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

007970

JUN 11 1990

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Trifluralin: Review of a rat metabolism study (Identification of metabolites in the urine samples)

Caswell No.: ~~890~~ 889 **HED Project No.:** 9-2174
MRID/Accession NO.: 412189-01 **EPA ID No.:** 59011
EPA Record No.: 251682

TO: C. Grubbs, PM Team 74
Registration Division (H7508C)

FROM: Whang Phang, Ph.D. 6/8/90
Pharmacologist
HFAS/Tox. Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head 6/8/90
and
Marcia van Gemert, Ph.D. 6/8/90
HFAS/Tox. Branch II/HED (H7509C)

In response to the requirements stated in the Registration Standard (April, 1987) for Trifluralin, the registrant had submitted a metabolism which characterized and ~~identified~~ identified the metabolites in the urine of rats treated with radiolabeled trifluralin. This study has been reviewed, and a DER for it is attached. The conclusion is as follows:

Groups of rats (5/sex) received 3 consecutive doses (300 mg/kg) of radioactive trifluralin by gavage. The analytical results indicated that, intact trifluralin was not detected in the urine samples of either male or female rats. As many as 30 to 40 metabolites were present in the urine, and individually most of these metabolites represented less than 1 to 2% of the total radioactivity. A similar spectrum of metabolites was found in the urine samples of both males and females.

The data obtained from this study are useful, but the study can not be classified according to the core-guidelines for a metabolism study. However, the study, which was conducted properly and scientifically, achieved its objectives.

Reviewer: Whang Phang, Ph.D. 6/8/90
HFAS/Tox. Branch II (H7509C)

Secondary Reviewers: K. Clark Swentzel, Section Head K. Clark Swentzel 6/8/90
HFAS/Tox. Branch II (H7509C)

DATA EVALUATION REPORT

CHEMICAL: Trifluralin; 2, 6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine

STUDY TYPE: Metabolism (Identification of metabolites)

Caswell No.: 890 **HED Project No.:** 9-2174

MRID/Accession NO.: 412189-01 **EPA ID No.:** 59011

EPA Record No.: 251682

Sponsor: Elanco Products Co.

Testing Facility: Lilly Research Laboratories, Greenfield, IN

Citation: Magnussen, J. D., Identification of the urinary metabolites of ^{14}C-trifluralin in rats. Lilly Research Laboratories; Lab. Proj. ID No.: Experiment ABC-0433. August 16, 1989.

Conclusion: The objective of this study was to characterize and to identify the metabolites in the urine of trifluralin treated rats as specifically required by the Registration Standard for trifluralin (April, 1987).

Groups of rats (5/sex) received 3 consecutive doses (300 mg/kg) of radioactive trifluralin by gavage. The analytical results indicated that, intact trifluralin was not detected in the urine samples of either male or female rats. As many as 30 to 40 metabolites were present in the urine, and individually most of these metabolites represented less than 1 to 2% of the total radioactivity. A similar spectrum of metabolites was found in the urine samples of both males and females.

The data obtained from this study are useful, but the study could not be classified according to the core-guidelines for a metabolism study. However, the study, which was conducted properly and scientifically, achieved its objectives.

Material and Methods

Test article: Uniformly ring labeled ^{14}C-trifluralin
Lot 553-VE9-116; Radiopurity >98.0%
specific activity: 8.95 μCi/mg.
Prior to the initiation of the study, the radio-

labeled trifluralin was mixed with an appropriate quantity of unlabeled compound from Lot 554AP2 to yield a final specific activity of 0.25 μCi/mg (550 dpm/μg).

Test animals: 8-9 weeks old Fischer 344 rats which weighed 195-223 gm for males and 141-156 gm for females. These animals were obtained from Harlan Industries, Cumberland, IN, and they were the same strain as those used in the chronic feeding study.

Study design:

The objective of this study was to characterize and to identify the metabolites in the urine of trifluralin treated rats. This information is needed to fulfill the requirements for the metabolism study as stated in the Registration Standard for Trifluralin (April, 1987).

The selection of the dosage for this study was based upon the urinary tract effects seen in 300 mg/kg rats in a chronic feeding study with Fischer rats (MRID No. 00044337). Groups of 5 rats/sex were treated with radiolabeled trifluralin by gavage at 300 mg/kg for 3 consecutive days. Radiolabeled trifluralin (1782.58 mg) was dissolved in 58 ml of corn oil to yield an approximate concentration of 30 mg ^{14}C-trifluralin/ml, and each animal received 1.0 ml/100 gm body weight. The animals were housed individually in the metabolism cages.

Urine and feces samples of each animal were collected at 24 hour intervals following treatment except the last sample which covered only a six hour interval. The volume of each urine sample was measured, and the sample was then stored frozen until analysis. Six hours after the third dose, the test animals were sacrificed, and the liver was collected, pooled according to sex, and froze at -20 °C. It should be noted that since the objective of this study was to identify the metabolites in the urine, the feces samples were collected as another source of metabolites and were not analyzed at this time. The urine samples from each collection period were pooled according to sex for analytical purposes.

The radioactivity in raw urine samples was determined by liquid scintillation counting. The initial characterization of the radioactivity in the urine was achieved by silica gel column chromatography which removed significant quantities of nonradioactive contaminants. Radioactive fractions were pooled and subjected to thin layer chromatography (TLC) for separation and identification of metabolites. High pressure liquid chromatography (HPLC) was also used to determine the metabolite. When sufficient amounts of the radioactive material was available after subjected to TLC and HPLC, the pooled sample was further analyzed with nuclear magnetic resonance spectroscopy and/or mass spectroscopy for

confirming the structure of any metabolite.

Results

a. The values of the total radioactivity in the urine samples were excerpted from the report and presented in Table 1. The urinary radioactivity level of the pooled sample contained the highest level at 24-48 hours sampling period. This result was expected because this sampling period was within 24 hours of the second treatment and within 48 hours of the first dose.

There seems to be a large difference between the volume of the pooled urine samples and the sum of the volumes of the individual animals in each sampling period as presented in Table 1. This reviewer telephoned the study director, Dr. J. Magnussen to inquire about this discrepancy. He informed this reviewer that the foot note No. 2 of Table 1 (Table 3 in the submitted report) is missing an additional sentence which explain that this differece is due to pooling of the water rinses of individual sample container.

b. Radioactive residues characterization: The results of silica gel column chromatography were presented in Figures 1 & 2. The elution patterns of the 48-hour pooled samples of male and females were similar. Both contained two major peaks which were designated as fractions F and G. Fraction F contained approximately 34.4% of the total radioactivity in male rat urine and 29.5% of the total radioactivity in female rat urine while Fraction G contained 48.8% and 47.1% of the total radioactivity in male and female 48-hour urine samples, respectively. Fraction G residues were found to be rather polar. Using a solvent system developed for polar conjugates (butanol/water/glacial acetic acid), the TLC results showed that this fraction composed of seven or more radioactive zones with no one zone representing more than 16.8% of the radioactivity of the Fraction G of either male or female urine.

There were five other minor fractions which contained 0.6% to 8.8% of the total radioactivity in urine samples of male or females (Figures 1 & 2).

c. Identification of metabolites: Based upon the analyses with TLC, it was estimated the there were as many as 20 to 30 metabolites in fractions A to F and 10 to 20 metabolites in Fraction G. Since the quantity of some of these metabolites was less than 1% of the total urine radioactivity, it would be difficult to isolate and identify each metabolite. It was decided to isolate, to identify, and to confirm the chemical structure of any metabolite which cochromatographed on TLC with any of the reference compounds and which was

present in quantity of 1% or more of the total residue. Following this rationale, some of the metabolites were identified with TLC by comparing the R_f value of the reference compound with that of the isolated metabolite. The report indicated that the chemical structure of some of the isolated metabolites was confirmed by NMR analysis, but the actual NMR results were not included in the report. The isolated metabolites and their percentage of the total residues in urine were excerpted from the report and presented in Table 2 and the compound designations and the structures of the reference compounds were presented in Figure 3.

Among all the metabolites identified, the metabolite F1 was 14 to 15 % of the total radioactive residues in the urine. Further analysis indicated that the F1 metabolite was consisted of two major components (F1A and F1B) and one or more minor components as shown in Table 2. The chemical structures of the components designated as F1 metabolite could not be easily determined.

Discussion

The analytical results indicated that, following oral administration of radiolabeled trifluralin, intact trifluralin was not detected in the urine samples of either male or female rats. The silica gel column chromatograph results showed similar elution patterns for the urine samples of both males and females. The isolation and identification results showed that in the urine samples there were as many as 30 to 40 metabolites, and individually most of these metabolites represented less than 1 to 2% of the total radioactivity.

Based upon the compound identification results, the author proposed that there might be at least four different types of metabolic reactions involved. The proposed scheme for various pathways was excerpted from the report and presented in Figure 4. One reaction involved dealkylation of one or both propyl groups. A second reaction might be a reduction of one or both nitro groups to the corresponding amines. A third possibility involved cyclization to yield various benzimidazole metabolites. The fourth metabolic pathway might involve conjugation. It was estimated that 76 to 80% of the radioactive residues in the urine could be conjugates. N-acety-lated metabolites might also be present, but the bulk of the other conjugates were most likely sulfates or N-glucuronides.

trifluralin

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Pages 6 through 12 are not included.

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TOXCHEM NO. 890 Trifluralin

CITATION	MATERIAL	MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT #
Metabolism - RAT Lilly Research Lab Project No.: Experiment ABC-0433	^{14}C - Trifluralin Specific Activity: ~~378~~ 8.95 μCi/mg	41218 9-01	This is a special study which was design to ident. ~~ify~~ the metabolites in urine ~~pimples~~. ~~The~~ Rats ~~were~~ received 300 mg/Kg of ~~the~~ radiolabeled trifluralin. There were approximately 30 - 40 metabolites found in the urine samples of both males + females. The study is scientifically sound, and the results ~~are~~ valid.		N/A

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