(7-30-85) MRID 152211

CASWELL FILE #11

8.1-85

004723

CHEMICAL: Alachlor

TEST MATERIAL: ^{14}C Alachlor

STUDY/ACTION TYPE: Metabolism: Identification of Metabolites in Urine

STUDY IDENTIFICATION: The Metabolites of Alachlor in Monkey Urine Obtained from Dermal Penetration Studies (Studies #MSL 3386 conducted in 1981 and submitted to the Agency on January 5, 1982). Monsanto Special Report #MSL-4609, RD #596, compiled by S. R. Muench, March 20, 1985. Accession Nos. 257283 and 258593.

NOTES:

1. The above report has the following information that appears to be different from the above documentation:
Report#: MSL-3386
Project#: 7824
Date: February 1984
Authors: C. L. Livingston and W. R. Purdum

2. The original study was performed by H. I. Maibach of the University of California, Study #MA-81-261, November 28, 1981, submitted in January 5, 1982, (Accession #070592, reviewed by EPA in a memo dated July 20, 1982, pages 18 and 19); and an addendum which included the data requested by this reviewer because they were missing from the original report. The addendum was submitted on July 29, 1982, as Special Report MSL-1983, R.D.396 dated July 27, 1982, (Accession #247937, reviewed by EPA in a memo dated March 23, 1983, pages 4, 5,and 6).

STUDY DIRECTOR: The study director for this section of Maibach's original study was not identified.

DATE INITIATED: Initial date of treatment: 1981 (However, the samples of urine used in this study were frozen for 2 to 3 years before analysis).

DATE SUBMITTED: March 26, 1985

REVIEWED BY: Amal Mahfouz

Amal Mahfouz 7/30/85

CONCLUSION:

In this study three major metabolites were identified in monkey's urine: two mercapturic acid conjugates and one conjugate of thioacetic acid. The three metabolites were clearly identified as DEA derivatives, and they apparently were detected at a similar ratio in both urine samples collected from the intramuscular test and the dermal test. The average total percentages of these metabolites (three monkeys in each test) are 69.87 $\pm$ 4.30% and 61.70 $\pm$ 1.55% in the intramuscular and dermal test, respectively.

It is not clear if these percentages refer to the level of radioactivity in the analyzed urine samples or refer to the total amount of the dose recovered in urine (71.4% and 15.6% of the administered dosages in the intramuscular study and the dermal study, respectively). In the absence of this information, it is not possible to verify these findings.

Although this reviewer agrees with the registrant that the major metabolites in the monkey's urine in both the intramuscular test and the dermal test are conjugates of metabolites which contain the DEA moiety, several issues presented in the discussion section of this review compromise the quantitative data obtained from this study. This study remains classed as Core Supplementary (pilot study, see Discussion Section).

Material and Methods

The materials and methods used for identification of metabolites in urine were evaluated and a copy is attached as Appendix #1.

This reviewer has the following comments:

1. It appears that the urine collected from each monkey (which was left over after ^{14}C -radioanalyses by Dr. Maibach's Laboratory in 1981) was sent frozen to the metabolism laboratories of Monsanto. These samples were kept frozen for two years until further metabolite analyses were performed. Monsanto's report #MSL-3386

of February 1984 did not clearly describe the conditions under which these samples were stored. The Agency was concerned about the stability of these samples after this lengthy storage period and dicussed these concerns with the registrant. In a letter dated July 3, 1985 (Accession # 258593), Monsanto indicated that these samples have been stored under deep freeze conditions shortly after the time of collection. However, The registrant's letter still did not clearly indicate when these samples were deep frozen. In view of the fact that the study was not designed to identify and quantify the metabolites in excreta, it remains quetionable if timely precautions were taken to adequately freeze these samples before any significant degredation occurred.

2. It appears that a very low concentration of metabolites and a limited volume of urine was available for analysis. Thus, the registrant decided to identify the metabolites in the fraction of urine that contained the highest radioactivity. Although this is an acceptable practice, this reviewer cannot consider this study design as valid without the identification and quantification of metabolites in the rest of the urine samples. The fact that the registrant knew that he would have access to more adequate samples from the new monkey studies makes the present study suspect.

Due to the above discussed deficiencies, the data obtained from this study are considered by this reviewer as qualitative data or preliminary information (pilot study).

Results:

Three major metabolites were identified in this study in both the intramuscular and dermal tests. Table #1 below reflects the average percentage of these metabolites which were identified in the urine of 3 monkeys from each test (the data are reproduced from Table #1 of the submitted report).

TABLE I

HPLC/LSC Histogram Data for the Major Urinary Metabolites

Dose Group	Peak No.*	Percentage of Distribution[a]
intramuscular (0-4 hour samples)	1	14.0. ± 3.45
	2	31.16 ± 5.49
	3	24.71 ± 3.31
	total percentage	69.87 ± 4.38

Table I (continued)

Dose Group	Peak No. *	Percentage of Distribution[a]
topical (24-48 hour samples)	1	12.07 ± 6.09
	2	32.98 ± 5.70
	3	16.65 ± 3.34
	total percentage	61.70 ± 1.55

[a] Data represents the average ^{14}C-radioactive distribution and the calculated standard deviation from samples from three monkeys in each dose group.

* Peak number:

P_1:3[(2-[(2,6-diethylphenyl-N-(methoxymethyl) amino]-2-oxoethyl)thio]-2-(acetylamino)-propanoic acid

P_2:3-[(2-[(2,6-diethylphenyl)amino]-2-oxethyl)thio]-2-(acetylamino) propanoic acid

P_3:2-[(2-[2,6-diethylphenyl)-N-(methoxymethyl) amino]-2-oxoethyl)thio]acetic acid

Discussion

A dermal penetration study in monkeys was performed by Dr. Howard I. Maibach for Monsanto Company in 1981 (study #MA-81-261, 11/28/81). The protocol of that study included two sections: a preliminary intramuscular test and a percutaneous test. In both tests the elimination of ^{14}C-labeled alachlor/ metabolites was monitored in urine for 5 days. In the intramuscular test, the elimination was also monitored in feces.

The intramuscular test indicated that 71.4% of a single dosage was eliminated in urine and 5.7% in feces; 22.9% of the initial dosage was not accounted for. Most of the elimination occured within the first 24 hours of the initial treatment. In the dermal test, all data were corrected for a parenteral excretion factor of 71.4% based on the urine excretion data in the intramuscular test. It was determined that 15.6% of the dermal dosage was excreted in urine within 5 days of the initial treatment; most of the elimination occured within the first 2-3 days of treatment. The half life was 15.2 hours in the intramuscular test and 31 hours in the dermal test.

The study was initially submitted to the Agency as a completed study on January 5, 1982. However, this reviewer noted that the dermal test was not included in the report and the registrant was asked to submit the missing data (see memo of July 20,1982, pages 18 and 19). On July 29, 1982, Monsanto submitted the dermal absorption section of the study, however, due to several deficiencies, the study was classed as Core supplementary (see my review of March 23, 1983. page 4, 5, and 6).

There was no indication in the protocol or in the submitted study that the urine would be appropriately saved for further qualitative or quantitative identification of metabolites. In April 1984 the registrant submitted a new protocol for pharmacokinetics studies in monkeys as a part of a protocol for biomonitoring applicator dermal exposure. If a decision was made to identify metabolites in 2 to 3 year old urine samples, that decision was not discussed with the Agency. Furthermore, that decision appears to be incomprehensible in view of the deficiencies noted in the 1981 study and the projected availability of fresh urine samples in the new (IRDC, 1984) pharmacokinetics studies in monkey.

The registrant indicated in his letter of July 3, 1985, page 2 (second paragraph), that in the new 1984 monkey studies by IRDC, the sole purpose of the intravenous test was to define the kinetics of elimination of alachlor following an intravenous dose and that "no precautions were taken to collect and preserve the excrement samples under refrigerated conditions"; the registrant further indicated that "because of the possibility of further biodegradation over time by intestinal microflora under room temperature conditions, it was decided that metabolite

identifications would not be truly representative of metabolites in fresh excrement." This reviewer questions the rationale behind these statements in view of the following observations:

1. The registrant indicated in the methodology of the above mentioned new monkey studies that the urine in the intravenous test was collected for further identification of metabolites (see accession #256624, Part C, submitted to the Agency on January 30, 1985). However, these data were not reported in the study and no explanation was provided.

2. There is no explanation provided by Monsanto as to why appropriate provisions were not made to identify metabolites in that new intravenous study although it was clearly indicated in the method section of that study that metabolites would be analyzed.

3. In view of the difficulties in the study at hand relative to the length of the storage period, the limited amount of urine samples, and the very small amount of radiolabeled metabolites, samples from the new pharmacokinetic studies in monkeys offered a better alternative specimens for identification of metabolites in monkeys. The questions remain as to why these samples were not analyzed, and why precautions were not made to immediately freeze and analyze these samples although the method section (at least for the intravenous test) clearly indicated that these analyses would be performed in that study.

Finally, it is not clear from the submitted results if the percentages of metabolites identified in this study refer to the total amount of radioactivity present in urine or refer to the total amount of radioactivity in the analyzed urine samples. Also, the registrant did not report the amount of radioactivity present in the samples selected for analyses relative to the total amount of radioactivity initially recovered in urine in these studies (71.4% and 15.6% of the administered dosages in the intramuscular study and the dermal study respectively). In the absence of this information it is not possible to verify these findings.

Due to the above discussed deficiencies, the data obtained from this study are considered by this reviewer as qualitative data or preliminary information (pilot study).

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