PMSD/ISB

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SEP 20 1989

MEMORANDUM

SUBJECT: EPA No. 352-370: Methomyl: Generic Data for Reregistration. Metabolism of Methomyl in Lactating Goat. Response to Data Call. MRID No. 410483-01 DEB No.5210

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E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware has submitted a supplement to a study titled: " Metabolism of ^{14}C-Methomyl in the Lactating Goat. (AMR-22-80)" The supplemental study has been submitted in response to a data requirement in a 3(c)2(b) notice to du Pont as the registrant of methomyl. The Agency had requested additional identification of residues in the cyclohexane soluble fraction of liver.

DEB's review of the submission was performed in conjunction with the Dynamac Corporation review of the material (Paula A. Deschamp, program manager) done under contract with the EPA.

BACKGROUND

The Reregistration Guidance Document issued for methomyl in January 1982 identified animal metabolism of methomyl as a data gap. The registrant, E.I.Du Pont,in their initial response, argued that the animal metabolism had been delineated in a lactating goat metabolism study performed in 1976 and submitted as Report AMR-22-80. Specific questions of the Agency, however, remained.

These concerns were included among the requirements set forth in the data call-in notice of 3/27/87 to the registrants of methomyl:

> You must identify the residues in the cyclohexane soluble fraction that you reported in the goat metabolism study. The agency has not decided whether acetonitrile and acetamide should be regulated as part of the tolerance expression. Therefore any feeding study should include the analysis of these residues using validated analytical methodology.

At a meeting with representatives of du Pont on 6/18/87, DEB clarified the Agency's areas of concern as follows:

1. Identification of the cyclohexane-soluble metabolites found in animal tissues.

2. Quantitation of the acetonitrile level in milk and animal tissue.

3. Resolving the issue of possible acetamide presence in milk and animal tissues. If so, how much?

4. Develop validated methods for metabolites of Toxicological significance.

5. The need for a cattle feeding study.

(Taken from Sami Malak's memorandum of conference held 6/18/87 with du Pont.)

Du Pont's present submission is identified as a response to the data call-in notice of 3/87 and is specifically directed to the "identification of the residues in the cyclohexane soluble fraction extracted from the liver as reported in the original goat metabolism study."

The Residue Chemistry Chapter dated October 30, 1987 for the Methomyl Final Registration Standard and Tolerance Reassessment (FRSTR) requires additional data reflecting the metabolism of [1-^{14}C]methomyl in ruminants and poultry.

- Data depicting the metabolism of methomyl in ruminants and poultry. Animals must be dosed for a minimum of 3 days with [1-^{14}C]methomyl at a level sufficient to make residue identification and quantitation possible. Milk and eggs must be collected twice daily during the dosing period. Animals must be sacrificed within 24 hours of the final dose. The distribution and characterization of residues must be determined in milk, eggs, liver, kidney, muscle, and fat. Specific analyses for acetonitrile and acetamide must be included. Samples

from these studies must also be analyzed using all proposed data collection and enforcement methods (including Method 1 in the PAM, Vol. II, Pesticide Reg. Sec. 180.253) to ascertain that the methods are capable of adequately recovering and quantifying all residues of toxicological concern.

CONCLUSIONS

1. The registrant has responded adequately to the specific requirement of the data call-in notice requesting identification of radioactive metabolites of methomyl in the cyclohexane-soluble extract of goat liver. Preliminary data from a recently completed cow metabolism study was included to support this conclusion.

2(a). The qualitative nature of the residue in animals is not adequately understood. Unidentified non-extractable ^{14}C-residues accounted for high percentages of the total radioactivity in goat liver (72.2%) and cow liver (87.2%). Also, the submitted data do not include an account of radioactivity loss during sample fractionation and no attempt was made to prevent volatilization of acetonitrile during the extraction procedure.

2(b). Additional data are required depicting the distribution and characterization of terminal ^{14}C-residues, including specific analyses for acetonitrile and acetamide, in milk, eggs, kidney, muscle, and fat of poultry and ruminants.

2(c). The capillary GLC/MS analytical procedures presented in Attachment II (report No. AMR-22-80, supplement 1) are adequate for the determination of acetamide in cow tissues and milk. The detection limit of the method is 0.1 ppm, and recovery of acetamide from samples of cow liver fortified at 10 ppm and milk fortified at 100 ppm was 70 and 97%, respectively.

RECOMMENDATIONS

1. The specific requirement of the data call-in notice for methomyl relating to the nature of metabolites in the cyclohexane fraction of goat liver should be considered as satisfied.

2. E.I. duPont de Nemours and Co., Inc. should be notified that these data do not fulfill the outstanding data gap specified for animal metabolism in the FRSTR.

It should be noted that duPont has submitted a protocol for completion of a cattle feeding study. This protocol was reviewed be amended to include metabolites A, B, and C, additional data reflecting these compounds will be required

by DEB (J. Stokes (DEB #2565, dated 8/18/87). Some preliminary data from a study conducted under the protocol were included in this submission. The registrant should be informed that an additional study may be required if the animal metabolism data reveal additional residues of concern in studies currently in progress.

DETAILED CONSIDERATIONS

Lactating Goats:

Details of the in-life portion of this study are presented in original Report No. AMR-22-80 (MRID 00063418) and are briefly described below. A lactating goat received [1-^{14}C]methomyl (specific activity 1.20 μCi/mg; >99% pure) orally for 10 days (two doses per day in capsule form) at a level equivalent to 20 ppm in the diet, for a total of 443 μCi. The animal was sacrificed 24 hours after the final dose. The goat liver was freeze-dried and a portion was stored frozen at -25 C for approximately 10 years until analysis. Aliquots of freeze-dried goat liver tissue were combusted and analyzed for total radioactivity by LSC. Liver samples were extracted according to the method of Bligh and Dyer (1959). Freeze-dried goat liver was extracted with chloroform:-methanol:water (1:2:0.8), centrifuged, and the supernatant mixed with chloroform:water (1:1), bringing the chloroform:methanol:water ratio to 2:2:1.8. The mixture was blended, centrifuged, and the methanol:water and chloroform phases were removed and aliquots analyzed for total radioactivity by LSC. The unextractable portion was air-dried, weighed, ground to a powder, and aliquots were analyzed by combustion/LSC. The chloroform extract (total lipid fraction) of goat liver was saponified under nitrogen with 0.5 M potassium hydroxide in methanol:water (1:1), cooled, and extracted with petroleum ether to separate the neutral lipid fraction. The methanol:water phase was acidified with hydrochloric acid and free fatty acids were partitioned into chloroform. Aliquots of the petroleum ether, methanol:water, and chloroform extracts were analyzed for total radioactivity by LSC. Extracts were concentrated and analyzed by HPLC using a Zorbax ODS column for analysis of methomyl, methomyl oxime, and long chain fatty acids, an Aminex HPX-87H column for analysis of acetamide, acetic acid, and acetonitrile, and a Zorbax NH_2 column for analysis of glycerol and glycerol 3-phosphate. Radioactive compounds were identified by comparison of retention times with those of chromatographed standard compounds.

Of the total administered radioactivity, ca. 1.5% (5.97 ppm methomyl equivalent) was recovered from unextracted freeze-dried goat liver. Of the total radioactive residue (TRR; expressed as ppm methomyl equivalents) in goat liver, 0.93 ppm (15.6%) was methanol:water-soluble, 0.5 ppm (8.4%) was chloroform-soluble, and

4.31 ppm (72.2%) was unextractable. Following saponification of the chloroform extract, 0.07 ppm (1.2% of the TRR) was petroleum ether-soluble, 0.05 ppm (0.8% of the TRR) was chloroform-soluble, and 0.22 ppm (3.8% of the TRR), was methanol:water-soluble.

Table 1. Distribution of [^{14}C]methomyl metabolites and terminal residues in liver from a goat and a dairy cow following oral doses[1].

Residues	Total Radioactive Residues			
	Goat Liver		Cow Liver	
	%	ppm[2]	%	ppm
methanol:water soluble				
Glycerol	13.4	0.80	10.8	7.5
Glycerol-3 phosphate	0.5	0.03	0.9	0.5
Acetic acid	1.1	0.07	1.4	1.0
Acetamide	0.8	0.04	0.1	0.1
Acetonitrile	ND[3]	ND	ND	ND
Methomyl	ND	ND	ND	ND
Methomyl oxime	ND	ND	ND	ND
Unidentified	3.6	0.21	3.5	1.9
Petroleum ether soluble				
Neutral lipid fraction	1.2	0.07	0.1	0.1
Chloroform soluble				
Fatty acid fraction	0.8	0.05	1.3	0.8
Non-extractable	72.2	4.31	87.2	57.2
Total identified	19.4	1.15	13.2	9.1
Total unidentified	74.2	4.43	92.10	60.0

1. Cows received [^{14}C]methomyl at 80 ppm in the diet for 28 consecutive days. Goats received [^{14}C]methomyl at 20 ppm in the diet for 10 consecutive days.

2. ppm methomyl equivalents.

3. Nondetectable; the method detection limit was 0.01 ppm (reported as <0.1% of the TRR).

HPLC analysis of the initial methanol:water extracts of goat liver and the methanol:water extracts of the chloroform phase following saponification identified four radioactive components by comparison of retention times with those of standard compounds (Table 2). The combined methanol:water fractions accounted for 19.4% of the TRR (1.15 ppm) in goat liver and consisted of glycerol at 13.4% (0.8 ppm), glycerol-3 phosphate at 0.5% (0.03 ppm), acetic acid at 1.1% (0.07 ppm), and acetamide at 0.8% of the TRR (0.04 ppm). Unidentified components in the combined methanol:water fractions

accounted for 3.6% of the TRR (0.21 ppm). Methomyl and methomyl oxime were not detected (<0.1 ppm) in methanol:water extracts. In addition, no acetonitrile was detected in the methanol:water fraction; however, no attempt was made to prevent its volatilization during the extraction procedure.

HPLC separation of long-chain free fatty acids in the saponified chloroform extracts isolated radioactive compounds which eluted at retention times corresponding to fatty acids ranging from C_{14} to C_{20}. ^{14}C-Residues in the non-extractable portion of goat liver and in the petroleum ether phase (neutral lipid fraction) following saponification of the initial chloroform extracts were not characterized.

Lactating dairy cows: Data are also presented from a cattle feeding/metabolism study currently in progress (duPont report No. AMR-898-87) in which lactating Frisian dairy cows received [1-^{14}C]methomyl (initial specific activity 66.9 μCi/mg, diluted with non-radiolabeled methomyl to 0.15 μCi/mg; 97.7% pure) orally for 28 days (in capsule form) at 80 ppm in the diet. The animals were sacrificed ca. 16-24 hours after the final dose. A sample of liver from a single cow was frozen and shipped to the lab within one week where it was stored frozen at -20 C for approximately 6 weeks until analysis. Cow liver was extracted and analyzed using the same procedures described previously for goat liver.

Of the total administered radioactivity, ca. 2.9% (65.6 ppm methomyl equivalents) was recovered from unextracted cow liver. Of the total radioactive residue (TRR; expressed as ppm methomyl equivalents) in cow liver, 9.4 ppm (14.3%) was methanol:water-soluble, 2.9 ppm (4.5%) was chloroform-soluble, and 57.2 ppm (87.2%) was unextractable. Following saponification of the chloroform extract, 0.1 ppm (0.1% of the TRR) was petroleum ether-soluble, 0.8 ppm (1.3% of the TRR) was chloroform-soluble, and 1.6 ppm (2.4% of the TRR), was methanol:water-soluble.

HPLC analysis of the initial methanol:water extracts of cow liver and the methanol:water extracts of the chloroform phase following saponification identified four radioactive components by comparison of retention times with those of standard compounds (Table 2). The combined methanol:water fractions accounted for 16.7% of the TRR (11 ppm) in cow liver and consisted of glycerol at 10.8% (7.5 ppm), glycerol-3 phosphate at 0.9% (0.5 ppm), acetic acid at 1.4% (1 ppm), and acetamide at 0.1% of the TRR (0.1 ppm). Unidentified components in the combined methanol:water fractions accounted for 3.5% of the TRR (1.9 ppm). Methomyl and methomyl oxime were not detected (<0.1 ppm) in methanol:water extracts. In addition, no acetonitrile was detected in the methanol:water fraction; however, no attempt was made to prevent its volatilization during the extraction procedure.

HPLC separation of long-chain free fatty acids in the final chloroform phase following saponification of the initial chloroform extracts of cow liver isolated radioactive compounds which eluted at retention times corresponding to fatty acids ranging from C_{14} to C_{18}. ^{14}C-Residues in the non-extractable portion of cow liver and in the petroleum ether phase (neutral lipid fraction) following saponification of the initial chloroform extracts were not characterized.

An analytical method developed for the determination of acetamide in cow tissues and milk is also presented (Attachment II to report No. AMR-22-80, supplement 1). Samples of milk and chopped cow liver were fortified with acetamide at 100 and 10 ppm, respectively. Milk samples were extracted with acetone and centrifuged; acetone was removed from the supernatant by rotoevaporation. Extracts were partitioned with hexane and the aqueous phase was centrifuged and diluted with methanol. Liver samples were extracted with acetone:water (95:5) in a blender, centrifuged after each extraction, and acetone was removed by rotoevaporation. Extracts were partitioned with hexane and the aqueous phase was extracted with ethyl acetate, evaporated, and redissolved in methanol. Milk and liver extracts were analyzed for acetamide by GLC (nitrogen-phosphorus detection) using a HP-20M (Carbowax 20M) capillary column. The presence of acetamide was confirmed by GC/MS. Recovery of acetamide from fortified liver and milk samples was 70 and 97%, respectively. The method detection limit was 0.1 ppm. Acetamide was detected in unfortified control samples of cow liver and milk at ca. 1 ppm.

In summary, the authors reported that the distribution of radioactivity in the methanol:water extracts, the chloroform extracts, and the non-extractable portions of the goat and cow livers is similar, and that this distribution is also similar to the distribution of radioactivity in the methanol extract, the ethyl acetate extract, and the nonextractable portion of goat liver in the original goat metabolism study (report No. AMR-22-80, 1976?; MRID 00063418). ^{14}C-Residues in the non-extractable protein matrix accounted for 81% of the TRR in the goat liver in the original study, and 72.2% and 87.2% of the TRR in goat and cow liver, respectively, in the present study. In the original study, 15.4% of the TRR in goat liver was methanol-soluble and 3.3% of the TRR was ethyl acetate-soluble; whereas in the present study, 15.6% and 14.3% of the TRR in goat and cow liver, respectively, was methanol:water-soluble, and 8.4% and 4.5% of the TRR in goat and cow liver, respectively, was chloroform-soluble. In addition, the ethyl acetate extract (3.3% of the TRR) in the original study was partitioned with water:cyclohexane and 98% of the radioactivity in this extract was cyclohexane-soluble. The authors reported that the cyclohexane extract in the original study and the chloroform lipid extract in the present study are equivalent in terms of both the nonpolar nature of the extracts and the amount of the TRR present in the extracts. In the present study, HPLC separated four methanol:-

water-soluble metabolites from both goat and cow liver following oral dosing of lactating goats and cows with [1-^{14}C]methomyl. The metabolites glycerol, glycerol-3 phosphate, acetic acid, and acetamide were identified by comparison of retention times with those of standard compounds. Following saponification of the initial chloroform extracts of goat and cow liver, chloroform-soluble free fatty acids isolated by HPLC had retention times corresponding to fatty acids ranging from C_{14} to C_{20}.

The available animal metabolism data do not adequately explain the qualitative nature of the terminal residue in animals because unidentified non-extractable ^{14}C-residues accounted for 72.2 and 87.2% of TRR in goat and cow liver, respectively and, although acetonitrile was not detected (<0.01 ppm) in methanol:water extracts of liver, no attempt was made to prevent its volatilization during the extraction procedure. The available animal metabolism data do indicate that methomyl is incorporated into natural products including glycerol, lipid, and protein. The authors proposed a metabolic pathway for methomyl in animals involving initial hydrolysis of methomyl to methomyl oxime and subsequent metabolism to acetonitrile. Acetonitrile is then metabolized to acetamide which is further hydrolyzed to acetic acid.

cc: Methomyl Reg. Std., RF., Circ., Reviewer, PMSD/IBS
RDI: PE:9/19/89:RL:9/20/89
H7509C:DEB:JG:jg:CM:2:Rm:803:557-1405:9/20/89.