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# Data Evaluation Report

Study type: Metabolism (85-1) Tox. Chem. No.: 003B

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Laboratory Project numbers: HRC/STR 18/88502; HRC/STR 18/89184; HRC/STR 18/89487; HRC/STR 18/89603; CTL/P/2809

Test material: [U-$^{14}$C]-Acetochlor

Synonyms: 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide

Testing Facilities: Huntingdon Research Centre Ltd.
Huntingdon, Cambridgeshire, England

ICI Central Toxicology Laboratory
Alderly Park, Cheshire, UK

Sponsor: ICI Central Toxicology Laboratory
Alderly Park, Cheshire, UK

Title of reports:
[1]: Laboratory Project No. HRC/STR 18/88502, "The Biokinetics of 14-C Acetochlor After Oral Administration to Rats at a Nominal Level of 10 mg/kg."

[2]: Laboratory Project No. HRC/STR 18/89184, "The Biokinetics of 14-C Acetochlor After Oral Administration to Rats at a Nominal Level of 200 mg/kg."

[3]: Laboratory Project No. HRC/STR 18/89487, "The Distribution and Excretion of Radioactivity after Oral Administration of 14-C Acetochlor at 10 mg/kg to Rats Pre-treated with Non-Radiolabelled Acetochlor."

[4]: Laboratory Project No. HRC/STR 18/89603, "The Metabolism of 14-C Acetochlor in the Rat after Oral Administration."

[5]: Laboratory Project No. CTL/P/2809, "Acetochlor: Biotransformation Study in the Rat."

Author(s): D.R. Hawkins, D. Kirkpatrick, G. Dean [1-4]; B.K. Jones [5]

Reports issued: [1-3]: February, April, June 1987; [4], June 1989; [5], March 1990

Conclusions:

In studies [1], [2], and [3], the the disposition and metabolism of $^{14}C$-acetochlor was investigated in male and female rats at a low oral dose (10 mg/kg), repeated low oral doses ( 10mg/kg x 14 days), and a high dose (200 mg/kg). Comparison of disposition data in bile duct cannulated and non-cannulated rats demonstrated that acetochlor was well absorbed after oral administration. Excretion was relatively rapid at the low dose, with a majority of radioactivity eliminated in the urine by 24 hours. At 200 mg/kg, urinary elimination of $^{14}C$-acetochlor derived radioactivity was decreased in male and female rats, while fecal (biliary) elimination was increased. At both 10 and 200 mg/kg, female rats eliminated a greater percentage of $^{14}C$ acetochlor derived radioactivity in urine than male rats. No effect was observed from repeated low oral dosing on the disposition of $^{14}C$-acetochlor in male or female rats.

Fecal elimination of $^{14}C$-acetochlor derived radioactivity was due to elimination *via* the bile, and was consistently less in female rats vs male rats at both the 10 and 200 mg/kg dose of acetochlor.

Residual $^{14}C$-acetochlor derived radioactivity was minimal in all dose groups, except in those tissues well-perfused with blood (heart, spleen, kidney, lungs, liver). This apparent accumulation of $^{14}C$-acetochlor derived radioactivity was due to binding of acetochlor and/or a metabolite to red blood cells, with a blood:plasma ratio of approximately 100 observed at 5 days post-dosing.

Urinary, biliary, and fecal metabolites of $^{14}C$-acetochlor were isolated and identified in studies [4]and [5] by TLC, HPLC, and LC/MS. The major biotransformation product in urine was the mercapturic acid conjugate of acetochlor after removal of the ethoxymethyl side chain. The percentage of this metabolite was decreased by approximately 50% in urine from rats dosed at 200 mg/kg; no other major alterations were observed in metabolite profile at the 200 mg/kg dose. Both glucuronide and glutathione conjugates of acetochlor were identified in bile, with no significant quantitative change in biliary metabolite profile with increasing dose. Fecal metabolites were difficult to identify. Enterohepatic recirculation of $^{14}C$-acetochlor derived radioactivity is suggested from these studies, but the nature of the reactive species which binds to red blood cells was not identified in the present studies.

Core Classification: supplementary

This study does not satisfy the guideline requirements (85-1) for a metabolism study in rats. Justification for the omission of intravenous data is requested. In addition, percentage recovery from tissues and feces during processing is requested. As acetochlor is proposed for food use, the determination of the species bound to red blood cells may be toxicologically relevant.

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Purity of unlabeled acetochlor was also not stated in studies 1 through 3 and is requested.

# I. MATERIALS

## A. Test Material

### Metabolism Studies

[1]: $^{14}$C-Acetochlor
code no: CFQ 4196 (10 mg/kg dose)
batch analysis sheet: C/7499
Radiochemical Purity: 97.6% (3.0 mg/kg dose)
Specific Activity: 8.5 µCi/mg (10.0 mg/kg dose)

Unlabelled Acetochlor
batch 1+3
Chemical Purity: not stated

[2]: $^{14}$C-Acetochlor
code no: CFQ 4196 (200 mg/kg dose)
batch analysis sheet: C/7499
Radiochemical Purity: 98.9% (200 mg/kg dose)
Specific Activity: 0.5 µCi/mg (200 mg/kg dose)

Unlabelled Acetochlor
batch 1+3
Chemical Purity: not stated

[3]: $^{14}$C-Acetochlor
code no: CFQ 4196 (10 mg/kg x 14 days dose)
batch analysis sheet: C/7499
Radiochemical Purity: >98% (10 mg/kg x 14 days dose)
Specific Activity: 7.9 µCi/mg (10 mg/kg x 14 days dose)

Unlabelled Acetochlor
batch 1+3
Chemical Purity: not stated

### Metabolite Characterization and Identification Studies [4, 5]

Study [4] utilized samples of excreta obtained from the above listed metabolism studies [1-3]. In study [5], a separate group of rats was dosed for bulk collection and identification of acetochlor metabolites. Unlabelled acetochlor with a purity of 99.5% was used, while radiolabelled acetochlor (purity not stated; specific activity 0.6 GBq/mmol) was used.

B. Vehicles: Polyethylene glycol (PEG) 400 (Studies 1, 2, and 3)
PEG 600 (Study 4)

C. Test Animals: Species: rat
Strain: CD Sprague-Dawley
Source: Charles River, Margate, U.K.
Weights: study [1]: males, 199-264g; females, 190-224g.
study [2]: males, 189-211g; females, 205-224g
study [3]: males, 152-160g; females, 164-175g.
study [4]: males, 240-320g; females, 210-240g.

II. METHODS

A. Study Design

1) Metabolism Studies [1-3]

The bioavailability and disposition of $^{14}$C-Acetochlor was assessed in male and female rats following oral administration of the test compound. Rats received either a single oral dose of 10 or 200 mg/kg $^{14}$C-Acetochlor or 14 repeated daily doses of unlabelled test material at 10 mg/kg followed by a single radiolabelled dose of 10 mg/kg. Plasma concentrations following single oral doses of $^{14}$C-Acetochlor at 10 and 200 mg/kg were also determined from 0.25-360 hours post dosing. Dose groups were as follows:

| Study# | Dose (mg/kg) | Dose Route | Number of Animals Male | Female |
|---|---|---|---|---|
| 1 | 10 | Oral[a] | 5 | 5 |
| 2 | 200 | Oral[b] | 5 | 5 |
| 3 | 10 | Oral[a] | 5 | 5 |

[a]single dose
[b]14 daily unlabelled doses followed by one radiolabelled dose on day 15.

2) Metabolite Characterization and Identification Studies [4,5]

Metabolites of acetochlor in urine and feces from rats dosed orally with 10 and 200 mg/kg $^{14}$C-Acetochlor and from rats given repeated oral doses of 10 mg/kg acetochlor were characterized by TLC in study [4]. In study [5], male and female rats were orally dosed with either 10 or 200 mg/kg $^{14}$C-Acetochlor for pooling of urine, feces, and bile samples and characterization of

metabolite profiles in these samples by TLC, with subsequent identification of metabolite structures by LC/MS or GC/MS. A proposed pathway for acetochlor biotransformation was proposed based on identification of metabolites in the various excreta (Study 5).

C. Experimental

1) Metabolism Studies [1-3]

a. Animal Husbandry

Animals were acclimated to the laboratory environment for at least 14 days before dosing. Male rats were approximately 9 weeks old and female rats 12 weeks old at the time of dosing. Animals were given food (LAD 1 pellets, Labsure, Croydon, U.K.) and water *ad libitum*. Conditions of animal housing were not provided.

b. Dosing

For the single oral doses of 10 and 200 mg/kg $^{14}C$-Acetochlor, appropriate amounts of labelled and unlabelled acetochlor were dissolved in polyethylene glycol (PEG) 400 to give final solution concentrations of 4mg/ml or 84 mg/ml for the 10 and 200 mg/kg dose groups, respectively. Dose volume was 2.5 ml/kg. For the repeated low dose oral study (10 mg/kg x 14 days), 2 batches of non-radiolabelled acetochlor were prepared, each sufficient for 7 days dosing. Stability of acetochlor had been demonstrated in the dose solution for up to 17 days (page 16 of Study [3]), but data were not provided.

The quantity of radioactivity received by each rat was determined from liquid scintillation counting in triplicate of duplicate aliquots of dose solution equivalent to the volume received by the rats which was diluted in 200ml acetonitrile.

A pilot study was conducted at the 10 mg/kg dose level to determine if a five-day collection period was sufficient for excretion studies, and if significant amounts of $^{14}C$-acetochlor were eliminated via expired air. Results (Table 1, page 22 of Study [1]) showed that the five day period was sufficient and that negligible amounts of radiolabel were excreted in expired air.

Rats were housed individually in glass metabolism cages during the single dose studies. In the repeat dose studies, rats were housed in groups of 5 for the first 12 days of the study, until 2 days before administration of the radiolabelled dose, when they were transferred to individual glass metabolism cages.

c. Sample Collection and Analysis

Studies [1-3]:

Urine was collected on solid $CO_2$ at 6, 12, and 24 hours following dosing, and after 24 hours was collected at 24 hour intervals up to 5 days. Feces were collected every 24 hours for 5 days. Cages of treated animals were rinsed with tap water following sacrifice of the animals. Following sacrifice by cervical dislocation, the liver, kidneys, heart, lungs, brain, testes or ovaries, spleen, uterus, g.i. tract plus contents, and samples of bone marrow, muscle, and fat

and the entire skin and fur were removed and stored with the carcass at -15 °C until analysis. Blood was also removed by cardiac puncture at sacrifice, and part of the sample was retained as whole blood, while plasma was obtained from the remainder.

Samples of feces homogenates, tissues, plasma, and whole blood were mixed with cellulose powder and analyzed for radioactivity by combustion in a sample oxidizer followed by liquid scintillation counting. Aliquots of urine, cagewash, carcass digests, and skin digests (1ml for each) were mixed with MI-31 special scintillator cocktail and counted by liquid scintillation counting.

In plasma experiments, samples were withdrawn into heparinized tubes and centrifuged for immediate analysis of plasma radioactivity.

d. Statistics

No statistical analysis was reported in Studies [1-3].

2) Metabolite Characterization and Identification Studies [4,5]

a. Animal Husbandry

Husbandry data apply only to study [5], as study [4] utilized samples of excreta from studies [1-3]. In study [5], animals were housed in groups in stock rat cages prior to dosing. Rats were provided with food (pelleted PCD diet, Special Diet Services Ltd, Essex) and water *ad libitum*. Animals were acclimated to the room conditions for at least 3 days prior to use in temperature and humidity controlled rooms.

**Note:** The diet given these rats may not be identical to the diet given the rats in studies [1-3]. This may or may not influence metabolism of acetochlor, depending upon the relative similarity of the 2 diet formulations.

b. Dosing:

As in (a) above, dosing applies only in study [5]. Four dose solutions (I-IV) were prepared by mixing the appropriate amounts of labelled and unlabelled acetochlor in PEG 600 for the following purposes:

I: 200 mg/kg acetochlor, for urinary metabolite collection in 8 male rats.
II: 200 mg/kg acetochlor, for urinary metabolite collection in 6 female rats.
III: 10 mg/kg acetochlor, for biliary metabolite collection in 2 male rats.
IV: 200 mg/kg acetochlor, for biliary metabolite collection in 2 male rats.

Rats were transferred to stainless steel group metabolism cages upon dosing, except bile duct cannulated rats, which were housed in individual glass metabolism cages.

c. Sample Collection and Analysis:

Urine and feces were collected for up to 3 days over solid $CO_2$. Bile from bile duct cannulated rats was collected for up to 3 days at room temperature. Urine and bile samples were either diluted or weighed for counting by LSC in duplicate. Fecal samples were homogenized in a similar weight of magnesium sulphate for sample oxidation and subsequent counting by LSC.

Samples of urine or bile were pooled by sex and dose for analysis by TLC. Radioactivity from portions of pooled urine was extracted by elution from Bond Elut C8 (study 5) or C18 (study 4) columns. Samples of urine subjected to ß-glucuronidase/sulfatase enzyme hydrolysis were also applied to Bond Elut columns for extraction of radioactivity.

Feces (pooled over 24 hours from each sub-group of five animals of the same sex) were extracted sequentially with ethyl acetate, acetonitrile, and acetonitrile:water (7:3, v/v). The ethyl acetate extract was evaporated to near dryness under a stream of nitrogen. Acetonitrile in the final 2 extraction solvents was extracted and the aqueous residue processed by sorbent extraction as described for urine above. Normal phase TLC was then carried out using a variety of solvent systems described in both study [4] (page 12) and study [5] (page 17).

Chromatographic correspondence between reference compounds and radioactive metabolites in excreta samples was achieved by co-chromatography of reference compound with sample extract.

Solvent extract samples of urine and bile were also subjected to HPLC with both UV and radiochemical detection. Three different separation procedures were employed for complete metabolite separation and identification, as listed on pages 19-20 of study [5]. Resolved metabolites from HPLC were subjected to thermospray MS, using a VG LC-MS thermospray/plasmaspray interface. GC-derived mass spectra were obtained using electron impact MS. Standards of synthesized acetochlor metabolites (pages 47-49 of study [5]) were used for comparison to biological samples for mass spectral analysis.

D. Compliance

A signed statement of no data confidentiality claims was provided with all studies.

A signed statement of GLP compliance (40 CFR 160.35) was provided with all studies.

A signed statement of quality assurance was provided with all studies.

A signed statement of EPA flagging criteria was provided with studies 1,2,3, and 5.

## III. RESULTS

### 1) Metabolism Studies

The stability of the dose solution for the repeated low-dose study was stated to be 17 days in study [3] (page 16 of report). However, there were no confirmatory data to support this statement.

Verification of dose for rats in the 10 and 200 mg/kg dose groups (studies 1, 2, and 3) was presented by liquid scintillation counting of dose solution aliquots. In study [5], the dosing syringe was weighed prior to and following dosing of each rat to obtain the precise dose received by each rat (page 41 of study [5]. Recovery of radiolabel from urine after sorbent extraction and enzyme incubation was reported in study [4]; this recovery was between 98-99%. No data were presented on recovery of radiolabel from feces or tissues in any study.

#### a. Absorption

In male rats dosed orally with a single dose of 10 mg/kg $^{14}$C-acetochlor, 59.6% of the dose was excreted in urine by 24 hours, while the total percentage of the dose excreted in urine was 70.6% at 120hr. In female rats at this dose level, 66.5% was excreted in urine in 24 hours, while a total of 77% of the dose was excreted in 120hr. Thus, urinary excretion of $^{14}$C-acetochlor derived radioactivity was slightly higher in females at the 10 mg/kg dose level. This is reflected in the lower fecal excretion of $^{14}$C acetochlor derived radioactivity in female rats (Table 2, below). Repeated oral dosing at the 10 mg/kg dose level produced a similar pattern of urinary and fecal elimination in male and female rats.

At the 200 mg/kg $^{14}$C acetochlor dose level, 43.1% of the total dose was eliminated in urine by 24 hours in male rats, and a total of 51.6% was eliminated by this route in 120hr. In female rats at this dose level, 51.6% of the total dose was eliminated in 24 hours in urine, and 64.7% was found in urine at 120hr. This decreased urinary elimination at the 200 mg/kg dose as compared to the pattern seen at 10 mg/kg was accompanied by an increase in fecal elimination of $^{14}$C-acetochlor derived radioactivity.

Although a significant amount of radioactivity was observed in feces at both doses of $^{14}$-C acetochlor, this radioactivity was determined to be of biliary origin in experiments conducted in study [5], where bile duct cannulated rats were administered similar oral doses of acetochlor. Thus, absorption of acetochlor at both 10 and 200 mg/kg was apparently complete.

#### b. Distribution

Tissue levels of $^{14}$C-acetochlor derived radioactivity were negligible (between 0.01-0.05% of the total dose) at all dose levels, with the exception of the following, as summarized in the table below (**Table 1**):

**Table 1**

Distribution of $^{14}$C-Acetochlor Derived Radioactivity in Male and Female Rats[a]

| | LDM | LDF | PCM | PCF | HDM | HDF |
|---|---|---|---|---|---|---|
| g.i. tract | 0.49(0.55)[b] | 0.27(0.19) | 0.36(0.41) | 0.18(0.15) | 3.3(0.19) | 3.6(0.15) |
| liver | 0.50(0.3) | 0.58(0.22) | 0.45(0.23) | 0.38(0.18) | 8.7(0.25) | 7.6(0.16) |
| blood | 4.58(1.79) | 6.18(2.01) | 4.41(1.46) | 4.72(0.91) | 112(2.05) | 105(1.87) |
| spleen | 0.77(0.02) | 0.92(0.02) | 0.85((0.02) | 0.74(0.02) | 19.7(0.02) | 19.3(0.02) |
| lungs | 0.65(0.03) | 1.02(0.05) | 0.73(0.03) | 0.75(0.03) | 15.7(0.04) | 16.7(0.04) |
| kidneys | 0.40(0.04) | 0.53(0.04) | 0.43(0.04) | 0.41(0.03) | 8.6(0.04) | 9.1(0.03) |
| heart | 0.94(0.04) | 0.84(0.03) | 1.11(0.04) | 0.83(0.03) | 23.3(0.04) | 19.6(0.03) |
| bone marrow[c] | 0.22 | 0.26 | 0.18 | 0.26 | 4.6 | 4.9 |

Abbreviations are: LD, low dose (10 mg/kg); PC, pre-conditioned dose (10mg/kg x 14days); HD, high dose (200 mg/kg).

[a]data represent the mean concentration (µg equivalents acetochlor/g) found at 120 hours post-dosing.

[b]percent total dose

[c]percentage for bone marrow not provided

As shown in **Table 1**, the concentration of radioactivity in tissues from administration of $^{14}$-C acetochlor was highest in those tissues well-perfused with blood. Few sex-dependent differences were seen. The concentration of $^{14}$-C acetochlor derived radioactivity was higher in male rats vs female rats at the 10 mg/kg single and repeated dose levels. Blood levels of $^{14}$-C acetochlor derived radioactivity were higher in female rats at the 10 mg/kg dose level vs male rats at this dose. Lung levels of $^{14}$-C acetochlor derived radioactivity were higher in female rats at the 10 mg/kg dose level vs male rats at this dose.

While the heart, spleen, lungs, and kidneys were all observed with significant amounts of $^{14}$-C acetochlor derived radioactivity on a µg/g tissue basis, the total percentage of a dose of $^{14}$-C acetochlor found in these tissues was less than 0.05% at all dose levels(**Table 1**). This is likely due to the presence of significant amounts of blood in these tissues, which as an organ showed the highest amount of radioactivity on a per gram tissue basis as well as percentage of total

radioactivity in pre-perfused tissues.

No apparent differences were observed in the distribution of $^{14}$-C acetochlor derived radioactivity between dose groups which would indicate accumulation of $^{14}$-C acetochlor derived radioactivity upon repeated dosing at 10 mg/kg, or altered distribution at the 200 mg/kg dose level.

c. Excretion

The excretion of $^{14}$C-acetochlor in urine and feces at both 3 and 200 mg/kg is summarized in the following Table:

**Table 2**

Excretion of $^{14}$C-acetochlor Derived Radioactivity in Male and Female Rats[a]

| | LDM | LDF | PCM | PCF | HDM | HDF |
|---|---|---|---|---|---|---|
| urine (+cage wash) | 70.6± 3.6 | 77.0± 7.1 | 65.0± 2.8 | 74.6± 1.8 | 51.6± 8.3 | 64.7± 5.5 |
| feces | 22.8± 2.8 | 13.2± 2.7 | 26.1± 2.5 | 16.5± 1.1 | 37.2± 5.7 | 26.9± 5.0 |
| carcass (mean) | 0.68 | 0.71 | 0.62 | 0.60 | 0.88 | 0.65 |
| Total (urine+ feces+ tissues) | 97.3 | 94.0 | 94.4 | 94.6 | 92.9 | 95.1 |

Abbreviations are: LD, low dose (3 mg/kg); PC, pre-conditioned dose (10mg/kg x 14days); HD, high dose (200 mg/kg).

[a]data represent the mean percent dose excreted at 120 hours post-dosing.

In all dose groups, >90% of a given dose of $^{14}$C-acetochlor was excreted within 5 days.Urinary excretion in males and females was largely complete in all dose groups by 24 hours. No apparent delay was observed in the high dose groups. However, as noted in Table 2, female rats in all dose groups showed higher percentages of urinary $^{14}$-C acetochlor derived radioactivity than males.

Fecal elimination of $^{14}$C-acetochlor derived radioactivity was a significant route of excretion, representing between 13 and 37% of a given dose in all dose groups. Male rats showed a higher percentage of fecal excretion than female rats in the single low dose groups and repeated low dose groups (Table 2), reflective of the decreased urinary elimination in male rats.

Fecal elimination of $^{14}C$ acetochlor derived radioactivity was increased at the 200 mg/kg dose level by 63% in male rats, and by 103% in female rats relative to that observed at the 10 mg/kg dose level. However, the percentage of a given dose of $^{14}C$ acetochlor eliminated by the urinary route was decreased by only 26% and 15% in males and females at the 200 mg/kg dose, indicating the possibility of enterohepatic recirculation of $^{14}C$ acetochlor derived radioactivity. This is supported by results from study [5], in which excretion of $^{14}C$ acetochlor derived radioactivity by the biliary route was examined in male rats given a single oral dose of 10 or 200 mg/kg $^{14}C$ acetochlor. Results (pages 45 and 46 of study [5]), indicated that greater than 80% of a given dose of $^{14}C$ acetochlor was eliminated *via* this route at both doses, while excretion in urine from non-bile duct cannulated male rats was approximately 70%.

d. Plasma Levels of 14-C Acetochlor derived Radioactivity

In studies [1], [2], and [3], the concentration of $^{14}$-C acetochlor derived radioactivity was measured from 0.25hr until 240 hours post-dosing. Peak plasma levels of $^{14}$-C acetochlor derived radioactivity occurred at 7 hours post-dosing in both male and female rats at the 10 mg/kg dose level. Plasma levels at 72 hours in both sexes were approximately 1/10 of peak plasma levels. A biphasic pattern of decline was observed in plasma levels of $^{14}$-C acetochlor derived radioactivity over the time course of blood radioactivity measurement, with plasma levels of $^{14}$-C acetochlor derived radioactivity consistently higher in female rats over the time course of radioactivity measurment. Estimated half life of elimination from inspection of Figure 3, page 33 of study [1] was 20 hours for both male and female rats.

Plasma levels of $^{14}$-C acetochlor derived radioactivity in rats subjected to repeated oral dosing at the 10 mg/kg dose level were not determined.

At the 200 mg/kg dose level, peak plasma levels of $^{14}$-C acetochlor derived radioactivity occurred at 12 hours post-dosing in both male and female rats. Peak concentration in female rats was considerably higher (41.9±9.6 µg equivalents/ml) than in male rats (25.0±4.6 µg equivalents/ml). Plasma concentrations in female rats remained higher for the duration of the plasma measurements. As with the 10 mg/kg dose level, plasma levels of $^{14}$-C acetochlor derived radioactivity fell significantly between 12- 48 hours. Estimated half life of elimination from examination of Figure 3, page 31 of study [2] was approximately 22 hours in males, and 30 hours in females.

2) Metabolite Characterization and Identification Studies ([4], [5])

a. Preparative TLC of urine

Urinary metabolites of acetochlor at 10 and 200 mg/kg were isolated from 0-24 hour urine in male and female rats in study [4], while pooled urine from 0-72 hours in male and female rats

given a single oral dose of 200 mg/kg $^{14}$-C Acetochlor. Metabolites in both studies were resolved by thin layer chromotography, and tentative identification made based upon co-chromatography with authentic synthesized standards of acetochlor metabolites. Urine was also subjected to treatment with ß-glucuronidase/sulfatase to determine the presence of glucuronide and/or sulfate conjugates.

In study [4], chromatographic analysis showed at least 15 radiolabelled compounds and no unchanged acetochlor. Rats in the repeated low dose groups (10 mg/kg x 14 days) showed no apparent change in the profile of urinary metabolites compared to the single dose groups at this dose level, but quantitative differences in urinary metabolites were observed in urine from rats at the 200 mg/kg dose level. In study [5], at least 12 radiolabelled components were identified from tlc chromatograms (page 30 of study [5]).

The major urinary metabolite identified in both study [4] and [5] was confirmed by mass spectrometry following resolution by hplc as the mercapturic acid conjugate of N-de-ethylated acetochlor (see Figure 1, attached, and page 23 of study [4]). This metabolite was given the designation **H1and J9** in study [4] (based on the use of two tlc systems to resolve urinary metabolites), and **U9** in study [5]. A significant percentage of urinary radioactivity was identified as polar compounds. Information on the relative percentage of the metabolite H1 (J9) and polar compounds in urine at the various doses is summarized as follows (Table 3):

TABLE 3
Proportion of H1 (J9) and Polar Compounds in Urine of Male and Female Rats Treated with 10 or 200 mg/kg Acetochlor[a]

| | 10mg/kg | | 10mg/kg x 14 days | | 200mg/kg | |
|---|---|---|---|---|---|---|
| component | males | females | males | females | males | females |
| polars | 21.1 | 19.3 | 20.2 | 21.9 | 16.2 | 15.8 |
| H1 | 22.1 | 29.8 | 22.1 | 28.3 | 8.9 | 10.5 |
| J9 | 24.8 | 32.3 | 27.3 | 37.2 | 12.1 | 15.9 |
| polars (after enzyme incubation) | 12.8[b] | | | | | |

[a]data from Table 5, page 25 of study [4]. Results are expressed as % dose.
[b]enzyme incubation performed only on male rats at the 10 mg/kg dose level.

As shown above, metabolite H1(J9) accounted for between 22-32% of a dose of acetochlor in the urine at 10 mg/kg. The level of this metabolite was not significantly affected by repeated oral

dosing at 10 mg/kg. However, at the 200 mg/kg dose, the percentage of H1(J9) in the urine was decreased in male rats by 60%, and in and female rats by 65%. There was no other obvious increase in the percentage of other urinary metabolites to account for this decrease, with the exception of the appearance of a metabolite of acetochlor in which the terminal chlorine atom was replaced by a hydroxyl group. This metabolite represented 4% and 12% of the dose of acetochlor in urine at the 200 mg/kg dose level.

Incubation of urine with glucuronidase/sulfatase resulted in a decreased percentage of polar metabolites in urine (from 22% to 13%), indicating the presence of minor amounts of glucuronide conjugates in urine.

Remaining urinary metabolites of acetochlor were minor, constituting between 1-5% of the total dose (Table 5, page 25 of report [4]). Comparison of the total percentage urinary metabolites with urinary recovery of radioactivity showed that there was no significant amount of urinary radioactivity unaccounted for in metabolite analysis.

b. TLC of bile

The profile of biliary metabolites in male rats given a single oral dose of 10 or 200 mg/kg $^{14}$-C acetochlor is summarized below:

TABLE 4

Biliary Metabolites of Acetochlor[a]

| | 10mg/kg | | 10mg/kg x 14 days | | 200mg/kg | |
|---|---|---|---|---|---|---|
| component | males | females | males | females | males | females |
| B5 | 3.8 | - | - | - | 3.7 | - |
| B7 | 8.9 | - | - | - | 13.8 | - |
| B9 | 30.2 | - | - | - | 41.1 | - |
| B15 | 5.7 | - | - | - | 3.6 | - |

[a]data from Table 1, page 39 of study [5].

As shown, four metabolites were isolated from bile of male rats. Two metabolites, B9 and B7, appeared to constitute the major biliary metabolites at both doses of acetochlor, while metabolites B5 and B15 constituted a smaller percentage of biliary radioactivity. Remaining biliary metabolites were resolved into at least 10 minor components, each of which represented less than 5% of the total dose.

The major metabolite, B9, was confirmed as the O-glucuronide of de-ethylated acetochlor following derivatization with diazomethane, purification of this derivative by hplc, and confirmation by mass spectrometry (figure 7, page 36 of study [5]). Metabolites B7 and B5 were identified by mass spectrometry following purification as the glutathione conjugate of acetochlor (B7) and the de-acetylated glutathione conjugate of acetochlor (B5).

Metabolite B15 was identified by LC-MS as the mercapturic acid conjugate of acetochlor (Figure 1, attached).

c. TLC of fecal extracts

TLC quantitation of fecal extracts of acetochlor dosed rats at 200 mg/kg showed complex patterns of components which were difficult to identify even when co-chromatographed. In summary, ethyl acetate extracts contained mainly non-polar compoments, while acetonitrile:water extracts contained a high percentage of polar material.

Five bands (A-E) were distinguished in fecal metabolite analysis by tlc. Band A co-chromatographed with acetochlor, although two reference compounds (the sulphonylmethyl and thiomethyl derivatives of acetochlor) also co-chromatographed with band A. However, the total radioactivity of band A accounted for no more than 2% of the dose in 0-24 hour fecally excreted radioactivity.

Band B was not identified, and accounted for 3% and 4% of a dose of acetochlor in males and females, respectively.

Band C contained approximately 2% (male) or 1% (female) of a dose of acetochlor, and co-chromatographed with the sulphoxymethyl derivative (reference compound 13, page 23 of study [4].

Band D co-chromatographed with the cysteine conjugate of acetochlor formed after removal of the ethoxymethyl side chain (compound pictured above U9, Figure 1, attached).

Band E co-chromatographed with reference compound 16, the mercapturic acid conjugate of acetochlor and the major urinary metabolite, as described above for urine.

## IV. DISCUSSION

In this study, the disposition and metabolism of acetochlor was investigated in male and female rats. Data were presented in studies HRC/STR 88502 (study [1]), 89104 (study [2]), and 89407 (study [3]) demonstrating the absorption, distribution, and excretion of $^{14}C$- acetochlor in male and female rats at a single low oral dose (10 mg/kg), repeated low oral doses (10mg/kg x 14 days) and a single high oral dose (200 mg/kg). In studies HRC/STR 18/89603 (study [4]) and

CTL/P/2809 (study [5]), the biotransformation and identification of acetochlor metabolites in urine, bile, and feces was investigated in excreta samples from rats used in studies [1-3], or in rats administered single oral doses of 10 and 200 mg/kg (study [5]).

Absorption of a dose of $^{14}C$-acetochlor appeared complete at both 10 and 200 mg/kg by comparison of excretion data in bile duct cannulated and non-cannulated rats (studies [1-3] and [5]). While a significant percentage of $^{14}C$-acetochlor derived radioactivity was found in feces of rats (between 13-26% at the 10 mg/kg dose level, and between 26-37% at the 200 mg/kg dose level), it was demonstrated in bile duct cannulated rats that fecal excretion of $^{14}C$-acetochlor derived radioactivity was greatly diminished (between 3-7%, pages 45 and 46 of study [5]). Thus, fecal radioactivity was of biliary origin and did not represent unabsorbed acetochlor.

Excretion of a dose of $^{14}C$ acetochlor in urine was relatively rapid at the 10mg/kg dose level, with the majority of radioactivity (59-67%) eliminated in the urine by 24 hours. At the 200 mg/kg dose level, a lower percentage of $^{14}C$-acetochlor was eliminated in urine by 24 hours (43-51%), but a greater percentage was found in feces at this same time point. Although this altered pattern of elimination may be the result of enterohepatic recirculation of radioactivity eliminated in bile at the high dose, there was no apparent difference in the percentage of $^{14}C$-acetochlor derived biliary radioactivity eliminated at both doses (pages 45-46 of study [5]). In addition, examination of the time course of urinary elimination (Figure 1, page 31 of study [1] and page 29 of study [2]) shows that a much smaller percentage of $^{14}C$ acetochlor derived radioactivity was eliminated in urine between 0-12 hours. Thus, some delay in urinary elimination of $^{14}C$ acetochlor derived radioactivity is apparent. At all doses examined, the percentage of $^{14}C$ acetochlor derived radioactivity eliminated in urine of female rats was somewhat higher than that of male rats, with a corresponding decrease in fecal elimination (Table 2 of this review).

The concentration of $^{14}C$-acetochlor derived radioactivity in tissues examined at 120 hours showed that radioactivity was concentrated in those tissues receiving the greatest amount of cardiac output (heart, lungs, liver, kidneys, and spleen; Table 1). Few sex- or dose-dependent differences were noted, but at each dose level, the greatest concentration of $^{14}C$-acetochlor derived radioactivity was found in whole blood. This is reflected in the ratio of whole blood to plasma radioactivity, which exceeded 100 at all doses in both sexes. Thus, binding of acetochlor or a metabolite to red blood cells is extremely likely from review of these data. Identification of the chemical species responsible for binding was not performed in the present studies. However, it should be noted that conjugation with glutathione (see Table 3, above) is a major biotransformation pathway for acetochlor as shown by the profile of urinary metabolites, indicating the formation of a potentially reactive electrophilic species capable of such binding.

Metabolites of acetochlor in urine, feces, and bile were characterized and identified in studies [4] and [5]. From the results of these studies, the ethoxymethyl side chain and the chlorine atom of acetochlor were identified as major reaction sites for biotransformation. It is proposed, from analysis of acetochlor metabolites in bile and urine, that initial conjugation with glucuronic acid and glutathione occurs in liver, with subsequent excretion of the glucuronide conjugate (B9), the mercapturic acid conjugate (B15), and the glutathione conjugate of N-dealkylated acetochlor

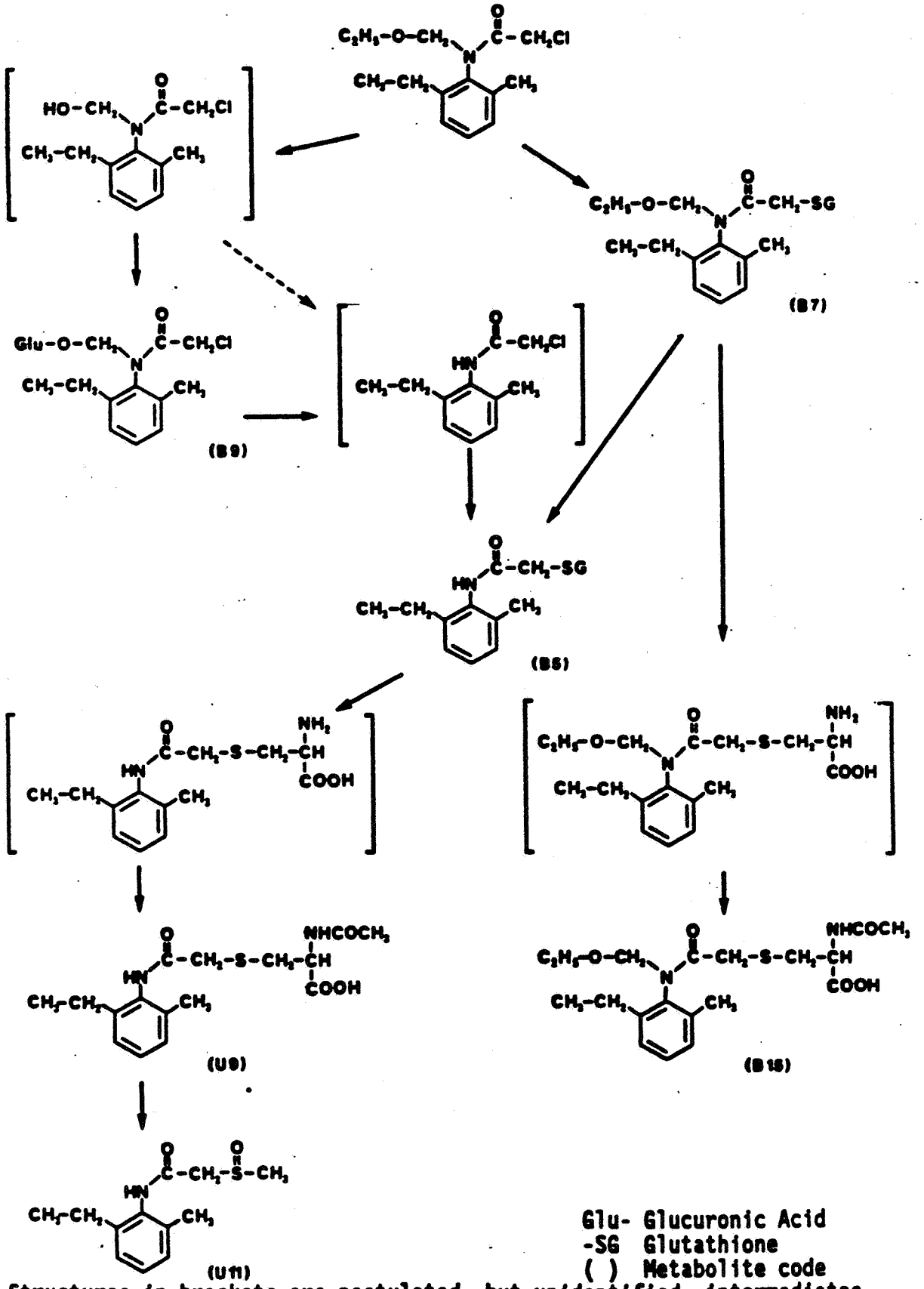
(B5) in bile. The two glutathione conjugates are reabsorbed, with further metabolism to the mercapturic acid conjugate which is excreted in urine. Since the amount of mercapturic acid metabolite in urine exceeded the combined amounts of glutathione conjugates in bile, the additional marcapturic acid found in urine is proposed to come from hydrolysis of the glucuronide conjugate in the gut, with subsequent reabsorption and metabolism to the glutathione conjugate which is then excreted in urine as the mercapturic acid conjugate.

The limited data presented on *in vivo* plasma kinetics of acetochlor demonstrated that the elimination of $^{14}C$-acetochlor derived radioactivity in plasma was at least biexponential (page 33 of study [1]), page 31 of study [2]). While it is possible to approximate the half life of elimination for $^{14}C$-acetochlor by inspection of the graphical data, the observation that acetochlor and/or a metabolite binds to red blood cells does not give much meaning to an evaluation, as $t_{1/2}$ would likely be different for data on whole blood levels of $^{14}C$-acetochlor derived radioactivity over time. The lack of intravenous data does not make it possible to distinguish which chemical species might be responsible for binding to red cells, as binding likely occurs prior to the peak plasma levels reported in studies [1] and [2]. The avid binding of $^{14}C$-acetochlor derived radioactivity to red blood cells makes it likely that volume of distribution for acetochlor is small (limited to total body water).

Core Classification: supplementary

This study does not satisfy the guideline requirements (85-1) for a metabolism study in rats. Justification for the omission of intravenous data is requested. In addition, percentage recovery from tissues and feces during processing is requested. As acetochlor is proposed for food use, the determination of the species bound to red blood cells may be toxicologically relevant. Purity of unlabeled acetochlor was also not stated in studies 1 through 3 and is requested.

ACETOCHLOR: BIOTRANSFORMATION STUDY IN THE RAT
FIGURE 9
Proposed Pathway for the Biotransformation of Acetochlor




Glu- Glucuronic Acid
-SG Glutathione
( ) Metabolite code

Structures in brackets are postulated, but unidentified, intermediates