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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: PP#2F2716 (RCB #758) (Acc. #072376, 2/22/84)
Re-evaluation of The ^{14}C-fonofos Goat Metabolism Study.

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THRU: Charles L. Trichilo, Ph. D., Chief
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TO: William H. Miller, PM#16
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and

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BACKGROUND

In the Residue Chemistry Chapter of Fonofos (Dyfonate®) Registration Standard, under the topics of III. Nature of the Residue in Animals, A. Conclusion, 1. Status of Metabolism data (4/4/84, p.9), the first conclusion reads:

"Fonofos has been studied in vivo over the past decade in both rats and mice. However, there are no studies on livestock and, since many crops for which there are tolerance are used to feed both livestock and poultry, we will need a lactating ruminant (cow or goat) metabolism study and, in addition, we will require a study on poultry metabolism." This was considered a data gap (§171.4).

When the petitioner, Stauffer Chemical Company, submitted a tolerance petition for fonofos in or on potatoes (PP#2F2716) on 6/25/82, the above data gap was identified as Deficiency 1b in the memo of M. Nelson (9/15/82) as follows:

> "A large animal (lactating ruminant) metabolism study, presently lacking, is needed. Pending receipt and review of such a study, we cannot consider the nature of the residue in animals to be adequately delineated for purposes of this petition."

In response to the above memo (M. Nelson, 9/15/82), Stauffer Chemical Company submitted a volume of data (2/21/84) including a report entitled "Metabolism of O-ethyl S-phenyl Ethylphosphonodithioate (Dyfonate®) In The Lactating Goat" (Acc. #072376). RCB's Comments/Conclusion on this goat metabolism study were given in the memo of Martha J. Bradley (6/13/84) which reads:

> "The submitted metabolism study was apparently designed to study the absorption and elimination of Dyfonate®, and does not identify the tissue residues which are our greatest concern. The petitioner should conduct a new lactating ruminant metabolism study and should consult the Residue Chemistry Guidelines for the nature of the residue in livestock for the proper procedures (such as animals should be dosed daily for at least three days and animals should be sacrificed within 24 hrs. of cessation of dosing)."

Based on the above review, RCB concluded that this Deficiency 1b had not been resolved.

PRESENT CONSIDERATION

In a general response to the Fonofos (Dyfonate®) Registration Standard, Dr. Ralph L. Riggs (Starffer Chemical Company) sent a letter to William H. Miller (EPA) on 9/17/84. On page 4 and under the topics of Residue Chemistry and Magnitude of the Residue/Ruminant Metabolism Study (171.4), this letter reads:

> "The reviewer concluded that the study was designed to determine absorption and elimination and did not identify the tissue residues which were of greater importance to the EPA. We believe our report must have been misread since a great deal of emphasis was placed on the characterization of metabolites in the tissues. For example, the metabolites which occurred

in blood and milk through the first 48 hr. following dosing (after 48 hr, the ^{14}C in these fluids was too low to be characterized) were identified. The metabolites found in blood and milk would be expected to be the same as those occurring in tissues since these two fluids are in intimate contact with tissue cell protoplasm. Furthermore, the metabolites in goat muscle at the time of sacrifice were nearly completely identified. We request that this study be considered scientifically valid and removed from the list of fonofos data gaps."

RE-EVALUATION OF THE ^{14}C-FONOFOS GOAT METABOLISM STUDY

1. Previous Metabolism Studies

Before this goat metabolism study, ^{14}C-fonofos metabolism studies on rats, mice and potatoes have been conducted and reviewed by RCB (PP#2F2716).

2. The ^{14}C Goat Metabolism Study

The ^{14}C-fonofos goat metabolism study was initiated on May 24, 1976 and completed on June 16, 1980. It was conducted in two parts:

Part I: Four lactating goats were dosed as follows:

Goat A: Single dose with non-^{14}C fonofos at 2 mg/kg body weight

Goat B: Four doses with ^{14}C-fonofos at 0.5 mg/kg each (48 hrs. apart)

Goat C: Single dose with ^{14}C-fonofos at 2 mg/kg body weight

Goat D: Four doses with non-^{14}C fonofos at 0.5 mg/kg each (48 hrs. apart)

Goat B, C and D were euthanized 9 days after the last dosing and the following tissues were collected: liver and gall bladder, kidney, adrenal, heart, brain, mammary glands, striated muscle, subcutaneous fat, lung, ovary and uterus. During the 9 day dosing period, blood and milk samples were collected.

Part II: Goat E (which was actually Goat A in Part I) was kept for 50 more days after the Part I study and re-conditioned for 3 days before conducting a second single dose with ^{14}C-fonofos at 2 mg/kg body weight. Blood and milk samples were collected during the 9 day dosing period. This animal was not sacrificed upon termination of this experiment.

3. Methodology

The ^{14}C residues in excreta, blood, milk and tissue samples were determined by liquid scintillation counting. Activity in urine, feces, blood and milk was characterized using TLC and GC/MS techniques.

4. Recovery of Dosed ^{14}C During the 9 Day Period

Sample	Goat B (%)	Goat C (%)	Goat E (%)
Urine	57.21	45.25	76.60
Feces	15.70	20.60	20.86
Milk	1.25	0.70	1.75
Tissues	0.23	0.26	----
Total	74.39	66.71	99.21

These data indicate that the ^{14}C-fonofos administered was eliminated mainly in urine (45.25-76.60%) and feces (15.70-20.86%). Smaller amounts were excreted in milk (0.70-1.75%) or remained in tissues (0.23-0.26%) at the time of sacrifice which was 9 days after the last dosing. Note: The animals should have been sacrificed within 24 hours of cessation of dosing.

5. Tissue ^{14}C Residues

The percentages of the total ^{14}C residues in the edible tissues of goat B and C at 9 days after dosing are summarized below:

Tissue/organ	Goat B (4 doses)		Goat C (single dose)	
	%	ppm	%	ppm
Carcass	0.17	0.003	0.17	0.003
Fat internal	----	0.009	----	0.001
Fat subcutaneous	----	<0.001	----	0.002
Kidney	0.01	0.044	0.01	0.057
Liver	0.04	0.023	0.07	0.046
Striated muscle	----	<0.001	----	0.001

6. Urine Metabolites

The urine samples collected were combined into 9 pools for analysis. Of the 9 metabolites identified, the percentages of the 4 major metabolites are summarized below:

Urine Samples		Distribution of Activity in Urine as A Percent of terminal Activity			
		3-OH $PhSO_2Me$	4-OH $PhSO_2Me$	2-OH $PhSO_2Me$	$PhSO_2Me$
Goat B	0-48 hrs. after dose 1	55.8	19.9	1.8	2.0
	0-48 hrs. after dose 2	56.2	18.8	2.0	3.4
	0-48 hrs. after dose 4	54.9	18.9	1.2	4.0
Goat C	0-12 hrs. after dose 1	40.6	16.6	2.9	5.2
	12-48 hrs. after dose 1	51.3	20.1	1.8	5.0
	48-96 hrs. after dose 1	49.5	22.6	0.7	6.7
Goat E	0-12 har. after dose 2	45.0	17.5	1.9	4.1
	12-48 hrs. after dose 2	55.4	21.0	1.4	5.7
	48-96 hrs. after dose 2	56.2	17.6	0.7	4.7

7. Feces Metabolites

Methanol extraction of the feces recovered about 15% of the ^{14}C which was composed of essentially the same products observed in urine only in different proportions, but small amounts of fonofos and no conjugates were found.

8. Blood Metabolites

The blood samples collected were combined into 5 pools for analysis. Of the 7 metabolites ientified, $PhSO_2Me$ has the highest percentage as shown below:

Blood Samples		Distribution of Activity in Blood as A Percent of Terminal Activity			
		3-OH $PhSO_2Me$	4-OH $PhSO_2Me$	2-OH $PhSO_2Me$	$PhSO_2Me$
Goat B	6-48 hrs. after dose 1	0.9	0.2	0.0	97.2
	1.5-24 hrs. after dose 2	0.7	0.1	0.0	97.9
	1.5-48 hrs. after dose 4	0.6	0.1	0.0	96.9
Goat C	3-24 hrs. after dose 1	1.0	0.2	0.0	97.2
Goat E	3-32 hrs. after dose 1	0.6	0.0	0.0	98.6

9. Milk Metabolites

The milk samples collected were pooled into 3 fractions representing early, middle and late intervals of ^{14}C excretion. Of the 8 metabolites identified, $PhSO_2Me$ has the highest percentage as shown below:

milk Samples		Distribution of Activity Percent of Injected Dose			
		3-OH $PhSO_2Me$	4-OH $PhSO_2Me$	2-OH $PhSO_2Me$	$PhSO_2Me$
Goat B	6-48 hrs. after dose 1	0.0	0.0	0.0	0.27
	6-48 hrs. after dose 2	0.0	0.0	0.0	0.28
	6-48 hrs. after dose 4	0.1	0.0	0.0	0.35
Goat C	3-12 hrs. after dose 1	0.0	0.0	0.0	0.26
	24-48 hrs. after dose 1	0.0	0.0	0.0	0.34
	56-80 hrs. after dose 1	0.0	0.0	0.0	0.06
Goat E	6-12 hrs. after dose 1	0.0	0.0	0.0	0.62
	24-48 hrs. after dose 1	0.0	0.0	0.0	0.84%(=405 ppb)
	56-80 hrs. after dose 1	0.0	0.0	0.0	0.19

10. Carcass Metabolites

Carcass was the remains of the animal following removal of viscera, head and leg foreparts and included principally the muscle, bones and hide. 3-OH and 4-OH SO_2Me are the major metabolites as shown below:

Carcass Samples		Distribution of Activity in Carcass as A Percent of Terminal Activity				
		3-OH $PhSO_2Me$	4-OH $PhSO_2Me$	2-OH $PhSO_2Me$	$PhSO_2Me$	Unknown
Goat C	%	83.4	7.1	0.0	0.0	9.5
	ppb	1.50	0.13	0.00	0.00	0.17

11. The Overall Consideration of ^{14}C-Fonofos Goat Metabolism Study

Based on the above data, the petitioner reported that the magnitude of tissue residue, urinary excretion rates, rate of depletion of ^{14}C from milk and blood and the nature of the metabolites were virtually the same following both single and repeated doses.

RCB'S CURRENT COMMENTS/CONCLUSION

In accordance with EPA's specifications on animal metabolism studies, this ^{14}C goat metabolism study which was initiated on 5/24/76 and completed on 6/16/80 is not adequate to resolve the existing deficiency 1b spelled out in the RCB's 9/15/82 review of PP#2F2716.

RCB suggested in its 6/13/84 review of Amendment (2/21/84) to PP#2F2716 that the petitioner should consult the Pesticide Assessment Guidelines, Subdivision O, Residue Chemstry (EPA-540/9-82-023, Oct., 1982). Animals used for metabolism studies and dosed orally should be dosed daily for at least three days; and the dosed animals should be sacrificed within 24 hours of cessation of dosing for residue analyses and identification. The submitted data indicate that animals were treated with a single dose (goats A and C) or with 4 doses at 48 hours apart (goats B and D), and that goats B, C and D were euthanized 9 days after the last dosing. Also, since this experiment took more than four years to complete, the assurance of sample integrity is questionable.

RCB's major concern is that metabolites in edible tissues must be well characterized. Also, the study should represent a reasonable pre-slaughter interval, normally within 24 hours. After 9 days of the last dosing, as the experiment was carried out, it is expected that the level of activity would decrease and the nature of the residues may also change. RCB also does not concur with the petitioner's argument that "metabolites found in blood and milk would be expected to be the same in those occurring in the tissues since these two fluids are in intimate contact with tissue cell protoplasm." Significant variations in pesticide metabolites are often found in different tissues of the same animal. The petitioner also state that "metabolites in goat muscle at the time of sacrifice were nearly completely identified." RCB is not aware of any identification of muscle metabolites.

In summary, this study is inadequate for two important reasons: (1) The pre-slaughter interval (9 days) is too long; (2) Metabolites in edible tissues (liver, fat, kidney, muscle) are not well identified.

RECOMMENDATIONS

RCB concludes that its previous conclusion identified on page 2 in the 6/13/84 memo of M. J. Bradley is justified and that the existing deficiency 1b is still outstanding. The petitioner should be advised that a ruminant metabolism study is still needed. RCB suggests that a protocol be submitted to the Agency for comment before this study is started.

Also, the petitioner should be informed that other issues spelled out in Conclusions 2c, 3a, 3b, 3c, 4a, 4b and 4c of RCB's memos of 9/15/82 and 6/13/84 need to be resolved.

cc: R.F., Circu., W.T.Chin, TOX, PP#2F2716, EAB, EEB, FDA, PMSD-ISB
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