424875-01

DATA EVALUATION REVIEW

I. Study Type: fish bioaccumulation, supplement to xxx; guideline 165-4

II. Citation:

Leimkuehler, W.M. and Moore, K.S., Identification of Radioative Residues of Triazole-3,5-[$^{14}$C] Tebuconazole in the Nonedible Fraction of Bluegill Sunfish (Lepomis macrochirus), project No. FR030301. performed by Miles Inc. Agricultural Division, Stilwell, KS. received 9/24/92 under MRID# 424875-01.

III. Reviewer:

| | |
|---|---|
| Typed Name: | E. Brinson Conerly-Perks |
| Title: | Chemist, Review Section 3 |
| Organization: | EFGWB/EFED/OPP |

E. B. Conerly-Perks

IV. Conclusions:

This study provides the additional information requested in the previous review. No more data on fish bioaccumulation are required at this time. Metabolism during the period of time investigated does not result in breakdown of the "skeleton" of tebuconazole, but involves oxidation and conjugation.

V. Materials and Methods:

Abstract

Radioactive residues from bluegill sunfish (Lepomis macrochirus) exposed to [$^{14}$C] tebuconazole at a nominal rate of 60 ppb for 28 and 35 days were isolated and identified. This study is a repeat of a study done previously (Miles Report # 98037) where metabolite identification in the nonedible portion of the fish was inadequate. Total [$^{14}$C] residue was 9.2 and 11.3 ppm for the 28 and 35-day nonedible samples respectively. This is compared to 8.53 and 6.61 for the 28 and 35 day samples from the previous study. The nonedible fraction of the fish was extracted and used for metabolite identification. The diluter system was operated for a total of 7 weeks. Four toe six pan-sized bluegill (4 - 6 inch in length) were placed in each of the qeuaria (for 1 or 2 weeks) after the smaller fish were removed. The livers from these fish were excised and extracted, then used to facilitate metabolite identification.

In addition to the t-butyl hydroxy glucuronide and tebuconazole, eight remaining metabolites were identified by thermospray mass spectrometry including: 1) hydroxylated (ring or benzylic OH) t-butyl hydroxy tebuconazole sulfate, 2) t-butyl hydroxy tebuconazole sulfate, 3 and 4) glucuronides of hydroxylated (ring or benzylic OH) t-butyl hydroxy tebuconazole, 5) glucuronide of tebuconazole t-butyl acid, 6) tebuconazole t-butyl acid, 7) t-butyl hydroxy tebuconazole and 8) hydroxylated (ring or benzylic OH tebuconazole.

Experimental

test substance -- triazole-3,5-[$^{14}$C] tebuconazole, spec. act. 19.7 mCi mmol, radiochemical purity 100% (TLC), diluted to 0.7 mg/ml and 2.8 mCi/mmol with unlabelled tebuconazole in ethanol solution. The solution was prepared fresh each week and stored in the refrigerator.

test system -- the test solution was applied by a proportional dilutor system to a final concentration in the aquaria of a nominal 60 ppb. At initiation of the test, approximately 95 two to three gram fish were added to each aquarium (four treatment and one control). After the small fish were removed, pan-sized fish were added. Test fish (small) were removed at 28 or 35 days, and control fish were removed at 35 days. Large fish were exposed for one or two weeks.

extraction -- 28-day samples of fish were extracted using acetonitrile and acetonitrile/ water. 35-day samples were not processed. Livers from pan-sized fish were processed similarly.

analysis
total $^{14}C$ -- combustion followed by liquid scintillation counting

specific compounds -- thermospray MS

VI. Study Author's Results and/or Conclusions:

STUDY AUTHOR'S DESCRIPTION OF RESULTS

[The following is not verbatim.] Results (shown in figures 4, 5, and 6, attached) represent results from the previous study, the 28-day current study, and the liver extract from the pan-sized fish. The metabolite patterns are similar. The most noticeable difference is that peak 3 of the original study actually appears as two separate components. Table 2 (attached) compares the present and previous studies, and table 3 (attadhed) lists name and peak number of the identified metabolites.

Figure 19 (attached) depicts the proposed pathway for tebuconazole metabolism in fish, which may be summarized as "oxidation followed by conjugation". There appears to be no degradation of the basic structure [within the context and conditions of the study].

STUDY AUTHOR'S CONCLUSIONS

The results of the current tebuconazole fish metabolism study closely matched the results of the previous study (MILES Report No. 98037 in both magnitude of the $^{14}C$-tebuconazole equivalent residue and metabolite distribution. No breakdown of the basic tebuconazole structure was witnessed. All metabolites in question in the non-edible fraction were characterized or identified.

VII. Reviewer's Comments:

1) Although the current and previous studies differ in some of the details, the overall picture is similar.

VIII. CBI Information Addendum: attached

Table 2. Comparison of the Distribution of [$^{14}$C] Tebuconazole Metabolites from the 28-Day Nonedible Fraction in Two Fish Metabolism Studies.

| Metabolite No. | Miles Report No. 98037 (ppm) | | Current Study (ppm)[1] | |
|---|---|---|---|---|
| Metabolite 1 | 1.5% | 0.12 | 0.34 | 3.7 % |
| Metabolite 2 | 5.9 | 0.48 | 0.48 | 5.2 |
| Metabolite 3A | 7.2 | 0.58[2] | 0.38 | 4.1 |
| Metabolite 3B | | --- | 0.47 | 5.1 |
| Metabolite 4 | 1.6 | 0.13 | 0.27 | 2.9 |
| Metabolite 5 = t-BuOH + tcb gluc | 66.5 | 5.38 | 4.77 | 51.8 |
| Metabolite 6 | 2.6 | 0.21 | 0.31 | 3.4 |
| Metabolite 7 | 4.1 | 0.32 | 0.41 | 4.5 |
| Metabolite 8 | 1.6 | 0.13 | 0.38 | 4.1 |
| Metabolite 9 = parent | 9.1 | 0.74 | 1.39 | 15.1 |

[1] Ppm calculated by multiplying the HPLC peak percent times the residue level in the tissue. See Table 3 for identification.

[2] Only 1 metabolite was reported in Miles Report No. 98037.

total residues
previous — 5.09
present — 9.20



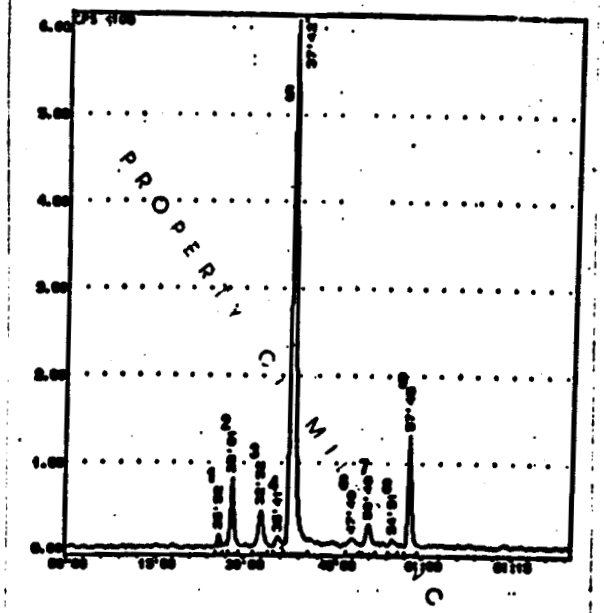


98037-1

Table 3. Identity of Metabolites in the Nonedible Fraction of Bluegill Sunfish Exposed to Triazole-3,5-[$^{14}$C]Tebuconazole

| Peak No.(Metabolite No.) | Metabolite |
|---|---|
| 1 | Dihydroxy Tebuconazole Sulfate |
| 2 | t-Butylhydroxy Tebuconazole Sulfate |
| 3A & 3B | Dihydroxy Tebuconazole Glucuronides |
| 4[1] | t-Butyl Acid Tebuconazole Sulfate |
| 5 | t-Butylhydroxy Tebuconazole Glucuronide |
| 6 | t-Butyl Acid Tebuconazole |
| 7 | t-Butylhydroxy Tebuconazole |
| 8 | Ring or Benzylic hydroxy Tebuconazole |
| 9[1] | Tebuconazole |

[1] Identified in Miles Report No. 98037.




re 4. HPLC radiochromatogram of [$^{14}$C]tebuconazole metabolites from the 28-day non-edible fraction from Miles Report No. 98037. The mobile phase consisted of water/ACN with 0.4% acetic acid.

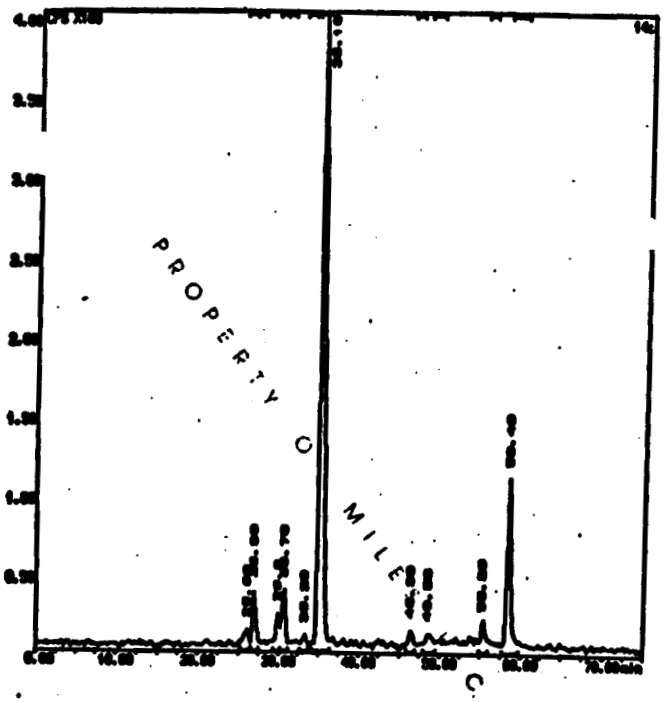



Figure 5. HPLC radiochromatogram of [$^{14}$C]tebuconazole metabolites from the 28-day extract of the non-edible fraction of bluegill sunfish. The mobile phase was water/ACN with 0.4% acetic acid.

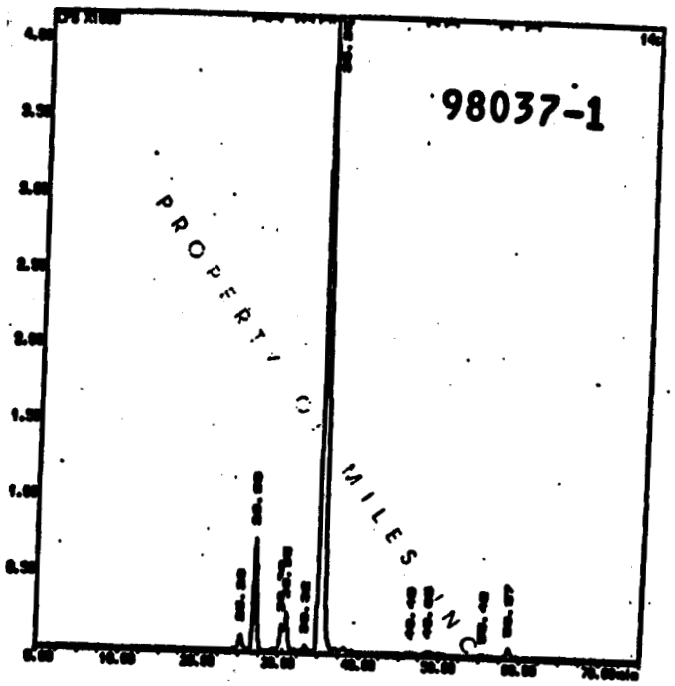



Figure 6. HPLC radiochromatogram of [$^{14}$C]tebuconazole metabolites from the extract of liver from pen-raised fish exposed to [$^{14}$C]tebuconazole for 14 days. The mobile phase was water/ACN with 0.4% acetic acid.

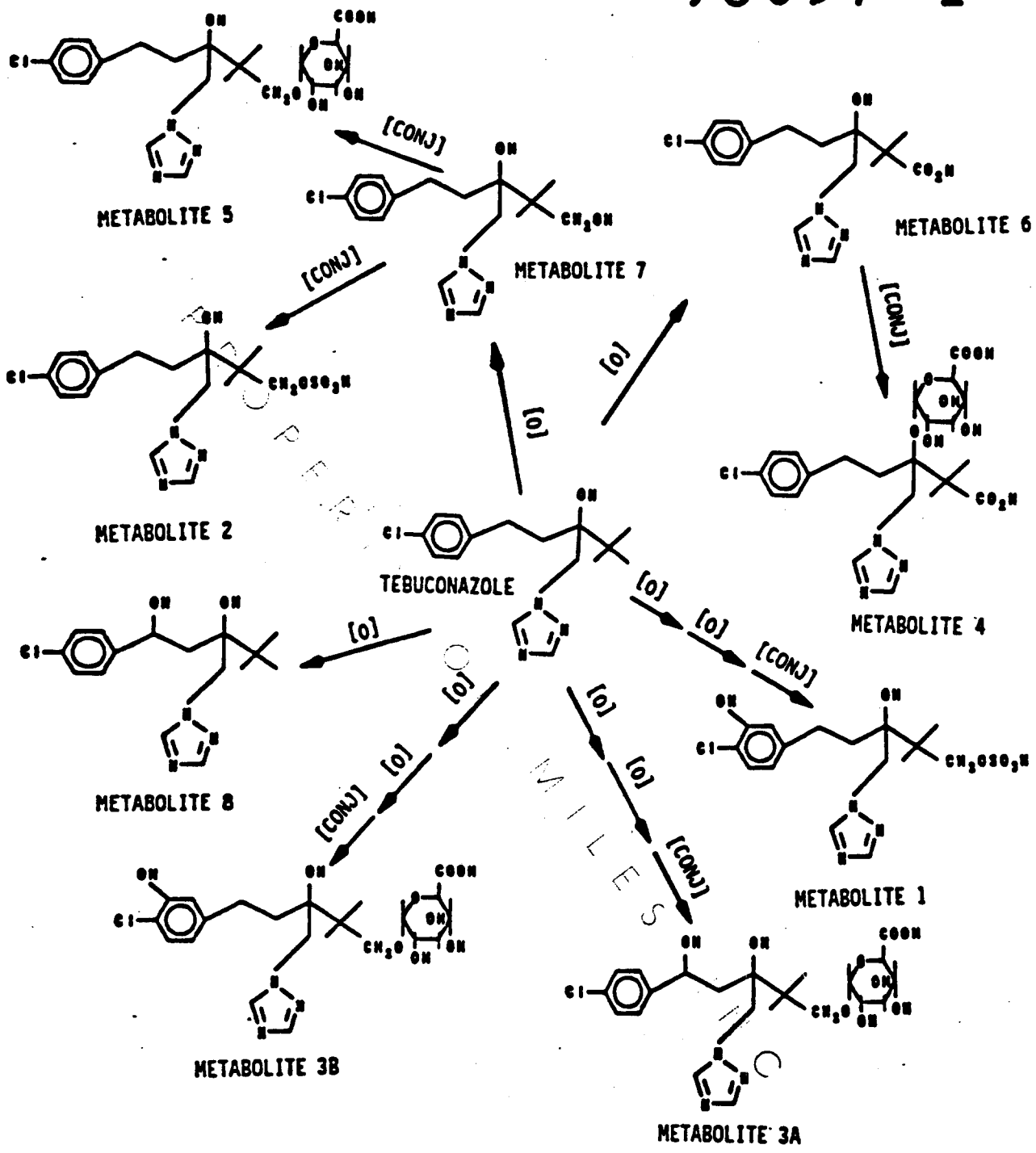



Figure 19. Metabolic Pathway of tebuconazole in Bluegill sunfish. [O] = oxidation. [CONJ] = conjugation. See Table 3 for list of names.