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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Thidiazuron. Additional Study Into the Nature of the Residue in Animals. MRID Nos. 42778001 and 42778002. Case No. 4092. CBRS No. 12009. DPBarcode D192068.

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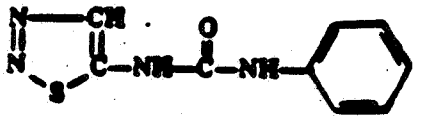

In support of reregistration, NOR-AM Chemical Company has submitted an addendum to thidiazuron animal metabolism studies previously reviewed in Phase 4 (2-2-93). This study was designed to identify previously characterized metabolites and a polar fraction.

Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) has one registered use as a cotton defoliant. In Phase 4 the registrant committed to conduct additional studies to fulfill requirements of nature of the residue-animals, residue analytical methods-animals, storage stability, and magnitude of the residue in meat, milk, poultry, and eggs.

Background

Currently, thidiazuron and its aniline containing metabolites are regulated. Tolerances ranging from 0.05 ppm to 0.4 ppm are established at 40 CFR 180.403 for residues in or on cattle, goats, hogs, horses, poultry and sheep fat, meat and meat by-products;

cottonseed; eggs; and milk.

Nature of the residue in animals studies were previously reviewed in Phase 4 (MRID No. 42529002 and MRID No. 42529003). Two metabolites (designated G and H) were found in significant amounts in most tissues. CBRS concluded in the Phase 4 review that additional effort must be made to identify metabolites G and H. Additionally, tissues from the metabolism study should be used for radiovalidation of data collection and enforcement analytical methods. Portions of this review are presented below.

MRID No. 42529002. [^{14}C-aniline] thidiazuron was fed to a lactating cow at a rate of 10 ppm for seven days. The animal was sacrificed within 24 hours of administration of the final dose. Radioactivity found was 0.05 ppm, 0.1 ppm, 1.5 ppm, and 1.0 ppm in fat, muscle, kidney and liver, respectively. Radioactivity reached a plateau in milk on the second day (0.2 ppm). Analysis was performed using HPLC and TLC. In liver, two unidentified components comprising 51% of the tissue radioactivity were characterized (designated G and H). Phenylurea, 4-hydroxythidiazuron, and thidiazuron constituted 13%, 4% and 2% of the total radioactivity, respectively. Partially extractable and unextractable residues comprised 3.0% and 19% of the total radioactivity, respectively. Components G, H, phenylurea, 4-hydroxythidiazuron, thidiazuron and a polar fraction were present in kidney extracts at 18%, 11%, 15%, 6%, 3%, and 23%, respectively, of the total radioactivity. In muscle, 58% was identified as thidiazuron, 2% as phenylurea, and 1% as 4-hydroxy-thidiazuron. Components G and H comprised only 13% of the muscle radioactivity combined. In fat, Component H comprised 46% of the fat radioactivity and component G was present at 7%. Phenylurea and 4-hydroxythidiazuron were identified at 10% and 2.3%, respectively. A polar fraction constituted 11% of the fat radioactivity. Only minor amounts were nonextractable. Thidiazuron , 4-hydroxythidiazuron, and phenylurea were identified in milk at 31%, 49%, and 3% of the milk radioactivity. Minor amounts of components G and H were characterized ($\leq$3%).

MRID No. 42529003. [^{14}C-aniline] thidiazuron was fed once daily to six hens for fourteen days at a rate of 8 ppm. Radioactivity did not plateau in eggs during this period. All tissues were first treated with protease and β-glucuronidase prior to extraction. Radioactivity found was 0.02 ppm, 0.27 ppm, 1.11 ppm, 0.66 ppm, 0.10 ppm, 0.10 ppm and 0.34 ppm in fat, gastrointestinal tract,

gastro-intestinal tract contents, liver, muscle, skin and blood, respectively. In liver, two unidentified components (designated metabolites G and H) comprised the majority of the residues (75%). No thidiazuron was detected. Phenylurea and 4-hydroxythidiazuron were detected at low levels. Two percent of the radioactivity was unextractable. The same two unidentified components (G and H) were present in muscle extracts (40%). Thidiazuron and 4-hydroxythidiazuron accounted for 2-3% of the muscle radioactivity and phenylurea accounted for 8%. Ten percent of the radioactivity was unextractable. In fat extracts, metabolite H comprised 60% of the radioactivity. Phenylurea, thidiazuron, and 4-hydroxy-thidiazuron accounted for 2 to 9% of the fat radioactivity. Nine percent of the radioactivity was unextractable. Phenylurea, thidiazuron, and component H constituted 16%, 20%, and 22% of the radioactivity in the egg, respectively. Partially extractable residues accounted for 24% of the egg radioactivity. Analysis was performed using HPLC and TLC. Metabolite H was not identified; however, it has been shown to cochromatograph with metabolite H found in cow liver.

Current Issues

MRID No. 42778002

Urine and kidney samples from the previous lactating cow study were used to identify component H and two polar fractions (designated fractions A and B). Extracted kidney samples were incubated in 10 M acid or base solutions, neutralized and analyzed to determine if the polar fractions degraded into known compounds; however, insufficient radioactivity was present to identify any of the components of fractions A or B. Two degradates were formed which accounted for approximately 38% of the tissue radioactivity but were not identified. Urine samples were then used and polar fractions A and B were characterized by HPLC using two columns. Fraction A resolved into four components and Fraction B resolved into six components using a TLC system. These components were not identified. The hydrolysis product of metabolite H from urine samples was derivatized by trimethylsilylation and trifluoroacetylation and analyzed using GC-MS. The derivatized product was identified as phenylurea in both processes indicating that metabolite H is a conjugate of phenylurea. The identity and distribution of radioactive residues among the milk and tissues is shown in Table A.

MRID No. 42778001

Hen excreta taken from the previous study were used in this study in an attempt to identify the polar fraction (designated fraction A/B) and metabolite G. Samples of excreta were analyzed directly using HPLC and fractions corresponding to the retention time of metabolite G and fraction A/B were isolated via extraction with acidic or basic solutions. The samples were then neutralized and analyzed by HPLC and TLC. Fractions A and B could not be resolved; therefore, the registrant analyzed the entire fraction. This fraction was further broken down into five components(designated A/B1-A/B5) which were not identified. The hydrolysis product of metabolite G was identified as 4-hydroxythidiazuron by cochromatography with a reference standard in HPLC and TLC systems. This indicates that metabolite G is a conjugate of 4-hydroxy-thidiazuron. Table B below displays the identity and distribution of radioactive residues among the eggs and tissues.

Table A. LACTATING COW - % Tissue Radioactivity

	Liver	Kidney	Muscle	Fat	Milk
Thidiazuron	2	3	58	---	31
Phenylurea	13	15	2	10	3
4-hydroxythidiazuron	4	6	1	2	49
Component G (phenylurea conjugate)	15	18	---	7	<3
Component H (4-hydroxythidiazuron conjugate)	36	11	13	46	<3
Polar Fraction	22	23	---	11	11
Total	92	76	74	76	<100

Table B. LAYING HEN - % Tissue Radioactivity

	Liver	Muscle	Fat	Eggs
Thidiazuron	---	2	2	20
Phenylurea	9	8	9	16
4-hydroxy thidiazuron	5	3	4	---
Component G (phenylurea conjugate)	14	18	11	---
Component H (4-hydroxythidiazuron conjugate)	64	22	56	22
Polar Fraction	3	17	11	13
Total	95	70	93	71

Conclusions

The nature of the residue in animals is sufficiently understood. Most of the radioactivity was extractable (>80%). Attempts were made to release unextractable residues in cow liver, cow kidney and hen liver; however, release of unextractable residues in hen muscle, which accounted for 13% (0.01 ppm) of the tissue radioactivity, was not attempted. The registrant's attempt to identify/characterize low level polar fractions was unsuccessful; however, these fractions were negligible (<0.02ppm) with the exception of cow kidney (0.35 ppm). Metabolites G and H were sufficiently characterized as conjugates of 4-hydroxythidiazuron and phenylurea, respectively.

cc: Reviewer(F. Fort), List D File, RF, SF, Circ.
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