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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 (July 15, 1987) by Centre International d'Etudes du Lindane (CIEL) to Data Gap §171-4 (Nature of the Residue in Livestock Poultry as Identified in the Residue Chemistry Chapter of the September 30, 1985 Lindane Registration Standard - (RCB No. 3315)
MRID No. 402713-01

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THRU: Charles L. Trichilo, Ph.D., Chief
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TO: Amy Rispin, Chief
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and

George LaRocca, PM 15
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and

Toxicology Branch (Attn: 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'Connor (McKenna, Conner, and Cuneo) and a radiolabeled goat metabolism study, "Metabolism Study of ^{14}C-Lindane Fed or Topically applied to Lactating Goats."

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

Summary of Remaining Data Gaps, re: 171-4 - Poultry Metabolism

Data Gap 171-4 - (Poultry Metabolism) is only partially fulfilled.

- o The registrant needs to provide raw data and GLC chromatograms.
- o Lindane metabolites need to be confirmed by GC/MS.
- o Since lindane residues did not plateau in eggs after 4 and 6 days of dosing, a combination long-term ^{14}C-lindane metabolism/feeding study is needed after TB has given an input as to what residues are of special toxicological concern. A deference will be sent to the Toxicology Branch. The registrant may provide a protocol for this study.

Recommendations

1. RCB recommends that the registrant first provide raw data/calculations, GLC chromatograms, and GC/MS confirmations of all metabolites for RCB's review.
2. Secondly, after TB has given an input as to what residues are of special toxicological concern in poultry then the registrant should provide a protocol outlining a long-term combination ^{14}C-lindane metabolism feeding study.

RCB's Comments/Conclusions, Re: Poultry Metabolism

1. Tentatively, the nature of the residue is relatively understood over a period of 4 days. After data normalization to 100 percent, the registrant reported the identifications for

most of the residue as outlined in Table 4 that follows in this review. However, the registrant is required to submit raw data/ calculations, GLC chromatograms, and confirmations of terminal residues by GLC/MS in order to strengthen his report.

2. For long-term exposure of lindane to poultry, the nature of the residue is not adequately understood. In all of the studies, no plateau of ^{14}C was reached in egg yolk after dosing the hens for 4 and 6 days. For example, in Group A (1.3 ppm dosing level) the following residues were observed in egg yolk: Day 1, < 0.01 ppm; Day 2, 0.04 ppm; Day 3, 0.15 ppm; Day 4, 0.24 ppm; Day 5, 0.45 ppm; and Day 6, 0.83 ppm. In view of this, RCB concludes that when the registrant carries out his long-term poultry feeding studies, he should use ^{14}C-lindane. It will be necessary to see when and if a plateau can be reached in chicken eggs.

3. In the future long-term ^{14}C-lindane poultry feeding studies, enough hens should be dosed wherein tissues of some hens should be subjected to necrospy around 15 days and some tissues subjected to necrospy after 30 days of dosing. If the results for the 15-day and 30-day necropsies are similar, the studies need not to go beyond 30 days. If the results from the 30-day necropsy are higher than those observed for the 15-day necropsy, then the feeding study should go beyond 30 days. At each necropsy, effort should be made to release any possible bound lindane residue for analyses. The registrant may want to submit a protocol for the long-term ^{14}C-lindane poultry feeding study.

4. A Kuderna-Danish concentrator should be used during the concentration step instead of a rotary evaporator. This should prevent much loss of those metabolites of lindane that have early GLC retention times.

5. The registrant has identified 12 metabolites in poultry tissues by using GLC which by itself is not adequate for this purpose. Since

TLC was not successful as a confirmatory procedure, the registrant should confirm these metabolites by GC/MS. Once, that these confirmations have been received, RCB will defer to TB as to whether any or what residues are of special toxicological concern. Although no chlorophenols were reported in eggs or any tissues resulting from the 4-day metabolism study, they may need to be looked for in the long-term feeding study.

Note: An updated section of the relevant portion of Table A is attached to this review.

§158.125 Residue Chemistry

§171-4: Nature of the Residue (Metabolism) Poultry

The following additional data are required:

Tolerances have not been established for residues of lindane in poultry or eggs, however, it may be necessary to establish such tolerances if significant levels of lindane are found in poultry feed items. Labeling of all lindane end-use products with directions for use on livestock premises or farm buildings must bear a prohibition against application in poultry houses.

CIEL's Response (MRID No. 402713-01)

The petitioner has submitted ^{14}C-poultry metabolism studies reflecting oral dosing of White Leghorn laying hens.

Oral Dosing

In this study, the laying hens were assigned to the following groups:

Control - 4 hens
Low Dose - 4 hens
High dose - 6 hens
Group A - 2 hens
Group B - 2 hens

Each hen in the low-dose group received a daily ^{14}C-lindane dose (equivalent to 1.2 ppm in the feed, 0.12

mg/kg body weight) in a gelatin capsule for 4 days. The high dose hens received a daily ^{14}C-lindane dose equivalent to 120 ppm in the feed or 11.05 mg/kg/body weight for 4 days, and Group A was given 6 daily doses of 0.125 mg/kg body weight equivalent to 1.31 ppm in the feed. All of the chickens were necropsied within 12 hours after the final doses. Group B hens received 6 daily doses of 0.125 mg/kg body weight equivalent to 1.31 ppm lindane in the feed and then placed on a withdrawal period for 6 days prior to necropsy. Representative samples of breast and thigh tissues, liver, kidney, gizzard, heart, blood, skin and fat were collected from all hens. Eggs were collected daily throughout the study.

In a protocol previously submitted to RCB, the petitioner calculated the dietary burden of lindane imposed upon poultry to be 1.28 ppm (see RCB's June 12, 1986 review of Protocols for Lindane Metabolism Studies. . .). In this study, the low dose (equivalent to 1.2 ppm in the feed) was interpreted to be a 1X feeding level, and the high dose (equivalent to 120 ppm in the feed) was interpreted to be a 100X feeding level.

Experimental Processes

Radioactivity Analyses for Total Residues

This report indicates that egg and excreta samples were homogenized by blending, aliquoted, and then combusted. Tissue samples were blended with dry ice and aliquoted for combustion. Samples were analyzed for ^{14}C by using a Beckman 3801 liquid scintillation spectrometer. The results are summarized below:

Table 1. Total ^{14}C-Residues in Eggs

Dose Group	Study Day	Yolks (ppm)	Whites (ppm)
Low dose	1	< 0.01	< 0.01
1X	2	0.03	< 0.01
(1.2 ppm)*	3	0.10	< 0.01
	4	0.19	< 0.01
High dose	1	< 0.01	< 0.01
100X	2	1.54	0.11
(120 ppm)*	3	5.13	0.16
	4	10.83	0.21

Group A (1.3 ppm)*	1	< 0.01	< 0.01
	2	0.04	< 0.01
	3	0.15	< 0.01
	4	0.24	< 0.01
	5	0.45	< 0.01
	6	0.83	< 0.01
Group B (1.3 ppm)*	1	< 0.01	< 0.01
	2	0.05	< 0.01
	3	0.16	< 0.01
	4	0.23	< 0.01
	5	0.36	< 0.01
	6	0.54	< 0.01
Off feed (^{14}C)	7	0.69	< 0.01
	9	0.72	< 0.01
	10	0.90	< 0.01
	11	0.74	< 0.01

*Equivalent to ppm in feed.

Table 2. Total ^{14}C Residues in Chicken Tissues

	*PPM Found			
Samples	Low Dose (1.2 ppm)	High Dose (120 ppm)	Group A (1.3 ppm)	Group B (1.3 ppm)
Liver	0.32	11.65	0.39	0.15
Kidney	0.20	10.73	0.28	0.26
Gizzard	0.13	7.86	0.12	0.04
Skin	0.72	49.93	0.92	0.41
Breast	0.02	1.44	0.05	0.02
Thigh	0.16	11.81	0.22	0.11
Fat	1.26	96.98	2.83	1.05
Heart	0.24	20.66	0.12	0.04

*PPM found for the Low Dose, High Dose, and Group A reflect analyses wherein the chicken were necropsied within 12 hours after the final dose. PPM found for Group B reflect analyses of chicken tissues after a withdrawal period of 6 days.

The data indicated that averages of 38%, 30%, 44%, and 63% of ^{14}C were recovered in the excreta of the Low Dose, High Dose, Group A, and Group B, respectively.

Identification and Quantification of Extractable Residues
Extraction and Clean Up

To test the extraction of organosolvent efficiencies, sample aliquots (2 to 10 g) of the homogenized low dose egg yolks and white, excreta, and tissues were extracted with ethyl acetate and the extracts were quantitated for ^{14}C by liquid scintillation counting.

High dose tissue, egg, and excreta samples were extracted with hexane followed by reextracting with ethyl acetate. All extracts, except for skin and fat samples, were then cleaned up on a Florisil column. Skin and fat samples were first carried through an acetonitrile--hexane partition before subjection to a Florisil column clean up. The Florisil columns were eluted with 100 mL volumes of the following solvents in order: hexane, 50% ethyl acetate in hexane, 50% ethyl acetate in hexane, ethyl acetate, and methorol. The hexane eluate fractions from the hexane extracts of high-dose samples were not evaporated prior to analyses by GLC. The petitioner indicates that the ethyl acetate extracts were evaporated by rotary evaporation. Except for the hexane eluates, we assume that the other eluates (fractions) from the Florisil column were also evaporated by rotary evaporation.

The percent extraction of ^{14}C from the eggs, excreta, and tissue samples of the low and high dose groups are summarized below:

Table 3. Percent Extracted

Sample	Low Dose	High Dose
Liver	100	89
Thigh	133	105
Breast	---	90
Fat	144	141
Skin	68	80
Yolk	111	96
Heart	152	---
Excreta	79	74

Percent extraction of ^{14}C from the Group A and Group B studies was not reported.

Thin Layer Chromatography (TLC)

The petitioner reports that concentrated extracts were examined by one dimensional TLC using the following development systems:

System I - Methylene chloride/ethyl acetate 95/5 v/v
System II - Toluene/acetone 75/25 v/v
System III - Ethyl acetate/toluene/acetic acid 50/45/5 v/v/v

However, the petitioner indicates that the TLC work is not reported here since nearly all volatile components were lost during the concentration or during TLC plate development. Thus, he said that his TLC analyses only confirm the presence of lindane.

Gas Liquid Chromatography (GLC)

Sample extracts, potential degradation products, and lindane standards were analyzed by GLC equipped with a 3% OV-17 on Gas Chrom Q 100/120 mesh column and an electron capture detector.

Results and Discussion

GLC identification of lindane and its metabolites were done only on the High Dose hens. For egg white, heart and breast, the data submitted showed all (100%) of the terminal residues as being lindane after normalization. The distribution data on the other edible commodities are summarized in Table 4 on thatfollows.

Discussion of the Identification Work

The registrant indicated, "The combined ethyl acetate extracts were evaporated by rotary evaporation to near dryness and solvent exchanged to hexane."

This reviewer has experienced that when working with organochlorinated compounds such as lindane and its metabolites, one should not use rotary evaporation unless a Snyder column is inserted between the sample flask and the solvent trap. It would probably be better to use only a Kuderna-Danish concentrator [see Rapid Method for Chlorinated Pesticide Residues in Meat and Meat Products by J.H. Onley and P.F. Bertuzzi (JAOAC, Vol. 49, 370 (1966) and Residues in Eggs from Low Level Feeding of Five Chlorinated Hydrocarbon Insecticides to Hens by J.G.

Table 4. Identification of Lindane Metabolites From High Dose (100 X) Extracts a (Refer to Table 5 of the Registrants Submission)

Component	Retention Time (min)	Distribution of Metabolites[b] Egg Yolk % (ppm)	Thigh % (ppm)	Liver % (ppm)	Skin % (ppm)	Fat % (ppm)
Dichlorobenzenes	0.67			9.54		
1,3,5-Trichlorobenzene	0.80			6.44 (0.74)		
1,2,4-Trichlorobenzene	0.97	0.63 (0.07)	1.96 (0.23)	19.37[c] (2.3)	3.40	3.50
1,2,3-Trichlorobenzene	1.13		0.33 (0.04)	0.87	0.75	0.60
1,2,4,5 or 1,2,3,4-Tetrachlorobenzene	1.60[d]		17.65(2)	2.30	2.18	3.10
1,2,3,4-Tetrachlorobenzene or Tetrachlorocyclohexene	2.03[d]		4.41 (0.52)	4.37	1.10	0.97
2,3,4,5,6-Pentachlorocyclohexene	2.78	4.20 (0.45)	4.62 (0.55)	3.76	4.76 (2.4)	6.13 (5.9)
1,2,3,4,5-Pentachlorobenzene	3.45	0.13	0.02		0.18	0.10
Hexachlorocyclohexene	4.43	0.62 (0.07)	0.19 (0.02)	0.53	1.70	0.60
Hexachlorobenzene	7.81			1.35		
Lindane	11.84	94.45(10)	70.83(8)	51.45(6)	86.0(43)	85.0(82)
	TOTAL	100.0	100.0	100.0	100.0	100.0
Amount of Initial Radiocarbon Identified[e]		121.3	109.1	76.0	66.4	101.5

[a]Data normalized to 100% for relative distribution.
[b]GC data for all Florisil fractions pooled for total quantitation
[c]Levels confirmed by reanalysis of fresh samples.
[d]Could not separate by GC.
[e]Actual percent of tissue, organ, egg or excreta radioactivity identified.

Cummings, et al. (JAOAC, Vol. 49, 354 (1966)]. For all further work with regard to lindane, the registrant may want to use the preceding apparatus and techniques in order to prevent loss of lindane and its metabolites.

The registrant concluded the following:

> In conclusion, based on analysis of low (IX) and high (100X) dose tissue, organ, and egg samples, it is suggested that poultry samples should be analyzed only for lindane. All the metabolites in this study could only be identified in the exaggerated dosing study (100X) - high dose group. It follows that volatile metabolites would be eliminated during poultry processing and home cooking, leaving only lindane for potential human exposure.

The 100X dosing residue data indicate that many of the metabolites in various poultry tissues and eggs would not exist at levels higher than 0.02 ppm during a 4-day metabolism/feeding study when considering a possible 1X dosing level.

However, after prolonged feeding, for example 15, 30, or more days, we can not say from observing this 4-day High-Dose study that very substantial levels of lindane metabolites will not exist in eggs and the tissues of poultry. After a 4-day dosing period about 51 percent of the parent compound remains in liver. It is noteworthy that in all studies, Low Dose, High Dose, Group A, and Group B where there was continuous dosing of lindane, throughout periods of 4 or 6 days, no plateau was reached for total residues in egg yolk (see the preceding Table 1 in this review). These findings are also in agreement with a poultry feeding study published in JAOAC, Vol. 49, 370 (1966). Probably more knowledge could have been obtained if the registrant had dosed his hens until a plateau was reached in eggs and then carried out the necropsy step.

Finally, without some residue data, RCB cannot agree with the registrant that volatile metabolites would be eliminated during poultry processing and home cooking, leaving only lindane for potential human exposure. Incidentally, some people eat raw eggs. As previously discussed if one uses the Kuderna-Danish concentrator during the various concentrations, lindane and most of its metabolic residues should stand a better chance of being saved. Since all of the metabolites presented in Table 4 above will appear on the same gas chromatogram along with lindane, a search for any of them in eggs and poultry tissue should not be a great burden upon the registrant when he is carrying out his future poultry feeding studies. Although no chlorophenols were reported in eggs or any tissues resulting from the High Dose 4-day metabolism study, they may need to be looked for in the long-term feeding study. These compounds were found in the ruminant (goat) metabolism studies submitted by the registrant. Appropriate GLC chromatograms and raw data relating to the analyses of lindane and its metabolites were not submitted in this report.

Attachment: Partial Table A-Generic Data Requirements for Lindane

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

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Attachment to RCB's Review of Partial Response (July 15, 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