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

AUG 24 1988

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Partial Response (June 9, 1988) by Centre International d'Etudes du Lindane (CIEL) to Data Gap Section 171-4 (Magnitude of the Residue in Poultry and Eggs) as Identified in the Residue Chemistry Chapter of the September 30, 1985 Lindane Registration Standard - (RCB No. 4034) MRID No. 406605-01

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The law firm of McKenna, Conner, and Cuneo has submitted a partial response to Residue Chemistry (section 158.25) data gaps cited in the Lindane Registration Standard (September 30, 1985) on behalf of its client, the Centre International d'Etudes du Lindane

(CIEL) and its three members holding U.S. registrations for the insecticide lindane: Rhone-Poulenc Ag Company (representing Rhone-Poulenc Agrochemie), E.M. Industries, Inc. (representing Shell Agrar GmbH & Company, KG), and Inquinosa (Industrias Quimicas del Noroeste, S.A.). The submission consists of a cover letter from C.A. O'Connor (McKenna, Conner, and Cuneo) and a feeding study entitled: Lindane Tissue and Egg Residue Study in Poultry, dated February 5, 1988.

The pertinent data gap cited in the Registration Standard will be restated below, followed by CIEL's response and DEB's comments/ conclusions.

Summary of Deficiencies That Need Resolution, Data Gap re: Magnitude of the Residue - Residue Studies (Section 171-4)

1. The metabolism of lindane in poultry and the toxicological importance of its metabolites are not yet adequately understood.

2. Additional crop residue and processing data are needed to properly evaluate potential lindane residues in animal feed items (see Dietary Exposure Branch, formerly Residue Chemistry Branch, chapter of the Lindane Registration Standard, September 30, 1985) and poultry commodities.

3. More detailed information on the analytical methodology utilized is required.

Recommendation

1. DEB recommends that the registrant complete his crop residue and processing studies so that calculations for expected secondary residue levels in poultry commodities can be finalized.

2. DEB recommends that the registrant resolve those issues relating to poultry metabolism after which decisions can be finalized as to whether or not some metabolites need to be regulated.

3. DEB recommends that the registrant be prepared to analyze reserved samples (if supported by storage stability data) and/or carry out new feeding studies if any lindane metabolites need to be regulated.

DEB's Comments/Conclusions

1. A decision as to the adequacy of the poultry feeding study cannot be made until outstanding issues on poultry metabolism (see DEB's [RCB's] memorandum of March 24, 1988, pertaining to poultry metabolism study), including the importance of the metabolites, and potential lindane residues in animal feed items and analytical methodology are resolved.

2. Additional crop residue data on apples, grapes, and tomatoes required by the September 30, 1985 Lindane Registration Standard and corresponding processing studies are needed to further evaluate potential lindane residues in animal feed items (i.e., apple, grape, and tomato pomace) and the expected levels of secondary lindane residues in poultry and eggs.

3. The poultry feeding study indicates that lindane residues accumulate in poultry and eggs. New tolerances for lindane/metabolites in poultry fat, meat, meat byproducts, and eggs may be required after outstanding issues relating to poultry metabolism and potential lindane residues in animal feed items are resolved.

4. Analysis of reserve samples and/or a new poultry feeding study may be required if any metabolites are of toxicological concern. Storage stability data should be made available to support any metabolites that may be of toxicological concern.

5. A more detailed discussion of the analytical procedures utilized for extracting and analyzing lindane residues in tissue organs and eggs is required, including information as to whether a Kuderna-Danish concentrator or rotary evaporator was utilized. Additional extractions and analysis of reserve samples may be necessary.

DETAILED CONSIDERATIONS

Section - 158.25 Residue Chemistry

Section 174-4 - Magnitude of the Residue (Poultry and Eggs)

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

- o 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

The petitioner has submitted a feeding study for lindane in poultry.

Lindane Tissue and Egg Residue Study in Poultry (MRID No. 406605-01)

A feeding study was conducted by Agrisearch, Inc., Frederick, MD, to measure and quantify eggs and tissues for residues of lindane resulting from the oral administration of lindane to White Leghorn hens, and to determine lindane effects on feed consumption, egg production, and general health. Seventy-five White Leghorn laying hens were acclimated for 1 week prior to initiation of oral dosing. Each hen was observed to be clinically normal during acclimation. The best 60 egg-producing hens were chosen for the study. Four hens were randomly assigned to each of 12 treatment groups and 6 hens were each assigned to 2 control groups. Each hen was individually housed in a layer cage 12 x 16 x 16 inches. The light cycle was set at 17 hours of light per day.

Technical lindane (715 g, Batch No. DA433) was received from Rhone-Poulenc Ag Company on June 4, 1987, as a white powder with a stated purity of 99.5 percent. Agrisearch analysis of the technical lindane showed a purity of 99.8 percent. The stability of lindane under the conditions of storage was determined from extra capsules prepared and stored (-15 degrees C) with dose capsules. Oral dose calculations were based on a 120 g daily ration per hen and the maximum theoretical dietary contribution of lindane to poultry feed per an April 28, 1987 office memorandum of the registrant as follows:

RAC	Tolerance (ppm)	Dry Down Factor (Pomace)	ppm in Pomace	% In Diet Laying Hen
Apple	1.0	8	8	5
Grape	1.0	4.3	4.3	5
Tomato	3.0	20	60	2

The above data were used to calculate the maximum amount of lindane in the diet as follows:

Feed Item (Pomace)	Lindane in Diet (ppm) Laying Hens
Apple	0.40
Grape	0.22
Tomato	0.66
TOTAL	1.28

Based on the maximum expected intake of Lindane residue, the following feeding levels are proposed:

Species	ppm in Feed		
	1X	3X	10X
Laying Hens	1.5	4.5	15

Accordingly, the following table summarizes the dose groups used in the study:

Treatment Group	No. of Hens	Oral Dose Level (ppm)	(ug)	Days on Test
1	6	Control	0	28
2	6	Control	0	60
3	4	1.5	180	28
4	4	1.5	180	28
5	4	1.5	180	60
6	4	1.5	180	60
7	4	4.5	540	28
8	4	4.5	540	28
9	4	4.5	540	60
10	4	4.5	540	60
11	4	15	1800	28
12	4	15	1800	28
13	4	15	1800	60
14	4	15	1800	60

Each daily dose was prepared by adding the appropriate amount of lindane, dissolved in acetone, to a gelatin capsule containing poultry feed. The acetone was allowed to evaporate and the capsules

were sealed and stored frozen. Capsules were prepared weekly and extra capsules were kept for dose check analysis. Each hen received a single capsule daily at the morning sampling and feeding period. The capsule was administered with a drop of K[1/] jelly and the neck of the bird was massaged.

Egg production for each hen was measured daily and the average egg production was 88.2 percent for all hens. The eggs were counted and collected at the morning sampling and dosing period. Egg samples were taken on days 0, 1, 3, 7, 14, 21, 25, and 28 of the 28-day study and on days 35, 42, 49, 56, and 60 of the 60-day study. All eggs for a dose group were deshelled, composited by group, and homogenized by shaking. The egg samples were stored frozen at -15 degrees C pending analysis.

Necropsy was performed after sacrifice by exsanguination. Tissue samples were composited by group (four hens). The sacrifice schedule was 20 hours post 28-day dose for half the hens and 20 hours post 60-day dose for the remaining hens with body weights taken at sacrifice and prior to dosing. Tissue and organ samples included liver, kidneys, gizzard, breast muscle, thigh muscle, fat, and heart, and were placed on dry ice, and stored frozen at -15 degrees C.

All egg samples were allowed to thaw and were rehomogenized by shaking, and 10 g aliquots were taken for analysis. All tissue and organ samples were ground on dry ice using a Hobart food chopper or Wiley Mill. These samples were placed in the freezer until all dry ice had sublimed, where 10 g samples were weighed out for analysis.

The analytical procedures utilized were referenced as "Official Methods of Analysis" of the AOAC, fourteenth edition, 1984, entitled "Organochlorine and Organophosphorus Pesticide Residues, 29.001 through 29.049 (Ref. 3). In summary, tissue, organ, and egg samples were extracted into acetonitrile and made aqueous with saturated sodium chloride and the lindane partitioned into hexane. The hexane was evaporated and subjected to a florisil column clean-up procedure. The proper eluate from florisil was evaporated and redissolved in hexane for gas chromatographic analysis and electron

capture detection. The following table summarizes the lindane recoveries obtained from the control samples:

Lindane Recovery from Control Samples

Sample	Fortification (ppm)	Recovery (%)
Eggs	0.005	62-127
	0.05	69-123
	0.01	75-95
		Average: 97.2
Fat	1.0	90
	100	91
Liver	0.05	84
	0.5	87
	5	75
Breast	0.01	(A)
	0.1	78
Kidney	0.01	(A)
	0.5	88
	5	100
Gizzard	0.01	(A)
	0.10	84
Thigh	0.05	111
	0.5	90
	5	107
Heart	0.05	67
	.50	97
	5.0	84
		Average: 88.9

(A) Contaminated control at > 0.01 ppm.

Random capsules from weeks 1 and 6 were also analyzed for lindane content with an average of 101.9 percent of the expected lindane measured at all three dose levels.

No variations in egg production and no abnormal clinical or gross pathological signs were observed following the daily oral dosing of lindane to laying hens at 1.5, 4.5, and 15 ppm. Lindane residue levels in eggs were at a plateau by day 14 for all dose groups. Egg plateau residue levels were approximately 0.2, 0.55,

and 2.3 ppm for the 1.5, 4.5, and 15 ppm dose groups. The following table summarizes the egg lindane residue levels:

Egg Lindane Residue Levels

Low Dose, 1.5 ppm

Study Day	Group 3 (ppm)	Group 4 (ppm)	Group 5 (ppm)	Group 6 (ppm)	Average (ppm)
0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
1	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
3	0.018	0.021	0.027	0.025	0.022
7	0.097	0.092	0.121	0.131	0.110
14	0.229	0.199	0.196	0.242	0.216
21	0.205	0.143	0.174	0.221	0.185
25	0.191	0.163	0.184	0.219	0.189
28	0.221	0.157	0.203	0.239	0.205
35			0.204	0.248	0.226
42			0.203	0.247	0.225
49			0.223	0.309	0.266
56			0.238	0.302	0.27
60			0.254	0.349	0.301

Mid Dose, 4.5 ppm

Study Day	Group 7 (ppm)	Group 8 (ppm)	Group 9 (ppm)	Group 10 (ppm)	Average (ppm)
0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
1	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
3	0.046	0.054	0.051	0.033	0.046
7	0.212	0.255	0.301	0.295	0.258
14	0.621	0.521	0.657	0.639	0.609
21	0.577	0.631	0.633	0.571	0.603
25	0.633	0.716	0.781	0.561	0.672
28	0.577	0.537	0.649	0.592	0.588
35			0.711	0.681	0.696
42			0.323	0.655	0.489
49			0.444	0.469	0.456
56			0.476	0.545	0.510
60			0.528	0.574	0.551

High Dose, 15 ppm

Study Day	Group 11 (ppm)	Group 12 (ppm)	Group 13 (ppm)	Group 14 (ppm)	Average (ppm)
0	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
1	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
3	0.141	0.177	0.241	0.121	0.17
7	0.779	0.994	0.899	0.841	0.878
14	1.99	2.26	2.28	2.04	2.142
21	1.48	3.09	2.68	2.18	2.357
25	1.73	2.33	2.11	2.25	2.105
28	2.18	2.65	2.41	2.29	2.382
35			2.55	1.72	2.135
42			2.12	2.07	2.095
49			1.97	1.98	1.975
56			2.31	2.42	2.365
60			2.45	2.59	2.52

Group 1 and 2 controls were < 0.005 ppm, except Group 2 (Days 3 and 21) were at 0.024 ppm.

In general, dosing for 60 days did not increase lindane tissue and organ residue levels above those found after 28 days of dosing. Residue levels were approximately 10 times higher in the 15 ppm dose groups than in the 1.5 ppm dose groups. However, the 4.5 ppm dose group had residue levels less than three times those in the 1.5 ppm dose groups. Fat and fatty tissue contained the highest lindane residue levels. Residue levels were higher in thigh muscle (fatty tissue) than in breast muscle and the organs contained low residue levels. The following table summarizes the tissue and organ lindane residue levels:

Tissue and Organ Lindane Residue Levels

Group	Dose Level (ppm)	Days on Test	Thigh (ppm)	Breast (ppm)	Fat (ppm)	Heart (ppm)	Liver (ppm)	Kidney (ppm)	Gizzard (ppm)
1	0.0	28	<0.010	<0.007	<0.001	0.038	0.013	<0.012	0.007
2	0.0	60	0.01	0.007	<0.001	0.02	0.005	0.006	0.006
3	1.5	28	0.19	0.03	2.54	0.43	0.14	0.19	0.10
4	1.5	28	0.18	0.03	2.54	0.23	0.10	0.15	0.08
5	1.5	60	0.15	0.04	2.41	0.19	0.10	0.15	0.07

Tissue and Organ Lindane Residue Levels (Cont'd)

Group	Dose Level (ppm)	Days on Test	Thigh (ppm)	Breast (ppm)	Fat (ppm)	Heart (ppm)	Liver (ppm)	Kidney (ppm)	Gizzard (ppm)
6	1.5	60	0.18	0.03	2.67	0.21	0.11	0.21	0.05
7	4.5	28	0.35	0.07	7.04	0.37	0.46	0.38	0.29
8	4.5	28	0.37	0.12	8.46	1.4	0.55	0.71	0.34
9	4.5	60	0.60	0.08	9.72	1.0	0.33	0.41	0.31
10	4.5	60	0.43	0.08	8.11	0.75	0.17	0.45	0.24
11	15	28	1.21	0.40	27.4	2.17	0.83	2.51	1.00
12	15	28	1.49	0.32	27.9	2.35	0.72	1.55	0.88
13	15	60	1.57	0.33	28.6	3.07	0.86	2.18	0.53
14	15	60	1.36	0.34	27.1	2.67	0.95	1.96	1.10

Average Tissue and Organ Lindane Residue Levels

Dose Level (ppm)	Days on Test	Thigh (ppm)	Breast (ppm)	Fat (ppm)	Heart (ppm)	Liver (ppm)	Kidney (ppm)	Gizzard (ppm)
1.5	28	0.19	0.03	2.54	0.33	0.12	0.17	0.10
1.5	60	0.17	0.04	2.54	0.2	0.11	0.18	0.06
4.5	28	0.36	0.10	7.75	0.89	0.51	0.55	0.32
4.5	60	0.52	0.08	8.92	0.88	0.25	0.43	0.28
15	28	1.35	0.37	27.65	2.26	0.78	2.03	0.95
15	60	1.47	0.34	27.85	2.87	0.91	2.07	0.82

DEB Comments/Conclusions re: Poultry Feeding Study

The metabolism of lindane in poultry is not adequately understood at this time as discussed in DEB's (RCB's) March 24, 1988 review of CIEL's Partial Response of July 15, 1987 (memorandum of J. Onley) pertaining to poultry metabolism. Several issues, including the importance of the metabolites that need resolution, were outlined. Also, existing lindane tolerances for apples, grapes, and tomatoes of 1.0, 1.0, and 3.0 ppm are not supported by adequate residue data. Additional residue data on these commodities were required in the September 30, 1985 Lindane Registration Standard. Processing studies will also be necessary to formulate the appropriate feed additive tolerances (i.e., apple, grape, and tomato pomace). Until acceptable data are received, a final determination of potential lindane residue levels in animal feed items,

and the expected levels of secondary lindane residues in poultry and eggs, cannot be made. However, the current poultry feeding study does indicate that the existing uses of lindane may require that new tolerances be established for poultry, fat, meat, meat byproducts, and eggs.

The poultry feeding study in this submission allows for tentative conclusions on lindane only, without any metabolites. The data show that lindane residues accumulate in poultry fat, meat, meat byproducts, and eggs. The residue data on eggs indicate that lindane residues plateau after about 14 days in the mid- and high-dose groups (i.e., 4.5 and 15 ppm) but in the low-dose group (i.e., 1.5 ppm) lindane residues in eggs reach a lower plateau at 14 days and a second higher plateau at 60 days with lindane residue levels averaging 38 percent higher than the 14-day plateau. Lindane residues in eggs averaged .301 ppm after 50 days in the low-dose group and .609 and 2.142 ppm in the mid- and high-dose groups after 14 days.

Lindane tissue and organ residue levels were highest in fat with residue levels generally following a linear correlation with the dosage level. For instance, average lindane residue levels in fat for the 1.5, 4.5, and 15 ppm dosage levels were 2.54, 7.75, and 27.65 ppm, respectively, in the 28-day study. The second highest lindane residue levels were found in the heart with average lindane residues for the 1.5, 4.5, and 15 ppm dosage levels of 0.33, 0.89, and 2.26 ppm, respectively, in the 28-day study. Lindane residue levels in thigh muscle were from three to seven times greater than residue levels in breast muscle, likely attributable to thigh muscle's greater fat content. Thigh muscle lindane residue levels for the 1.5, 4.5, and 15 ppm dosage levels were 0.19, 0.36, and 1.35 ppm, respectively, for the 28-day study and 0.17, 0.52, and 1.47 ppm, respectively, for the 60-day study. Except for thigh muscle, tissue and organ lindane residue levels did not vary significantly from the 28- and 60-day feeding studies.

Although the analytical methodology utilized provided adequate recoveries from spiked samples, insufficient detail was provided. More information on the extractions and equipment used are needed. Information on whether a Kuderna-Danish concentrator or rotary evaporator was used and sample chromatograms are also needed.

Note: The registrant should reserve his poultry tissue and egg samples in the event that these samples need to be reanalyzed for lindane metabolites. However, the registrant must keep in mind

that any metabolite residue work done must be supported by storage stability data. If not, new feeding studies would be needed.

cc: Lindane Reg. Std. File - W. Boodee, PMSD/ISB, R.F., Reviewer/Otakie, G. LaRocca/PM#15, Circu., Lindane Subject File, TOX - Insecticide/Herbicide, A. Rispin - EFCD

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