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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: Methylthioacetate - In Vitro Metabolism Study With Rat and Rabbit

EPA ID No.: 239-2471
Record No.: 205004
MRID No.: 403225-01
Caswell Nos.: 584D and 2A
Proj. No.: 8-0210

FROM: Krystyna K. Locke, Toxicologist
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Hazard Evaluation Division (TS-769C)

TO: William H. Miller, PM 16
Insecticide-Rodenticide Branch
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THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Toxicology Branch/Hazard Evaluation Division completed an evaluation of the following study with methylthioacetate, an impurity in acephate:

- Comparative Esterolytic Activity with Methylthioacetate (SX-1724) in Various Tissues of the Rat and Rabbit. Chevron Environmental Health Center, Inc.; Project No. CEHC 2604. April 23, 1987.

This study was classified as Acceptable.

Attachment

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Reviewed By: Krystyna K. Locke, Toxicologist
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: Edwin R. Budd, Section Head
Section II, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: In Vitro Metabolism (Rat and Rabbit)

TOX Chem No.: 584D
MRID No.: 403225-01
Proj. No.: 8-0210

Accession No.: 403225-01

Test Material: Methylthioacetate (SX-1724); Purity: 99.4%

Study No.: CEHC 2604

Sponsor: Chevron Chemical Company
Agricultural Chemicals Division
Richmond, CA

Testing Facility: Chevron Environmental Health Center, Inc.
Richmond, CA

Title of Report: Comparative Esterolytic Activity with Methylthioacetate (SX-1724) in Various Tissues of the Rat and Rabbit

Author: D.W. Rosenburg

Report Issued: April 23, 1987

Conclusions:

In both species, liver microsomes had higher methylthioacetate (MTA) thioesterase activity than any other tissue or tissue fraction tested (kidney microsomes, plasma, and 10,000 and 105,000 xg supernatants). Thioesterase activity in rabbit liver microsomes was three to four times higher than that in rat liver microsomes. Very little of thioesterase activity was observed in plasma of both species, only 0.1 to 1.5 percent of the activity observed in their livers. It was concluded by the author of this report that the greater sensitivity of the rabbit, compared with the rat, to single doses of MTA could, in part, be explained in terms of kinetic differences between rabbit and rat liver microsomal thioesterases.

Classification of Study: Acceptable

Experimental Procedures

Three 10-week-old Sprague-Dawley Crl:CD®(SD)BR male rats and three 12- to 14-week-old New Zealand male rabbits were used in this in vitro study. The animals were:

1. Obtained from Charles River Breeding Laboratories, Inc., Portage, Michigan and R and R Rabbitry, Standwood, Washington, respectively;

2. Quarantined for 13 days before sacrifice;

3. Housed singly at temperatures of 22.0 to 22.7 °C and relative humidity of 30 to 62 percent; and

4. Fed unrestricted amount of food (Purina Certified Rodent Meal #5002 or Purina Certified Laboratory Rabbit Chow HF #5326) and water.

The effect of MTA on thioesterase activity in liver subcellular fractions, kidney microsomes, and plasma from both species was studied as follows:

1. The animals were sacrificed with CO_2.

2. The liver and kidneys were perfused in situ with 0.9% sodium chloride, removed, weighed, homogenized with 0.05 M potassium phosphate buffer (pH 7.4), and centrifuged at 10,000 xg for 20 minutes. The resultant supernatant was then centrifuged at 105,000 xg for 60 minutes and the pellet (microsomes) resuspended in 0.1 M potassium phosphate buffer (pH 7.4).

3. Blood was collected by cardiac puncture into a tube containing sodium heparin (anticoagulant) and centrifuged at 8800 xg for 10 minutes to obtain plasma.

4. Thioesterase activity was assayed in the 10,000 and 105,000 xg liver supernatants, liver and kidney microsomes, and plasma, using the spectrophotometric procedure of Ellman et al.[a] and MTA (instead of acetylcholine) as substrate.

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[a] Ellman, G.L.; Courtney, K.D.; Andres, V. Jr.; Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95

5. In the case of liver microsomes, enzyme kinetics were also studied. In these experiments, the effects of reaction time, temperature, protein concentration, MTA (substrate) concentration, and incubation with eserine (a competitive esterase inhibitor) or ethyl acetate (structurally similar to MTA) on MTA thioesterase activity were determined.

6. Liver, kidney, and plasma thioesterase activities were analyzed statistically using Student's t-test.[b]

Results

MTA Thioesterase Activity in Rat and Rabbit

Of the five fractions tested (10,000 and 105,000 xg supernatants, liver and kidney microsomes, and plasma), liver microsomes of both species had the highest MTA thioesterase activity. However, rabbit liver microsomes had significantly higher thioesterase activity than did rat liver microsomes. Expressed as μmoles product/min/mg protein, MTA thioesterase activity in rabbit and rat liver microsomes was 177.8 and 53.0, respectively.

Kidney microsomes from both species were similar in MTA thioesterase activity (25.7 in the rabbit and 29.7 in the rat), but the rat plasma had significantly more thioesterase activity than the rabbit plasma (0.77 and 0.20, respectively). However, plasma thioesterase activity was only 0.1 to 1.5 percent of the activity observed in liver microsomes (for details, see Attachment I.)

Kinetics of MTA Thioesterase

1. Hydrolysis of MTA was time- and protein-dependent in both species.

2. Eserine (1.0 mM), a competitive esterase inhibitor, reduced thioesterase activity by 48 and 15 percent in rabbit and rat liver microsomes, respectively.

3. Ethyl acetate (47 mM), structurally similar to MTA, reduced thioesterase activity by 15 and 43 percent in rabbit and rat liver microsomes, respectively.

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[b] Sokal, R.R.; Rohlf, R.J. (1981) Biometry. W.H. Freeman and Company, San Francisco, pp. 145-147.

4. A maximum rate of thioesterase activity was observed with MTA concentrations between 5 and 10 mM in the rat, whereas in the rabbit, thioesterase activity was still increasing at the highest concentration of MTA tested (25 mM). Using the Lineweaver-Burk plot of velocity (μmoles product/min per mg protein) versus substrate concentration (mM), the Km and Vmax were determined for thioesterase in liver microsomes as follows:

   Km(mM) = 13.4 (rabbit) and 2.25 (rat)
   Vmax (μmoles product/min per mg protein) = 343.6 (rabbit) and 74.0 (rat)

5. Exposure of rabbit and rat liver microsomes to 45 °C for 15 minutes reduced thioesterase activity only by 7 percent in each species. However, an exposure to 60 °C reduced thioesterase activity by approximately 95 percent in the rabbit and 63 percent in the rat.

According to the above data, rabbit and rat microsomal thioesterases have different kinetic properties. It was concluded that the observation that rabbit liver microsomes hydrolyzed MTA three to four times faster than did rat liver microsomes provided a partial metabolic basis for understanding the greater sensitivity of the rabbit to MTA, compared with the rat and other species. Toxicology Branch agrees that the results of this study should be kept in mind while evaluating toxic effects of single doses of MTA in rats and rabbits.

Classification of Study: Acceptable

Quality Assurance statement, dated April 30, 1987, was submitted.

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