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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Lindane dermal application metabolism study protocol. RCB #514 No RD Accession No.

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Cynthia Deyrup

THRU: Charles L. Trichilo, Ph.D., Chief
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TO: Amy Rispin
Science Integration Staff
Hazard Evaluation Division (TS-769C)

and

George La Rocca
Registration Division (TS-767)

The law firm of McKenna, Conner, & Cuneo has submitted a partial response to Residue Chemistry (§158.125) data gaps cited in the Lindane Registration Standard (9/30/85) on behalf of its client, CIEL, the Centre International d'Etudes du Lindane [Rhone-Poulenc, Inc.; Celamerck GmbH & Co., KG and its US affiliate, E.M. Industries Inc.; and Inquinosa (Qimocos de Noroeste SA Industries)]. The submission consists of a protocol for a radiolabeled dermal application metabolism study for RCB's review; also, the cover letter from C.A. O'Connor (McKenna, Connor, & Cuneo) to George LaRocca, RD, addresses concerns the registrant raises on the Residue Chemistry requirements cited by the Registration Standard. The issues raised by the registrant will be summarized below followed by RCB's Comments and Recommendations.

§158.125 Residue Chemistry

171.4

Registrant's Concern No. 1

The Registration Standard requested an animal metabolism study on a lactating ruminant. CIEL considers the requirement for a spray application to a lactating ruminant to be inappropriate because approved labeling prohibits treatment of lactating animals. Therefore CIEL requests a waiver so as to limit the dermal metabolism study to non-lactating ruminants. Also CIEL proposes to use a spray or patch application instead of a dip because of the hazards and expense of handling the large amounts of radioactive material required for a dip application.

RCB's Comments and Recommendations

According to the EPA Index to Pesticide Chemicals, dermal applications of lindane may be made to goats, and there is no restriction against using lindane on lactating goats. Therefore RCB recommends that a lactating ruminant be included in the dermal application metabolism study. In any case, RCB notes that a lactating cow has been included in the protocol submitted for the dermal application study in order to compare the data to that obtained from orally treated lactating goats.

In RCB's judgment, an appropriately conducted metabolism study using a patch or spray application could substitute for a study using a dip application.

Registrant's Concern No.2

The Registration Standard requires submission of the plant storage stability data before completion of the plant metabolism study. Since storage stability data are needed on residues of toxicological concern as identified in the metabolism study, and since the storage stability data are needed for the validation of plant residue data, CIEL proposes to submit storage stability data with the plant residue data (48 months after receipt of the Lindane Registration Standard).

RCB's Comments and Recommendations

RCB considers it logical to delay the submission of storage stability data until the residues of toxicological concern have been identified.

Registrant's Concern No. 3

CIEL plans to conduct the animal metabolism, feeding, and residue studies so that samples are not stored for an appreciable time.

2

CIEL therefore requests a waiver from the requirement of submitting storage stability data on these substrates.

RCB's Comments and Recommendations

Currently there are no storage stability data available for residues of lindane or its metabolites in animal commodities--not even for the parent itself. Although the registrant intends to conduct his experiments so that animal commodities are not stored for an appreciable period, the possibility exists that some contingency could arise which would necessitate storage. Therefore, RCB recommends that the requirement for storage stability data for residues of lindane in animal commodities not be waived. Since other residues of toxicological concern (besides lindane) may be found as a result of the animal metabolism studies, RCB suggests that the radiolabeled tissues from the metabolism studies could be valuable in generating storage stability data for these residues as well as for validating any analytical methodology which may need to be developed for these residues. Because of enforcement considerations, RCB recommends that potential problems relating to storage stability in animal commodities be identified as soon as possible. However, the current due date for storage stability data is 12 months after receipt of the Standard (i.e., 6 months before the animal metabolism data are due). Since residues of toxicological concern must first be identified before storage stability data can be generated on them, RCB also recommends that storage stability data for residues of toxicological concern in animal commodities be submitted with the animal metabolism data or, if it is necessary to develop new analytical methodology, with the new analytical method.

Registrant's Concern No. 4

CIEL requests an 18 month extension (i.e., 36 months after the receipt of the Standard) for the submission of the feeding study results because the animal metabolism studies must be completed before the analyses of animal tissues from the feeding studies can be carried out.

RCB's Comments and Recommendations

Radiolabeled animal commodities are very useful in the development of the analytical methodology used to generate the residue data. The efficiency of the extraction technique can be readily checked, and the efficacy of the analytical methodology can be validated. Therefore RCB recommends that the due date for the submission of data from the animal feeding studies be extended for 12 months (not 18 months) following the due date of the animal metabolism data (i.e., 30 months after receipt of the Standard). RCB considers 12 months an adequate period to carry out the feeding study (in which animals are dosed for one month or until residue levels have plateaued in milk or

eggs) and, if necessary, the development of appropriate analytical methodology.

Registrant's Concern No. 5

CIEL also requests an 18 month extension on the due date for submitting animal residue studies which involve application of the material by means other than oral ingestion.

CIEL proposes to use a dip application to satisfy the Agency's request for residue studies reflecting various dermal applications. The dip application should lead to the highest residues in meat and milk. CIEL proposes to dip cattle and hogs.

CIEL requests a waiver from conducting a feeding study on lambs; CIEL maintains that residue data on cattle should be translatable to lambs.

RCB's Comments and Recommendations

These animal residue studies involve dermal applications of lindane. These studies should not take as long as the feeding studies in which animals should be fed until residues plateau in milk or eggs. Therefore RCB recommends that the due date for the residue data reflecting dermal applications be extended to 6 months after the due date for the animal metabolism data (i.e., 24 months after receipt of the Standard).

RCB agrees that the dip application would represent the worst case for dermal application and recommends that the dip application would be adequate for the purpose of assessing the levels of residues arising in animal commodities from dermal treatment.

However, unshorn lambs should be subjected to dipping, in addition to the cattle and hogs. The available residue data, though scanty, indicate that the highest residues were found in unshorn lambs, which exhibited lindane levels of 21-51 ppm in the fat 3 weeks after treatment. In a meeting with RCB, representatives of Rhone-Poulenc have agreed to dip unshorn lambs (memo of R. Perfetti, 2/10/85).

The registrant was not specifically asked to conduct a feeding study using lambs in the Lindane Registration Standard because residue data from cattle would be translatable to lambs.

In response to the animal metabolism data gap cited by the Lindane Registration Standard, CIEL has submitted a protocol for a lindane metabolism study reflecting dermal application. The protocol is summarized below.

Protocol for Determining the Metabolic Fate of ^{14}C-Lindane Applied Topically to Beef Cattle

Although, according to CIEL, dermal use of lindane is restricted to non-lactating ruminants, a cow, as well as a steer, will be included in this study in order to compare the results of dermal application to those obtained from orally dosing a lactating goat. The specific activity of the ^{14}C-lindane used to treat the cow and steer will permit the detection of 0.01 ppm total ^{14}C-residues in tissues and 0.002 ppm in milk. The ^{14}C-lindane will be formulated as an emulsifiable concentrate in an aqueous suspension and applied to a shaved area of the animal's back. About 10% of the total body area will be treated so that no run-off will occur. Since 1/10 of the animal will be treated, only 1/10 of the label dose will be used for each application. A typical dosage rate would be 1-2 gallons of a 0.06% lindane solution. This rate translates to 0.45 g ^{14}C-lindane per application.

The animals will be treated twice at 7-day intervals in accordance with the label recommendations. Total milk will be collected twice daily and immediately before slaughter and will be frozen as soon as possible. The animals are to be slaughtered 48 hrs after the second application by exsanguination after stunning. Tissue samples of liver, kidney, omental and renal fat, and muscle will be collected. Tissues will be homogenized, then radioanalyzed by combustion or liquid scintillation counting. The skin and subcutaneous fat from the application site will be removed, weighed and stored frozen until analysis. The longissimus dorsi muscle under the application site will also be sampled and stored frozen until analysis.

Tissues containing greater than 0.1 ppm ^{14}C-residues, representative samples of milk (separated into skim milk and milkfat), and excreta will be extracted with various polar and non-polar solvents to determine levels of bound and extractable residues. The registrant will then attempt to isolate, characterize, and identify ^{14}C-residues extracted from edible tissue. Only extracts containing significant levels of radioactivity will be examined. Conjugated and non-conjugated metabolites will be separated by column chromatography, liquid-liquid partitioning, and/or thin layer chromatography. Hydrolysis by acid or enzymes (beta-glucuronidase; sulfatase) will be used to free conjugates. The non-conjugated metabolites and residues freed by hydrolysis will be further purified by column chromatography and/or preparative thin layer chromatography. Thin layer chromatography and/or HPLC of reference standards and the isolated residues will be used to characterize the ^{14}C-residues.

If possible, the identity of the major metabolites will be confirmed by GC/MS.

RCB's Comments and Recommendations

Although elsewhere in this submission the registrant had requested that only non-lactating ruminants be subjected to the dermal application (see Registrant's Concern No. 1), this protocol does include treatment of a dairy cow and analysis of its milk. Since dairy and non-dairy goats may be treated dermally with lindane, RCB advises including the dairy cow in this metabolism study as the protocol stipulates.

The registrant proposes to use 1/10 of the recommended dosage and to use lindane of a high enough specific activity to permit detection of 0.01 ppm ^{14}C-residues in tissue, and 0.002 ppm in milk. RCB assumes that these levels correspond to lindane equivalents. According to the Lindane Registration Standard, steers and heifers subjected to 1-6 sprays (2.27-13.62 g, assuming 2 gallons of spray) exhibited less than 2.5 ppm lindane in the fat one day after treatment. On the other hand, other studies cited by the Registration Standard indicate that cattle sprayed with 5.7 g lindane (assuming 2 gallons of spray) exhibited 10-20 ppm lindane in the fat one week after treatment. Extrapolating to the registrant's proposed dose of 0.454 g ^{14}C-lindane per application, the expected residues of lindane per se in fat could range from <0.5 ppm - 1.6 ppm. Other data cited in the Lindane Registration Standard indicate that cows treated with lindane sprays or smears (3.5 - 75.7 g per dose, assuming 2 gallons per treatment for the sprays) exhibited levels of 0.7 - 2.0 ppm lindane in milk 1-2 days after treatment. Extrapolating to the registrant's proposed dose of 0.454 g per application, the expected residue levels of lindane per se in milk would be 0.005 - 0.3 ppm. Although these projected levels are questionable because of the methodology used, the specific activity of the lindane to be used appears to be adequate to detect the expected levels of radioactivity in fat and milk from the proposed dosage rate.

Generally RCB recommends that the minimum dosage rate used in metabolism studies should approximate the use rate (1 X) for direct animal treatment; changes in the metabolic profile resulting from 10-fold differences in dosing, for example 0.1 X versus 1.0 X, are not unusual. Therefore RCB suggests that the registrant increase his dosage to reflect at least a 0.5 X application rate (instead of 0.1 X), if this increased dosage rate is feasible.

The registrant also proposes to examine only those tissues containing greater than 0.1 ppm total ^{14}C-residues. RCB assumes that this level corresponds to 0.1 ppm lindane equivalents. The registrant states, "The specific activity of the radiolabeled material is sufficient to allow the sensitivity of detection such that any residue values found can be easily corrected for the total seasonal dosage." If the proposed cut-off level of 0.1 ppm 14C-residues

already reflects the correction for the label seasonal dosage rate, RCB could consider the proposed cut-off level appropriate.

The registrant proposes to sacrifice the animals 48 hours after the second application. Generally, RCB recommends that animals be sacrificed within 24 hours after the last treatment. Several studies cited by the Lindane Registration Standard indicate that higher residue levels of lindane in milk were observed 24 hours after spraying than 48 hours after spraying. Therefore, the registrant will need to slaughter his animals 24 hours after treatment, instead of 48 hours after treatment.

The registrant mentions freezing samples of milk, urine, feces, skin, and underlying fat and muscle from the application site. RCB assumes that an oversight was responsible for the lack of a description of the storage conditions for liver, kidney, omental and renal fat, and muscle. RCB suggests that these tissues also be frozen as soon as practicable.

Other Considerations

Note to PM:

The Lindane Registration Standard cited as a data gap the open-ended application rates permitted with dermal applications. Although some labels recommend 2 applications, the End Use Product labels do not specify the number of treatments permitted to an animal per year. Appropriate action should be taken.

cc: Lindane Reg. Std. File, W. Boodee, PMSD/ISB, R.F., Reviewer-Deyrup, G. La Rocca-PM #15, Circu., Lindane Subject File
RDI:JHOnley:3/19/86:RDSchmitt:3/20/86
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