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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: Metabolism study with Metalaxyl (GL 85-1)

EPA P.C. Code: 113501
Tox. Chem. No.: 375AA
DP Bar Code: : D164959
Submission No.: S397172

TO: Carol Peterson/Linda Propst, PM Team 73
Reregistration Division (H7508W)

FROM: Nguyen B. Thoa, Ph.D. NT 04/05/93
Reregistration Section
Chemical Coordination Branch
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C) Roger Gardner 4-5-93 KPF 4/13/93

ACTION REQUESTED: Review metabolism study with metalaxyl (MRID No. 416645-01) submitted by CIBA-GEIGY Corp. as part of a reregistration package.

CONCLUSIONS: The absorption, distribution, metabolism, and excretion of metalaxyl were studied in groups of male and female Sprague-Dawley rats administered a single intravenous (i.v.) dose of 1.0 mg/kg, a single oral gavage dose of 1.0 or 200 mg/kg [^{14}C]-metalaxyl, or a 14-day repeated oral dose of 1.0 mg/kg unlabeled metalaxyl followed by a single dose of 1.0 mg/kg [^{14}C]-labeled metalaxyl on day 15. [^{14}C]-Metalaxyl was rapidly absorbed, distributed, metabolized completely, and eliminated in rats under all dosing regimens. Most of the radioactivity was recovered within 24 hours in the urine and feces (69.92-82.52% of the administered dose); recovery after seven days was essentially complete (95.90-110.01%). Urine was the primary elimination route in females, regardless of dosing route (65.63-74.12% of dose), while fecal elimination dominated in males of all groups (54.19-63.60% of dose).

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Seven days after dosing, the tissue residues of [^{14}C]-metalaxyl and possible metabolites totaled <1% of the administered dose, indicating that there is no appreciable bioaccumulation. Two dimensional thin layer chromatography (2D-TLC) of whole urine and fecal extracts resulted in 33 spots. Ten metabolites were identified based on comparison with synthetic standards and/or spectral data from the purified metabolites. Less than 1% of the parent compound was excreted, indicating that metalaxyl was completely transformed.

Urinary metabolites were predominantly (49.9-96.3%) conjugated, while the bulk of the fecal metabolites (46.6-78.8%) were unconjugated. Based on the identi-fied metabolites, three major pathways and one minor pathway of metalaxyl metabolism were proposed. Metalaxyl was biotransformed by demethylation, N-dealkylation, and oxidation, followed by glucuronide and sulfate conjugation.

STUDY CLASSIFICATION: Supplementary. This study may be upgraded if the following additional information is provided and is judged to be acceptable:

- On page 37, paragraph 1, line 6 of the study report, the study author stated, "Group III male rats still contained aqueous soluble residue (29.8%) possibly conjugated or polar anionic metabolites". A discussion of the causes of a significant percentage of radioactivity remaining in this fraction is required.

- The study author stated in line 6 of the second paragraph from the bottom of page 37 of the study report, "Two major metabolites, UM-23 and UM-26, maximally accounted (sic) for 23.3% and 8.3% of dose, respectively, are polar metabolites presumed intact glucuronide or sulfate conjugates since..." Because these metabolites in urine comprise 5% or more of the administered dose, reasonable efforts should have been made to identify the free metabolites corresponding to metabolites UM-23 and UM-26.

DATA EVALUATION REPORT

METALAXYL

Study Type: Metabolism in Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9302 Lee Highway
Fairfax, VA 22031-1207

Principal Author Lynne Haber Date 3/8/93
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Sanju Diwan

QA/QC Manager Sharon Segal Date 3/8/93
Sharon Segal

Contract Number: 68D10075
Work Assignment Number: 1-63
Clement Number: 91-196
Project Officer: James Scott

Guideline Series 85-1: Metabolism

EPA Reviewer: Paul Chin, Ph.D.
Review Section II,
Toxicology Branch I/HED H7509C
EPA Section Head: Marion Copley, DVM, DABT
Review Section IV,
Toxicology Branch I/HED H7509C

Signature: Paul Chin
Date: 3/17/93
Signature: Marion Copley
Date: 3/18/93

DATA EVALUATION REPORT 010164

STUDY TYPE: Metabolism in rats

EPA IDENTIFICATION NUMBER:

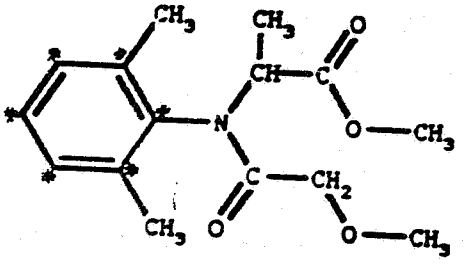
EPA P.C. Code: 113501

Tox. Chem. Number: 375AA

MRID Number: 416645-01

TEST MATERIAL: Metalaxyl

SYNONYMS: N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester; - Ridomil



* denotes the position of the ^{14}C label

SPONSOR: CIBA-GEIGY Corporation, Agricultural Division, P.O. Box 18300, Greensboro, NC

TESTING FACILITY: Biological Phase: Analytical Bio-chemistry Laboratories, Inc., 200 East ABC Lane, Columbia, MO

Analytical Phase: CIBA-GEIGY Corporation, Metabolism Department, 410 Swing Rd., P.O. Box 18300, Greensboro, NC

TITLE OF REPORT: 1. ABR-90079, "Characterization and Identification of Phenyl-[^{14}C]-Metalaxyl Metabolites in Rats." 108 pp.

2. Analytical Biochemistry Laboratories, Inc., Report No. 37613, "Metalaxyl: Rat Metabolism Studies: Tissues and Excreta." 109 pp.

AUTHORS: W. Itterly (Report 1); C.E. Jameson (Report 2)

REPORT DATES: October 19, 1990 (Report 1); August 20, 1990 (Report 2)

CONCLUSIONS: The absorption, distribution, metabolism, and excretion of metalaxyl were studied in groups of male and female Sprague-Dawley rats administered a single intravenous (i.v.) dose of 1.0 mg/kg, a single oral gavage dose of 1.0 or 200 mg/kg [^{14}C]-metalaxyl, or a 14-day repeated oral dose of 1.0 mg/kg unlabeled metalaxyl followed by a single dose of 1.0 mg/kg [^{14}C]-labeled metalaxyl on day 15. [^{14}C]-Metalaxyl was rapidly absorbed, distributed, metabolized completely, and eliminated in rats under all dosing regimens. Most of the radioactivity was recovered within 24 hours in the urine and feces (69.92-82.52% of the administered dose); recovery after seven days was essentially complete (95.90-110.01%). Urine was the primary elimination route in females, regardless of dosing route (65.63-74.12% of dose), while fecal elimination dominated in males of all groups (54.19-63.60% of dose).

Seven days after dosing, the tissue residues of [^{14}C]-metalaxyl and possible metabolites totaled <1% of the administered dose, indicating that there is no appreciable bioaccumulation. Two dimensional thin layer chromatography (2D-TLC) of whole urine and fecal extracts resulted in 33 spots. Ten metabolites were identified based on comparison with synthetic standards and/or spectral data from the purified metabolites. Less than 1% of the parent compound was excreted, indicating that metalaxyl was completely transformed.

Urinary metabolites were predominantly (49.9-96.8%) conjugated, while the bulk of the fecal metabolites (46.6-78.8%) were unconjugated. Based on the identified metabolites, three major pathways and one minor pathway of metalaxyl metabolism were proposed. Metalaxyl was biotransformed by demethylation, N-dealkylation, and oxidation, followed by glucuronide and sulfate conjugation.

STUDY CLASSIFICATION: Supplementary. This study may be upgraded if the following additional information is provided and is judged to be acceptable:

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reasonable efforts should have been made to identify the free metabolites corresponding to metabolites UM-23 and UM-26.

A. MATERIALS

1. Test Substance

The unlabeled test material (Lot number 585-0831, Ciba Geigy #B05344) was provided by Ciba-Geigy and described as a tan powder, with a purity of 95.3%.

Metalaxyl uniformly phenyl-labeled with [^{14}C] was provided by Ciba-Geigy. No rationale was presented for the choice of position of the radiolabel. The material used for the low-dose groups (GAN-XV-13) had a specific activity of 88.6 µCi/mg and radiopurity of 97.2%; the material used for the high-dose group (CL-XVIII-83) had a specific activity of 5.1 µCi/mg and a radiopurity of 97.3%.

2. Test Animals

Six- to eight-week old Sprague-Dawley rats age were obtained from Taconic Farms, Germantown, NY. Single or repeated oral gavage doses of metalaxyl were administered to groups of 5 males and 5 females. To determine the bioavailability of the test material, an additional group of 10 animals received an i.v. injection of 1 mg/kg metalaxyl. The male rats weighed 188-344 g and the female rats weighed 189-285 g at the time of the radiolabeled dosing of metalaxyl.

B. METHODS

1. The general health and condition of the animals were observed by a veterinarian. Rats were acclimated individually in stainless steel metabolism cages for at least 24 hours prior to dosing and maintained in the cages for the duration of the study. Rats received food ad libitum except for fasting 24 hours before radiolabeled dosing. Animals receiving the repeated doses of metalaxyl were not fasted. The diet was Purina Formulab 5008 (Bourn Feeds, Columbia, MO). Water was given ad libitum throughout the study. An analysis of the feed was conducted, but no mention was made of an analysis of the water.

2. Oral dosing solutions were prepared in distilled water:ethanol: polyethylene glycol (PEG 200) (1:1:1.3), and the i.v. dosing solution was prepared in isotonic saline. Radiolabeled solutions were prepared one or two days before dosing. Groups of 10 rats (5/sex) were given a single dose by oral gavage of 1 mg/kg (low-dose group) or 200 mg/kg (high-dose group) [^{14}C]-metalaxyl, or an oral gavage dose of 1 mg/kg/day of unlabeled metalaxyl for 14 days followed by a single gavage administration of 1 mg/kg [^{14}C]-metalaxyl on day 15. Another group of 10 rats received a single i.v. injection of 1.0 mg/kg [^{14}C]-metalaxyl via the tail vein. The rationale for the choice of doses was not reported. One male and

one female control animal associated with each dosing group received dosing vehicle only. All animals were observed for 7 days following the administration of the labeled metalaxyl, and then sacrificed.

Dosing volumes were calculated based on the average animal weight of a dosing group; actual administered doses were determined by weighing the dosing syringe before and after dosing. Where some animals within a dosing group differed markedly in weight (such as males versus females), average animal weights were calculated separately for each subgroup. Animals in the repeated-dose group were reweighed midway through the nonradiolabeled portion of the study and prior to receiving the radiolabeled dose, and dosing volumes were adjusted accordingly. The radiolabeled dosing solutions were analyzed by reverse phase high performance liquid chromatography (HPLC) after three months of frozen storage; radiochemical purity was found to range from 91.6 to 94.6%. HPLC analysis of the preconditioned dosing solution revealed that the animals in the repeated-dosing group received 1.36 mg/kg/day of unlabeled metalaxyl. Total radioactivity administered per animal was $4.2-7.5 \times 10^7$ dpm (low-dose groups) and $4.5-4.7 \times 10^8$ dpm (high-dose group).

3. Urine was collected over an ice bath 4, 8, 12, 24, and 36 hours after exposure to the labeled dose of metalaxyl, and daily thereafter. Feces were collected at 24, 36, and 48 hours after exposure to the labeled dose, and daily thereafter. Urine was analyzed for radioactivity before being frozen pending further analysis. Feces samples were homogenized with dry ice, sublimated to remove the dry ice and frozen pending analysis. Whole blood was collected by heartstick into heparinized containers. A sample was maintained as whole blood, and the rest was separated into plasma and red blood cells. Plasma and cellular fractions were separated, refrigerated before being analyzed, then stored frozen. On the seventh day after receiving the radiolabeled metalaxyl, rats were sacrificed by carbon dioxide euthanasia. Necropsies were conducted and major tissues were removed, rinsed with physiological saline, and frozen pending analysis. The tissues removed included the bone, brain, fat, gonads, heart, kidneys, liver, lungs, muscle, spleen, stomach and intestine. Urine, cage washings, plasma, and fat samples were analyzed in triplicate by liquid scintillation counting, after solubilization with Fluorosol for the latter two samples. Feces and other tissues were combusted in triplicate. Expired air was not collected.

4. For the characterization and identification of metalaxyl metabolites, the urines were pooled (0-36 hours) by dosing and sex groups. The pooled samples represented 82.2-90.1% of the total urinary excretion for each group. Feces (0-72 hours) were pooled by dosing and sex groups; pooled samples represented 94.8-97.8% of total fecal excretion. To compare metabolite patterns, urine or a methanol/water (80:20) extract of the feces from each pooled sample was subjected to two dimensional thin-layer chromatography (2D-TLC) on

silica gel plates. The following solvent systems (SS) were used (all v/v):

SS1: Chloroform:Methanol:Formic Acid:Water (83:13:1:1)
SS2: Ethyl Acetate:Ethanol:Glacial Acetic Acid (90:9.5:0.5)

Radioactive zones were detected using Kodak X-Omat AR film. Parent and standards were visualized with ultraviolet (UV) short wave light. Radioactive zones were quantitated in a scintillation counter. The reference standards used to identify metalaxyl and its metabolites are listed in Appendix A.

To analyze conjugated metabolites, urine pools and fecal extracts were first extracted with ethyl acetate to remove free metabolites. The aqueous phase was treated with β-glucuronidase, extracted with ethyl acetate to remove liberated metabolites, treated with aryl sulfatase, and again extracted with ethyl acetate. Each ethyl acetate extract was subjected to 2D-TLC with SS1 and SS2.

The female high-dose urine pool was chosen for isolating and analytically identifying metabolites, since it contained the largest amount of metalaxyl and metabolites and gave the same metabolic profile as the other pools. The urine was filtered and chromatographed on XAD-2 resin and eluted with methanol. The eluate was chromatographed on Lobar size B and C silica gel columns, resulting in 20 radioactive peaks. Additional chromatography steps followed, including rechromatography on Lobar columns, reverse phase HPLC on C-8 columns, and chromatography on a QAE anion exchange column. Ten metabolites, in addition to the parent compound, were identified by MS, proton NMR, and IR spectrometry. None of the data were analyzed statistically.

5. Protocol: The methods followed the study protocol.

C. REPORTED RESULTS

1. Elimination and Recovery: All dosage groups exhibited a sex-related difference in the elimination of metalaxyl. Feces was the predominant route of elimination in males of all groups, accounting for an average of 54.19-63.60% of the dose (Table 1). In contrast, females in all groups eliminated most of the test compound (65.63-74.12% of the administered dose) in their urine. Similar excretion patterns were observed from animals of the same sex in different dosage groups. The test material was rapidly eliminated; 69.92-82.52% of the administered dose was recovered in the urine and feces within 24 hours. Elimination was nearly complete seven days after dosing, with 0.30-0.57% of the administered dose remaining in the tissues or blood. Almost all of the radioactivity in the administered dose was accounted for, with total recovery ranging from 95.90 to 110.01%.

2. Distribution: Less than 1% of the administered dose was recovered in the tissues of any dosing group (see Table 2 for the distribu-

tion). The highest levels of radioactivity in the low-dose groups were found in the liver and intestine (10 and 45 ppb maxima, respectively). No major sex-related differences were seen in residue levels. Levels of total residue in high-dose tissues were higher than the levels found in low-dose and preconditioned low-dose tissues. The highest residue levels were in the intestine and the liver (3100 and 810 ppb, respectively, averaging the males and females together).

3. Metabolism

Characterization and quantitation of urinary metabolites: Pooled urine samples were subjected to 2D-TLC, resulting in 33 spots which were quantitated and compared with synthetic standards. Results from all dosing groups (as a percentage of administered dose) and the identity of the corresponding synthetic standards are shown in Table VI, Appendix B. The author reported that the one- and two-dimensional TLC metabolite profiles of urine from males and females were qualitatively similar regardless of dosage or route of administration, although there were quantitative differences. The author further reported that one-dimensional TLC showed a similarity between metalaxyl metabolites in rat urine and in urine from a lactating goat administered [^{14}C]-metalaxyl, with more polar and conjugate metabolites apparently present in the goat urine.

Relatively little of the radioactivity in the urine from the low-dose oral and i.v. groups was present as free metabolites; <16% was organosoluble (Table IV, Appendix B). Of the metabolites from the high-oral-dose group, 27.4% (male) and 49.2% (female) were organo-soluble, indicating a higher percentage of free metabolites at the high-dose level. Following glucuronidase and aryl sulfatase treatment, minimal to undetectable levels of metalaxyl metabolites from almost all groups were water soluble. The sole exception was the males in the preconditioned low-dose group, with 29.8% water-soluble radioactivity that the author hypothesized was due to conjugated or polar anionic metabolites. Glucuronidase treatment released as aglycones most of the radioactivity in the low-dose i.v. group (73.1% average) and the high-dose group (55.4% average). Most of the conjugated metabolites in the low-oral-dose groups were released as the free metabolites by aryl sulfatase. The author reported that no additional metabolites were evident by TLC after enzymatic hydrolysis, indicating that all conjugates were also present in the free form.

Metabolites identified by 2D-TLC were identified by comparison with standards and quantitated. Metalaxyl co-chromatographed with one of its metabolites, and could not be quantitated separately. However, the combined radioactivity in the two compounds accounted for ≤0.3% of the dose in the low-dose groups. In the high-dose group, the combined spot accounted for <0.1% of the dose in male urine, and 1.8% of the dose in female urine. The major identified metabolite (UM-11), accounting for 3.2-20.3% of the dose, co-chromatographed with standard CGA-107955 (Table VI, Appendix B). Two major

unidentified metabolites (UM-23 and UM-26), accounted for a maximum of 23.3 and 8.3% of the dose, respectively. They were presumed to be glucuronide or sulfate conjugates, since enzyme hydrolysis removed the corresponding TLC spots. A total of 19.6-51.4% of the radioactive material in the urine for all groups was identified. Glucuronide and sulfate conjugates of the identified metabolites accounted for the rest of the radioactive residue.

Characterization and quantitation of fecal metabolites: The author reported that the one- and two-dimensional TLC metabolite profiles of fecal extracts from males and females were qualitatively similar regardless of dosage or route of administration, and qualitatively similar to the profiles of urinary metabolites, although there were quantitative differences. The image quality of the TLCs reproduced in the report was too poor to permit an independent comparison, but the metabolite patterns (maps) of the high-dose male and female urine and fecal extracts do appear qualitatively similar.

Quantitation of 2D-TLC plates revealed that 0.2-0.8% of the administered dose was present in the feces as the parent compound or the co-chromatographing metabolite (Table X, Appendix B). Material co-chromatographing with CGA-107955 was the main fecal metabolite (FM-13) in females, accounting for 7.1-10.4% of the administered dose. In male rats, this spot co-chromatographed with both CGA-107955 and CGA-108905. The only other identified spots that accounted for >1% of the dose co-chromatographed with CGA-62826 (FM-12) and the two isomers of CGA-94689 (FM-2 and FM-3). Unidentified spots, accounting for 24.1-49.4% of the radioactivity in the fecal extract, were attributed to intact conjugates and/or free anionic metabolites.

The bulk (52% for low oral and i.v. dose males and 73.6% for the corresponding females) of the fecal metabolites, unlike the urinary metabolites, were organosoluble before enzymatic hydrolysis (Table VIII, Appendix B). The high-oral-dose group did not exhibit such a sex difference; the average for males and females was 61.7%. Glucuronidase treatment released most of the rest of the radioactivity (18.4-41.6%); aryl sulfatase hydrolysis released 3.8-11.6% of the radioactivity. The author assumed that all conjugated metabolites were also present in their free form.

Isolation and analytical identification of metabolites: Ten metabolites and the parent compound were purified from high-dose female urine and identified analytically by a combination of MS, NMR, and IR spectroscopy. Nine of the metabolites (M1-M8 and M10) had also been identified by co-chromatography with standards in 2D-TLC. One additional metabolite, M9, was the benzylic alcohol of M6. The metabolites and the corresponding standards are listed in Appendix A.

The study author proposed that metalaxyl is metabolized via three major pathways and one minor pathway. One pathway involves hydrolysis of the ether, followed by oxidation of the resulting

alcohol, ester hydrolysis, or N-dealkylation of the ester chain. A second pathway involves oxidation of an aromatic methyl to the benzylic alcohol, followed by oxidation to the benzoic acid or ester hydrolysis. The third major pathway proceeds by ester hydrolysis, sometimes followed by benzylic acid formation. The minor pathway occurs via hydroxylation at the meta position of the phenyl ring. Conjugation of the final products of all of the metabolic pathways was also proposed. The proposed metabolic pathway for metalaxyl in rats is presented in Figure 1.

A separate study[1] submitted by the sponsor found and identified eight rat urinary and fecal metabolites of metalaxyl. The parent compound accounted for 0.4% of the administered dose. Major metabolites were the compounds that were identified in the study reviewed above as CGA-107955 and CGA-94689. Urinary metabolites were partially conjugated with glucuronic acid. A published article[2] submitted by the sponsor describes the degradation of metalaxyl by the soil fungus Syncephalastrum racemosum (Cohn) Schroeter. The fungus transformed over 80% of metalaxyl in pure culture at 5 µg/mL and 50% of metalaxyl at 20 µg/mL in 20 days. The metabolites were identified as the compounds referred to in the reviewed study as CGA-100255 and CGA-94689.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

The author concluded that metalaxyl is rapidly and completely eliminated in the urine and feces, with 93-93% of an oral or i.v. dose being eliminated within 72 hours of administration. Urine was the predominant route of elimination in female rats (≈70%), while feces was the major elimination route in males (≈60%). No explanation was suggested for this sex-related difference. There were no other major sex-, route-, or dose-related differences in the rate or route of elimination. The similarity in elimination profiles of orally and intravenously administered metalaxyl indicated that metalaxyl was well-absorbed. The author also concluded that biliary excretion was suggested by the high fecal recovery in both males (59.4%) and females (35.7%) receiving an i.v. dose. Residual tissue radioactivity seven days after dosing with [^{14}C]-metalaxyl totaled 1% of the dose. The highest levels of radioactivity were found in the intestine and liver. No major sex- or dose-related differences in the tissue distribution pattern were found, but higher residues were found in all tissues of the high-dose group. Overall, the author noted that the observed tissue distribution pattern would suggest a localized dynamic enterohepatic circulation with metalaxyl metabolites moving through a conjugation, deconjugation, reabsorption, and reconjugation cycle.

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[1]Hambock, E., (1981). Metabolic Pathways of CGA 48 988 in the Rat.

[2]Zheng, Z., Liu, C.-Y., Freyer, A.J., and Bollag, J.-M. (1989). Transformation of Metalaxyl by the Fungus Syncephalastrum racemosum. Appl. Environ. Microbiol. 55:66-71.

Metabolites in urine and a fecal extract were characterized by TLC and sequential enzymatic hydrolysis. Two-dimensional TLC revealed a qualitatively similar pattern of 33 possible metabolites in urine and feces extract. Nine metabolites were identified on the basis of co-chromatographing with standards. Purification of metabolites and analysis of MS, NMR, and IR spectral data allowed the identification of these nine metabolites, the parent compound, and one additional metabolite.[3] Most of the urinary metabolites (49.9-96.8%) were present as glucuronide or sulfate conjugates, with glucuronide conjugates predominant in the i.v. and high oral dose groups, and sulfate conjugates more common in the low and preconditioned low oral dose groups. The bulk of the fecal metabolites (46.6-78.8%) were unconjugated; glucuronide conjugates were more common than sulfate conjugates, with no sex-, dose-, or route-related differences. Less than 1% of the recovered reactivity was due to unmetabolized metalaxyl.

Quality assurance statements for the analytical and biological phases of the study were signed and dated October 16, 1990, and August 29, 1990, respectively.

E. CONCLUSIONS BASED ON REVIEWERS' DISCUSSION AND INTERPRETATION OF DATA

The studies adequately described the absorption, distribution, metabolism, and excretion of [^{14}C]-metalaxyl in rats following low- and high-dose oral and repeated oral exposure, as well as single i.v. injection. The data indicate that labeled metalaxyl is well absorbed and eliminated. Fecal elimination predominates in males, and urinary elimination is the major route in females. The mechanism behind this sex-related difference is unclear. The oral absorption rate could not be determined because the peak blood levels were not measured in the study. Biliary excretion is suggested, especially in males, since ≈60% of the i.v. dose was eliminated in the feces. Therefore, the fecal elimination of metalaxyl following oral exposure is probably due to the unabsorbed compound and biliary excretion. The specific extent of these two processes in fecal elimination of metalaxyl was not evaluated. The low tissue levels of radioactivity, as well as the rapid elimination, demonstrate that bioaccumulation and retention of metalaxyl and/or its metabolites is low in rats. The largest residues were found in the intestine and liver, with no clear sex- or route-related differences. The high-dose group had the same tissue distribution, but higher residues in all tissues. Recovery of the radioactivity was acceptable (95.90-110.01%) for all dose groups. Almost all of the parent compound was metabolized following oral or i.v. dosing, resulting in 33 metabolites. No statistical methods were used to analyze the study results.

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[3] Note: the author erroneously reported in the summary that ten metabolites were identified by TLC and nine metabolites (including metalaxyl) were purified. We base our summary on the list of purified metabolites (pp. 17-35) and tables [illegible] and [illegible] (pp. 46, 47, 50, and 51).

Urinary and fecal metabolites were thoroughly characterized and found to follow similar patterns. The parent compound and ten metabolites were purified from high-dose female urine, and identified by comparison with synthetic standards and/or analytical methods. The other spots were attributed to glucuronide, sulfate, and possibly peptidic conjugates. Urinary metabolites were predominantly (49.9-96.8%) conjugated; glucuronide conjugates were predominant in the i.v. and high-oral-dose groups, while sulfate conjugates were more common in both of the low-oral-dose groups. The bulk of the fecal metabolites (46.6-78.8%) were unconjugated, with glucuronide conjugates being more common than sulfate conjugates in all dosage groups.

There appeared to be no major sex- or dose-related differences in the recovery of these metabolites in the urine or fecal extracts. Based on the identified metabolites, three major pathways and one minor pathway of metalaxyl metabolism were proposed.

These studies also showed that single oral administration of 1.0 and 200 mg/kg metalaxyl, as well as repeated dosing with 1.0 mg/kg/day, and single i.v. administration of 1.0 mg/kg, did not induce any apparent treatment-related clinical effects.

F. DEFICIENCIES

Major deficiencies are cited below:

- On page 37, paragraph 1, line 6 of the study report, the study author stated, "Group III male rats still contained aqueous soluble residue (29.8%) possibly conjugated or polar anionic metabolites." A discussion of the causes of a significant percentage of radioactivity remaining in this fraction is required.

- The study author stated in line 6 of the second paragraph from the bottom of page 37 of the study report, "Two major metabolites, UM-23 and UM-26, maximally accounted (sic) for 23.3% and 8.3% of dose, respectively, are polar metabolites presumed intact glucuronide or sulfate conjugates since..."

  Because these metabolites in urine comprise 5% or more of the administered dose, reasonable efforts should have been made to identify the free metabolites corresponding to metabolites UM-23 and UM-26.

Minor deficiencies in the study which did not affect the overall study results and conclusions are as follows: The author did not discuss the rationale for choosing the dose levels for this protocol. There was no discussion regarding the potential interference of the dosing vehicle to the kinetics of metalaxyl. Exhaled radioactivity was not measured, but the amount is not likely to be significant, considering the high total recovery. Urine samples were collected over an ice bath, rather than dry ice, and fecal samples were sublimed after mixing with dry ice, with the result that volatile metabolites could have been lost. Animals in the repeated dosing group were not fasted prior to dosing.

TABLE 1. Mean Percent Recovery of Radioactivity 7 Days After Administration of Metalaxyl to Rats

Dose Group	Sex	Percent of Administered Dose[a]				
		Urine + Cage Wash	Feces	Tissues	RBC + Plasma	Total[a] Recovery
1.0 mg/kg (intravenous)	Male[b]	44.09	59.38	0.37	0.015	103.86
	Female	65.63	35.68	0.42	0.017	101.75
1.0 mg/kg (single oral)	Male	35.00	62.13	0.31	0.011	97.45
	Female	66.79	34.66	0.56	0.013	102.02
1.0 mg/kg[c] (repeated)	Male	32.00	63.60	0.29	0.009	95.90
	Female	74.12	35.44	0.44	0.009	110.01
200 mg/kg (single oral)	Male	46.68	54.19	0.30	0.008	101.18
	Female	70.43	31.32	0.31	0.010	102.07

[a]Expired air was not measured for radioactivity.

[b]Five animals/sex.

[c]Animals were given 1.0 mg/kg/day unlabeled metalaxyl for 14 days and a single dose of 1.0 mg/kg [^{14}C]-metalaxyl on day 15.

Source: Tables 6-9, pp. 150-153.

TABLE 2. Distribution of Radioactivity in Tissues of Rats 7 Days After Administration of Metalaxyl

Tissue/Organ	Average Metalaxyl Residue in Tissues Expressed as ppb in Rats Dosed at:							
	1.0 mg/kg (single intravenous)		1.0 mg/kg (single oral)		1.0 mg/kg (repeated oral)		200 mg/kg (single oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Bone	0.29[a]	0.41	0.27	0.38	0.48	0.35	45	94
Brain	<MQL	<MQL	1.3	<MQL	0.2	0.28	55	100
Fat	0.36	<MQL	<MQL	2.1	<MQL	<MQL	380	670
Gonads	<MQL	1.1	<MQL	1.2	<MQL	0.99	28	250
Heart	<MQL	<MQL	<MQL	0.94	<MQL	0.47	98	140
Kidneys	1.6	2.8	1.2	2.3	1.7	2.1	160	280
Liver	5.4	10	3.7	9.0	4	8.2	640	980
Lungs	2.4	6.8	0.44	5.6	<MQL	3.7	120	200
Muscle	<MQL	<MQL	<MQL	0.34	<MQL	<MQL	66	110
Spleen	<MQL	2.9	<MQL	1.2	1.2	0.95	86	170
Stomach	0.8	0.32	<MQL	<MQL	1.3	2.0	71	150
Intestine	21	30	29	45	18	45	3530	2670
Carcass	1.3	1.8	0.28	3.0	2.3	2.6	180	1130
RBC	4	6	3	4	3	4	510	720
Plasma	0.7	1	0.5	1	0.3	0.5	50	63

[a]Each value represents the average of five rats.

<MQL = <Minimum Quantifiable Limit.

Source: Table 28; p. 172.

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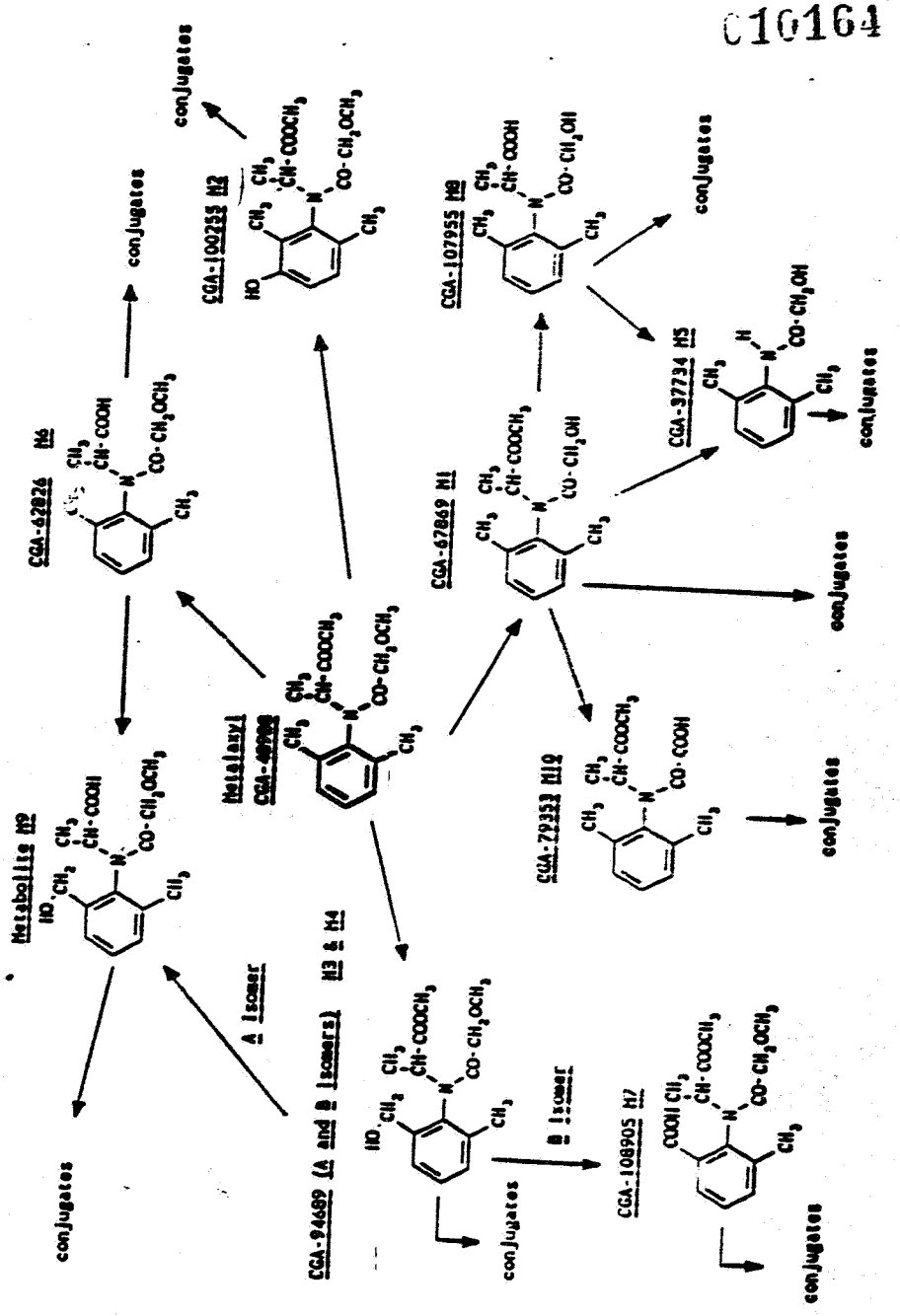

Figure 1. The proposed metabolic pathway of metalaxyl in rats.

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APPENDIX A

Figure 1. CHEMICAL NAMES AND STRUCTURES
(pp. 52-56)

Metalaxyl Sha # 113501

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