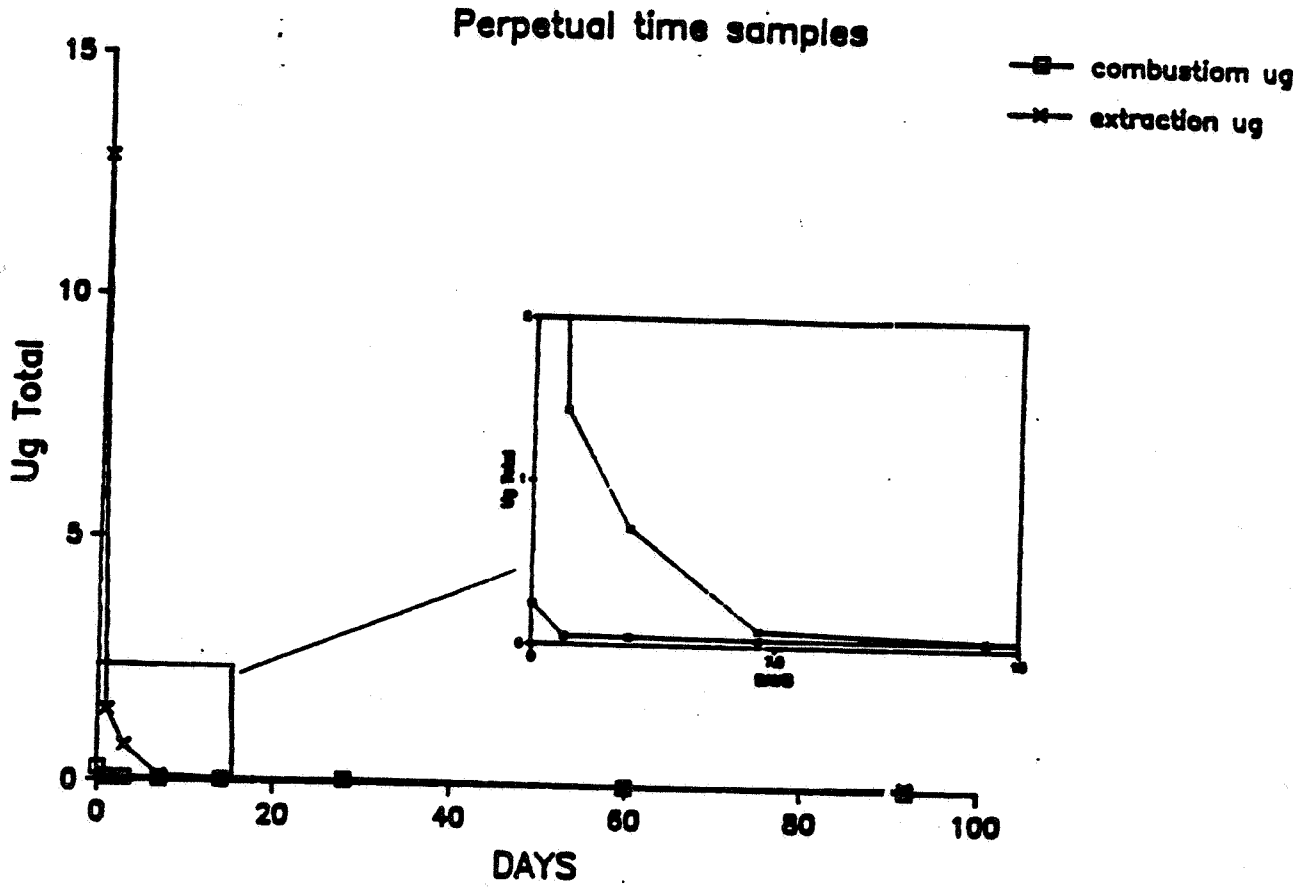
cc: with attachments,

Lindane Reg. Std. File/W. Boodee: PMSD/ISB: R.F., Reviewer/G. Otakie: G. LaRocca; PM#15, Circu., Lindane Subject File, TOX, Rispin-SIS

TS-769:RCB:CM#2:Rm 810:557-7324:G.Otakie:Typist/Kendrick; 3/17/88
Edited by MT, 3/22/88
RDI: M.J. Kovacs; 3/16/88: R.D. Schmitt; 3/16/88

ATTACHMENT 1
LINDANE REG. STD.
FOLLOW UP

FIGURE 4 Perpetual Residue Extracted and Bound



Accountability of ^{14}C Residues from Fruit and Foliage Samples Expressed as % of Total Residue (TR)

Sample	CH_3CN/H_2O ppm	%	+	2%HCl/MeOH ppm	%	=	TER ppm	%	+	Filter Cake (TBR) ppm	%	=	Total Residues ppm	%
0 Day Foliage	128	99.0		0.80	0.6		128.8	99.6		0.46	0.4		129.33	100
1 Day Foliage	55	99.1		0.36	0.7		55.4	99.0		0.10	0.2		55.5	100
8 Day Foliage	5.7	80.7		0.42	5.4		6.12	86.5		0.95	13.4		7.07	100
14 Day Foliage	2.8	74.9		0.47	12.6		3.27	87.5		0.47	12.5		3.74	100
21 Day Foliage	1.5	65.8		0.78	34.2		2.38	100		--	--		2.28	100
28 Day Foliage	0.85	61.1		0.20	14.4		1.05	75.5		0.34	24.5		1.39	100
28 Day Fruit	0.34	69.4		0.15	30.6		0.49	100		--	--		0.49	100
57 Day Foliage	0.41	42.3		0.56	57.7		0.97	100		--	--		0.97	100
57 Day Fruit	0.13	86.7		--	--		0.13	86.7		0.02	13.3		0.15	100
84 Day Foliage	0.46	59.0		0.17	21.8		0.63	80.8		60.15	14.2		0.78	100
84 Day Fruit	0.06	85.7		--	--		0.06	85.7		0.01	14.3		0.07	100
117 Day Foliage	1.2	52.2		0.11	4.8		1.31	57		0.94	43		2.3	100
117 Day Fruit	0.06	75.0		--	--		0.06	75.0		0.02	25		0.08	100
131 Day Foliage	0.44	61.1		0.05	6.4		0.49	68		0.23	32		0.72	100
131 Day Fruit	0.06	75.0		--	--		0.06	75		0.02	25		0.08	100

Note: All values are normalized (TR = TER + TBR)

ATTACHMENT 2
LINDANE REG. STD.
FOLLOW UP

ATTACHMENT 3
LINDANE REG. STD.
FOLLOWUP

Figure 2

Flow Diagram

Extraction and Fractionation of Apple Foliage and Fruit

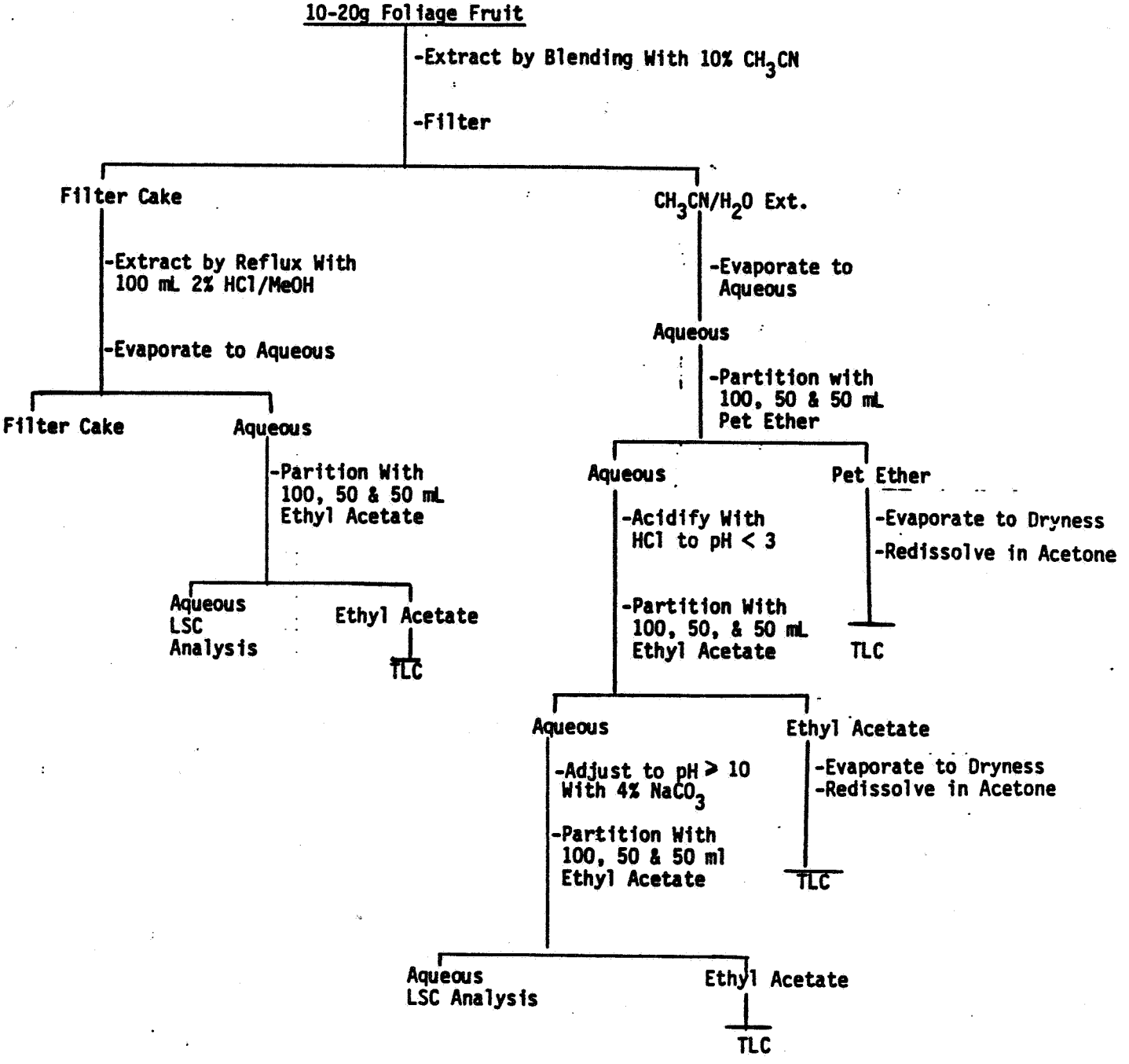


TABLE 5

EFFICIENCY OF PLANT TISSUE FRACTIONATION STEPS AS % OF TR

Sample	Extraction % of TR	TER	Organic Solvent Partitions				% of TR Spotted TLC
			Pet Ether	EtoAc pH3	EtoAc pH10	Aqueous	
0 Day Foliage:							
Initial Ext.	99	99.6	99.0	--	--	--	99.6
Filter Cake Ext.	0.6		--	0.6	--	--	
1 Day Foliage:							
Initial Ext.	99.1	99.8	99.1	--	--	--	99.8
Filter Cake Ext.	0.7		--	0.66	--	0.04	
8-Day Foliage:							
Initial Ext.	80.6	86.5	52.3	9.5	--	13.3	66
Filter Cake Ext.	5.9		--	4.2	--	1.7	
14-Day Foliage:							
Initial Ext.	74.9	87.5	40.1	12.8	--	24.6	63.5
Filter Cake Ext.	12.6		--	10.6	--	2.0	
21-Day Foliage:							
Initial Ext.	65.8	100	6.1	16.2	--	39.0	51.3
Filter Cake Ext.	34.2		--	29.0	--	5.2	
28-Day Foliage:							
Initial Ext.	61.1	75.5	12.9	18.0	--	32.3	41.8
Filter Cake Ext.	14.4		--	10.9	--	3.5	
57-Day Foliage:							
Initial Ext.	42.3	100	2.1	8.3	--	27.8	62.8
Filter Cake Ext.	57.7		--	52.4	--	5.3	
84-Day Foliage:							
Initial Ext.	59.0	80.8	1.6	25.5	1.6	30.3	44
Filter Cake Ext.	21.8		--	15.3	--	6.5	
117-Day Foliage:							
Initial Ext.	52.2	57	2.2	16.1	3.5	35.2	24.8
Filter Cake Ext.	4.8		--	3.0	--	1.8	
131-Day Foliage:							
Initial Ext.	61.1	68	3.3	16.7	4.4	36.7	27.5
Filter Cake Ext.	6.9		--	3.1	--	3.8	

ATTACHMENT 4
LINDANE REG. STD.
FOLLOW UP
PAGE-1

TABLE 5 (CONTINUED)

EFFICIENCY OF PLANT TISSUE FRACTIONATION STEPS
AS % OF TR

Sample	Extraction % of TR	TER	Organic Solvent Partitions				% of TR Spotted TLC
			Pet Ether	EtoAc pH3	EtoAc pH10	Aqueous	
28-Day Fruit:							
Initial Ext.	69.4	100	24.5	6.1	--	16.3	60.6
Filter Cake Ext.	30.6		--	30.0	--	0.6	
57-Day Fruit:							
Initial Ext.	86.7	86.7	26.7	6.7	--	33.3	33.3
Filter Cake Ext.	--		--	--	--	--	
84-Day Fruit:							
Initial Ext.	85.7	85.7	42.8	14.3	--	42.8	57.1
Filter Cake Ext.	--		--	--	--	--	
117-Day Fruit:							
Initial Ext.	75	75	25.0	12.5	--	37.5	37.5
Filter Cake Ext.	--		--	--	--	--	
131-Day Fruit:							
Initial Ext.	75	75	25.0	12.5	--	37.5	37.5
Filter Cake Ext.	--		--	--	--	--	

TABLE 6

METABOLITE DISTRIBUTION IN APPLE FOLIAGE AND FRUIT SAMPLES

Sample	TER Spotted as % of TR	Origin	Other Polar Metab.	Metabolite Distribution % of TR (Lindane Equivalents)									
				Lindane	RPI-L 06	RPI-L 20	RPI-L 21	RPI-L 25	RPI-L 26	RPI-L 27	RPI-L 29	RPI-L 30	Other Unknowns
Foliage:													
0 Day	99.6	1.6	7.4	77.0	--	--	--	--	--	--	--	--	13.0
1 Day	99.8	0.8	9.1	77.7	--	--	--	--	--	--	--	--	11.5
8 Days	66.0	13.6	8.3	38.6	--	0.2	--	0.2	0.3	--	--	0.3	7.2
14 Days	63.5	24.2	6.9	28.4	--	0.3	--	0.3	0.5	--	--	0.4	6.1
21 Days	51.3	34.3	3.8	3.8	0.2	--	--	--	--	--	--	--	2.9
28 Days	41.8	26.2	0.7	8.2	1.0	--	0.1	--	1.0	0.1	1.3	--	3.2
57 Days	62.8	52.0	1.3	2.4	0.8	0.2	--	0.2	0.8	--	1.3	0.2	3.6
84 Days	44.0	39.8	1.6	--	--	0.4	--	0.4	1.2	--	--	0.6	--
117 Days	24.8	15.6	0.1	5.1	0.8	--	--	--	0.1	--	0.1	--	--
131 Days	27.5	19.3	--	3.2	1.8	--	--	--	0.1	--	0.1	--	--
Fruit:													
28 Days	60.6	34.2	--	20.2	0.9	--	--	--	0.9	--	1.2	--	3.2
57 Days	33.3	3.5	--	23.3	0.9	--	--	--	--	--	1.3	--	3.6
84 Days	57.1	11.0	--	35.0	1.5 ✓	--	--	--	1.5	--	2,5	--	5.6
117 Days	37.5	12.4	--	15.4	9.5	--	--	--	0.1	--	0.1	--	--
131 Days	37.5	12.4	--	10.9	13.6	--	--	--	0.3	--	0.3	--	--

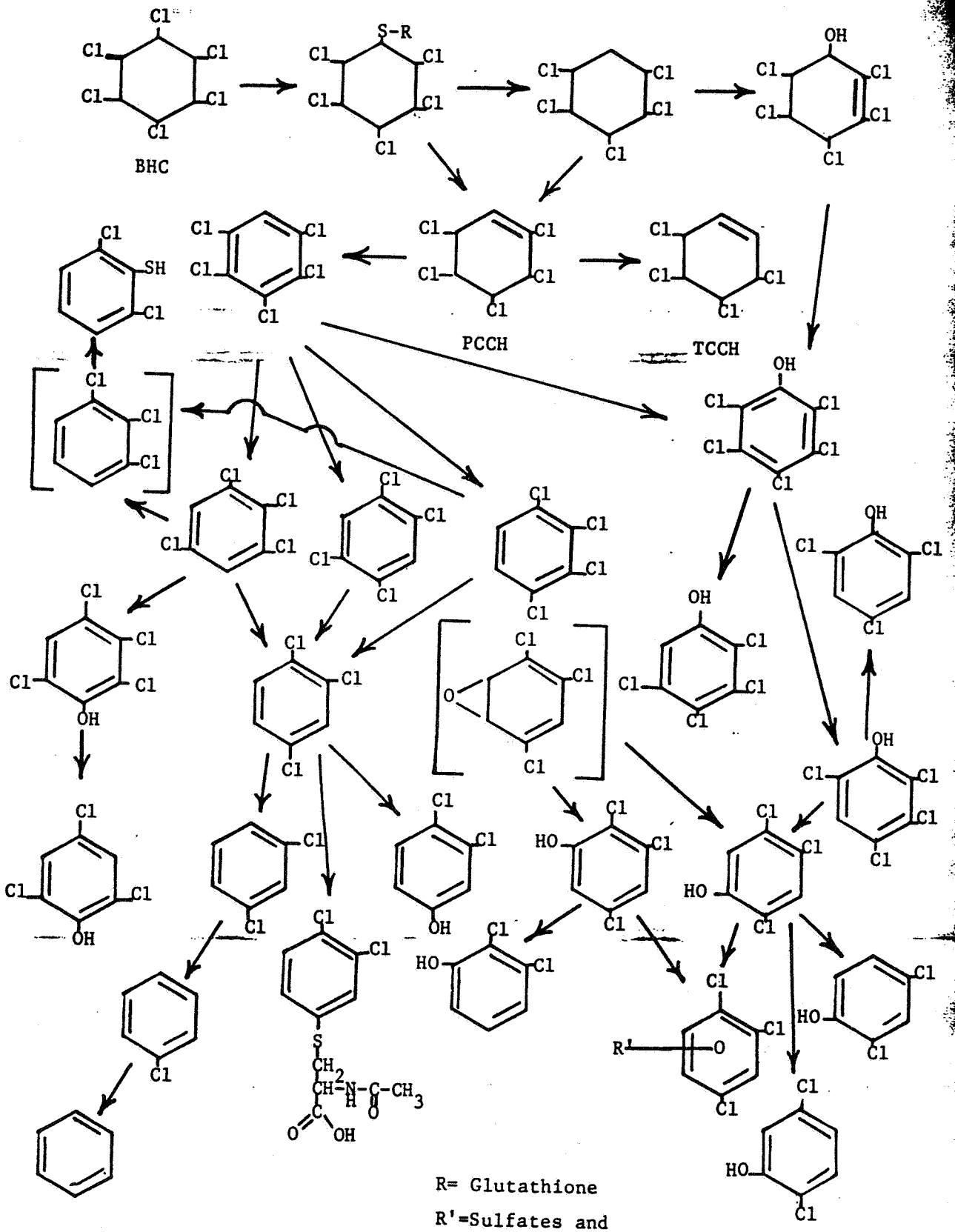
Metabolite Chemical Names:

RPI-L-06	Pentachlorophenol
RPI-L-20	2,3-Dichlorophenol
RPI-L-21	2,4-Dichlorophenol
RPI-L-25	2,3,5-Trichlorophenol
RPI-L-26	2,4,5-Trichlorophenol
RPI-L-27	2,4,6-Trichlorophenol
RPI-L-29	2,3,4,5-Tetrachlorophenol
RPI-L-30	2,3,5,6-Tetrachlorophenol

ATTACHMENT 5
LINDANE REG. STD.
FOLLOW UP

ATTACHMENT 6
LINDANE REG. STD.
FOLLOWUP

BHC METABOLITES - SUGGESTED RELATIONSHIPS



Attachment to RCB's Review of Partial Response (July 21, 1987) Relative To The September 30, 1985 Lindane Registration Standard

Table A
Generic Data Requirements for Lindane

Data Requirement	Composition[1]	Does EPA Have Data To Satisfy This Requirement?	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?	Timeframe For Data Submission[2]
§158.125 Residue Chemistry					
171-4 - Nature of Residue					
- Plants	PAIRA	Partially	MRID Nos. 404312-01, 404312-04, and 404109-02	Yes	24 Months
- Livestock (Poultry)	PAIRA and Plant Metabolites	Partially	MRID No. 402713-01	Yes	18 Months
171-4 - Residue Analytical Method					
- Plant and Animal Residues	TGAI and	Partially		Reserved	
171-4 - Storage Stability Data					
- Animal Commodities	PAI	No		Yes	18 Months
	PAI	No		Yes	48 Months
- Plant Commodities	Metabolites	No		Reserved	
171-4 - Magnitude of the Residue Residue Studies					
- Crop Group #1 Root and Tuber Vegetables					
o Crop 1 - Beets					
- Crop Field Trials	TEP	No		Yes	48 Months

LINDANE REG. STD. FOLLOW UP