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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#3F2884 and Chlorpyrifos Registration Standard. Dow's Response to DEB Review of March 9, 1988. MRID Nos. 406388-01 thru -03. DEB No. 3938.

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Background

Dow Chemical USA submitted additional metabolism studies and residue data in response to the EPA document entitled "Guidance for the Reregistration of Pesticide Products Containing Chlorpyrifos as the Active Ingredient", issued September 28, 1984. The initial DEB review of the additional data concluded that the plant and animal metabolism data were inadequate (see memo of S.H. Willett, dated 3/9/88). In PP#3F2884, Dow Chemical proposed to revise chlorpyrifos tolerances, which include the parent and 3,5,6-trichloro-2-pyridinol metabolites, in such a way that the amount of chlorpyrifos (parent compound) would be specified but that the combined residue level would not be different from the existing tolerance in order to reduce the concern over chlorpyrifos toxicity. DEB recommended for the revision of established tolerance expressions which separately express levels of parent and metabolites, but cautioned the registrant that tolerance levels may need to be changed at a later date if the additional metabolic work requested int the registration standard indicated other significant metabolites or residues (see DEB memo of S.H. Willett, dated 3/9/88).

Chlorpyrifos is the common name for the insecticide, O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate. Tolerances for this pesticide are established for combined residues of chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol (TCP) in 40 CFR 180.342.

Conclusions

1. DEB concludes that all matters concerning the metabolism of chlorpyrifos in plants and ruminant animals have been sufficiently addressed as a result of the data reviewed by DEB on March 9, 1988 and the additional information contained in this submission. The significant residues in plants and animals are chlorpyrifos and TCP.

2. DEB can also now conclude that the residue data submitted previously in response to residue chemistry data requirements outlined in "Guidance of the Reregistration of Pesticide Products Containing Chlorpyrifos as the Active Ingredient", specifically those data gaps described in footnotes 3, 4, 12, 13, 14, 15, 38, and 40, are adequate for the purposes of reregistration.

Recommendations

DEB continues to recommend for the establishment of the revised tolerance expressions for chlorpyrifos which separately express levels of chlorpyrifos and TCP (see also DEB's review of 3/9/88).

Present Considerations

The petitioner has submitted a response to the questions concerning the plant and animal metabolism studies that were raised in the initial data review (see DEB memo of S.H. Willett, dated March 9, 1988). The metabolism study deficiencies of that review will be restated below, followed by the petitioner's additional comments and DEB's response.

Deficiency, Data Gap I (Plant Metabolism)

Concerning the metabolic fate of chlorpyrifos in corn, it is noted that most of the ^{14}C-residues were not identified. Only 33 percent of the ^{14}C-activity was extracted with 75 percent acetone from green forage of which only 3 percent was identified. A second green forage sample extracted with 1 N NaOH contained 91 percent of the activity, but only 33 percent of that extract was identified as pyridinol. The petitioner should attempt to identify more of the ^{14}C-activity. The petitioner may need to

clean up the aqueous phase so that more of the extract can be looked at by TLC and HPLC. He should also subject the neutralized extract to TLC since there are some residues that appear early in the HPLC histograms supplied. In some instances conjoining bars have been attributed to 1 metabolite (see Figure 14, MRID No. 262241 lower graph). The petitioner should attempt to separate and identify each component. Identification of metabolites in corn fodder could also be greatly improved. For example extraction with 75 percent acetone solubilized only 23 percent of ^{14}C-activity and a total of 5 percent was identified as chlorpyrifos and pyridinol. While alkaline hydrolysis of the whole dry fodder solubilized 89 percent of the ^{14}C-activity only 54 percent of the activity was identified as pyridinol. Identification of components from the acetone and diethyl ether fractions was also insufficient.

The HPLC histogram (Figure 4, page 18, MRID No. 262241) of the ^{14}C-chlorpyrifos standard contains conjoining bars between 19 and 21 minutes. Could the shorter bar be a significant impurity? Perhaps the standard formulation should also be subjected to TLC. In the report on the metabolism of chlorpyrifos in/or sugar beets, the inadequacies are similar. An insufficient amount of the ^{14}C-activity in beet thinnings was solubilized by 75 percent acetone, and only a small fraction of the activity has been identified (see page 19, MRID No. 262243). Similarly, extraction of ^{14}C-activity from mature tops with acetone removed only 4 percent of the ^{14}C-activity. In the analysis of mature beets the petitioner has indicated that the analysis showed that pyridinol accounts for 36 percent of the beet residue, and the methoxypyridinol for 7 percent of the residue (page 9). The histogram implies that methoxypyridine accounts for 27.3 percent of the activity (in the ether phase extract). Methoxypyridine is not currently included in the tolerance expression. Here again the HPLC histogram of the formulation appears to contain 2 adjoining bars (page 17, Figure 4 - GH-C 1809). The petitioner is asked to explain these adjoining bars.

In the report on the early fate of chlorpyrifos on corn, soybeans, and sugar beets, the petitioner has not adequately identified compounds apparently present in the analysis (MRID No. 262242). Several conjoining peaks appear in the histograms, yet they have been attributed to only one compound (see pages 19, 21, and 24).

Overall the petitioner should attempt to identify more of the residues produced by metabolism of the insecticide in these crops. We suggest that attempts be made to improve the chromatography (i.e., better cleanup and additional solvent systems both HPLC and TLC). More compounds should be added to the standards profile. Additional histograms from control sample analysis obtained under the varying chromatographic conditions would aid in interpretation of results. In the table of results

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accompanying the histograms, ppm equivalents should be given as well as percentages.

RCB concludes that the above plant metabolism studies are not adequate for the purposes of reregistration. The nature of the residue is not adequately understood (see also Footnote 3, Guidance for Reregistration of Pesticide Products Containing Chlorpyrifos and the Active Ingredient).

Dow's Additional Response to Data Gap I (MRID# 406388-02)

The petitioner explains that the apparent conjoining bars in the HPLC histograms supplied can be attributed to the HPLC fraction collecting techniques.. Since compounds frequently elute from the column over a time period of a minute or more (depending on the chromatographic conditions chosen), it is not uncommon for a single component of an extract to be collected in more than one fraction.

Specifically, figure 14 of the corn study (Acc. No. 262241) shows the peak for chlorpyrifos eluting on top of a broad region of radioactivity in fractions 31 and 32. Since the majority of the radioactivity is in the 32 minute fraction, this is assigned as the retention time. The histogram program used assigns radioactivity to a peak until it reaches baseline or the increase of another peak is detected. When the increase in another peak is detected (as in 34), the radioactivity in the preceding fraction (33) is split between the two peaks using an integration method of dropping to the baseline. Therefore, the radioactivity in the 32 minute peak in the histogram has been summer over 29-32 minutes plus half of the radioactivity in the 28 and 33 minute fractions. The registrant states that the reason for running this particular histograms was to show the presence of chlorpyrifos in the extract. Although other minor peaks are present, the total level of chlorpyrifos present in this sample was 35% of an extract containing 7% of the residue in fodder which contained 4.1 ppm ^{14}C-chlorpyrifos equivalents. This equals 0.1 ppm chlorpyrifos in the fodder. Since all of the other components in the histogram are below that level, further characterization would not be possible even if better isolation and separation were achieved.

The HPLC histogram of formulated ^{14}C-chlorpyrifos in Figure 4 of the corn study (Acc.# 262241) and Figure 4 of the sugar beet study (Acc.# 262243) also contained conjoining bars. The petitioner states that these figures are identical since the same formulation was used in both studies. The conjoining bars are present because of the fraction collection technique as described previously. The petitioner has submitted a report on the synthesis of ^{14}C-chlorpyrifos in which the purity of the

radiochemical was determined to be >98.8% by reverse phase HPLC and >99% pure by TLC analyses (MRID# 406388).

The registrant has offered further explanation of their inability to specifically identify most of the ^{14}C-residues in the plant metabolism studies. Plant tissues (corn and sugar beets) were first extracted exhaustively with diethyl ether or acetone to release the parent compound and free metabolites which were not conjugated or incorporated into natural plant constituents. Tissues were then extracted with NaOH to attempt to hydrolyze or solubilize conjugated material (see acc. #s 262241 and 262243 for details of the extraction schemes). HPLC chromatography was conducted on the extracts and retention times of radioactive peaks compared to those of standards of possible metabolic products.

The registrant has submitted the following data summary as requested in the DEB memo of March 9, 1988.

TABLE I. ^{14}C-CHLORPYRIFOS RESIDUES IN CORN FORAGE, FODDER AND GRAIN

PLANT FRACTION	FORAGE		FODDER		GRAIN	
	%	ppm	%	ppm	%	ppm
Total ^{14}C	100	1.62	100	4.14	100	0.13
Chlorpyrifos	3	0.05	.7	0.03	<1.6	<0.002
Pyridinol	30	0.43	17	0.70	<1.6	<0.002
Methoxy	ND	ND	3	0.12	<1.6	<0.002
Polars	56	0.83	67	2.80		
Minor Peaks (sum)	11	0.16	4.5	0.18		
Insoluble	9	0.13	11	0.45		
Natural Plant Constituents					98.4	0.13

The majority of the radioactivity is primarily associated with very polar materials. Attempts to work with the polar fractions were made. However, as endogenous high molecular weight materials (proteins, polyssachrides, lipids, lignin, etc) were removed, so was the radioactivity. Therefore, the polar peaks appear to be associated with naturally incorporated ^{14}C. The significant nonpolar metabolites have been identified.

A similar data summary from the analysis of sugar beets treated with ^{14}C-chlorpyrifos has been submitted as requested by DEB.

TABLE II. ^{14}C-CHLORPYRIFOS RESIDUES IN SUGAR BEETS

PLANT FRACTION (sugar beets)	THINNINGS %	THINNINGS ppm	TOPS %	TOPS ppm	BEETS %	BEETS ppm
Total ^{14}C	100	0.67	100	0.05	100	0.22
Chlorpyrifos	1.5	0.01	---	---	0.3	.0007
Pyridinol	59	0.40	30	0.015	25.6	0.06
Methoxy	---	---	---	---	8.9	0.02
Polars	31	0.22	23	0.011	9.4	0.02
Minor Peaks (sum)	---	---	12	0.006	---	---
Insoluble	8.6	0.06	11	0.020	16.4	0.04
Sugar					40.5	0.04

The registrant concludes that the data for the residue analysis of beets are fairly well characterized with pyridinol representing the major metabolic product.

Although small amounts of radioactivity were released with organic solvents, solubilization of the radioactivity with caustic was fairly efficient in all cases and generally showed the pyridinol as the primary metabolic product. The petitioner concludes that all major nonpolar metabolites have been identified and a portion of the polar ^{14}C-residues represent conjugates which can be converted. The remainder of the polar material can be attributed to naturally incorporated ^{14}C.

DEB's Comments/Conclusions, re: Data Gap I

DEB concludes that the explanation submitted has sufficiently clarified matters concerning the metabolism of chlorpyrifos in plants. As a result of the submission of the plant metabolism data requested by DEB and the additional information presented here, this data requirement has been filled. The residues to be regulated in plants are chlorpyrifos and pyridinol (TCP).

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Deficiency: Data Gap II

The petitioner has not adequately explained several peaks appearing in several of the HPLC and TLC graphs. For example in Figure 10 (page 19) of Study No. 6148-103 (Acc.# 263124), the petitioner indicated that Fraction 9 of his ^{14}C-chlorpyrifos standard is chlorpyrifos. What is Fraction 10? Do we have adjoining compounds that could be separated by using different mobile systems? The petitioner is also requested to explain fully the broad peaks in Figures 12 and 15 and the fraction 4 peak in Figure 16 on pages 21, 24, and 25, respectively. Additional graphs for controls and standards would aid in this purpose.

RCB concludes that the nature of the residue in ruminants is not adequately understood at this time. However, RCB has previously concluded that the metabolism of chlorpyrifos in poultry has been adequately determined [see RCB's (M. Bradley) memorandum of January 21, 1987].

(See also footnote 4, Guidance for Reregistration of Products Containing Chlorpyrifos as the Active Ingredient)

Dow's Additional Response to Data Gap II (MRID# 406388-01)

The registrant explains here again that the conjoining peaks in the TLC graphs do not represent adjoining compounds, and the phenomenon observed is common when HPLC fractions are collected or TLC peaks are scraped in segments that are counted. In this study TLC bands of radioactivity typically occurred over 2-3 cm. Thus, in cases where the plates were scraped in 1 cm increments and counted, radioactivity was observed in adjoining segments.

Therefore fraction 10 in figure 10 (^{14}C-chlorpyrifos standard) of the animal metabolism study (MRID# 262341) is part of the chlorpyrifos peak that was split between fraction 9 and 10 in the analysis of the plate. ^{14}C-Linear scanning of the chlorpyrifos standard depicting more uniform peaks spanning about 2 cm were supplied in this submission (see attachments A-E). Similar arguments are applicable to Figures 12 (TLC graph for kidney extract) and 15 (TLC graph for muscle extract) in the animal metabolism study. The HPLC profiles of these extracts as shown in figures 6 and 9 of the animal metabolism study clearly represent the distribution of radioactivity in these tissues. The major components in both extracts are chlorpyrifos and the pyridinol (TCP). The two smaller peaks present in figure 9 in the HPLC graph for the muscle extract represent 5% and 13% of the total radioactivity in the extract, or 0.005 ppm and 0.012 ppm chlorpyrifos equivalents. Alkaline hydrolysis of the muscle extract demonstrated that these unidentified peaks could be

converted to the pyridinol and represented conjugates of the pyridinol (see figure 17, Acc.# 263124).

The registrant further explains that figure 16 on page 25 of the study is an HPLC profile depicting the distribution of radioactivity in an alkaline hydrolysis extract of the already extracted liver tissue. The unidentified peak in fraction 4 represents 1.17% of the total radioactivity in liver, or about 0.007 ppm chlorpyrifos equivalents. This level is too low to be identified, and can be characterized only as a very minor polar metabolite.

DEB's Comments/Conclusions; re Data Gap II

DEB now concludes that the study previously submitted adequately delineates the metabolism of chlorpyrifos in ruminants. The residues to be regulated are chlorpyrifos and TCP. As a result of the submission of this study and the additional information presented here, this data gap has been filled.

TS769C:DEB:SHW:shw-9/15/88:CM2:RM810:X1669
cc: RF, Circ., Willett, PP#3F2884, Reg. Std. File (Boodee), PM12, PMSD/ISB, Reto Engler-HED/SACB
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