004586

Study: The Metabolism of Acetochlor in the Laboratory Rat

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Sponsor/Contracting Lab.: Monsanto/Hazelton Raltech Inc.

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Reviewer: D. Stephen Saunders Jr., Ph.D. DSS 7/31/85 8/3/85

Test Compound

Homogeneous mixture of 12-C, 13-C, and 14-C-Acetochlor, lot no. 2179029, >98% a.i. Specific activity of 14-C label = 9.8 mCi/mmole.

Methods

The methods employed in this study were determined to be adequate by this reviewer. A photocopy of the submitted methods is attached to this review.

The study was conducted with Charles River CD SD rats, divided into four experimental groups: Group A received a single oral dose by gavage of 400 mg/kg radiolabeled acetochlor. Elimination of radiolabel by the pulmonary route was monitored in this group. Group B rats were given a single dose of 10 mg/kg, and Group C rats were given a single dose of 400 mg/kg. Group D received daily doses of 10 mg/kg for 14 days, followed by a single dose of 10 mg/kg of radiolabeled test substance. For all test groups, elimination of label was monitored for 7 days after the last dose.

Also, the structural formulas for several of the principal metabolites were identified by conventional analytical techniques.

Results

A. Excretion- Expired air was collected from animals of group A (400 mg/kg by gavage). These animals excreted an average of 0.04% of the administered dose over 7 days by exhalation. Because of the insignificant release by this route, expired air was not monitored in subsequent analyses.

In all of the treatment groups, acetochlor was rapidly excreted, as more than 70% of the administered dose was excreted within 48 hours (Table 1 of this review). The distribution of metabolites between urine and feces favored the fecal route in group B males (10 mg/kg single dose), however females excreted approximately equal amounts of label by either route. In contrast, animals from group C (400 mg/kg single dose) and group D (10 mg/kg repeated dose) excreted a larger proportion of the administered dose through the urine.

Table 1. Distribution of Excreted Dose (%)[a,b]

Group		Urine 0-2 days	Urine 0-7 days	Feces 0-2 days	Feces 0-7 days	Total 0-2 days	Total 0-7 days
B	Male	29.4	31.5	47.2	50.1	76.6	81.6
	Female	41.8	43.4	39.2	40.4	81.0	83.8
C	Male	42.5	46.7	26.0	28.9	68.5	75.6
	Female	46.5	49.7	24.7	26.6	71.2	76.3
D	Male	56.6	59.4	24.3	25.6	80.9	85.0
	Female	66.1	67.8	18.9	19.3	85.0	87.1

[a]data excerpted from submitted study.

[b]percent excreted days 0-2 calculated by reviewer.

Whole-body elimination of acetochlor was biphasic with a rapid and a slow phase. This type of excretion pattern is consistent with a two compartment model of distribution. Rapid excretion would be predicted from well-perfused organs such as heart, liver and kidney, and a longer half-life would be expected for excretion from tissues that do not receive as much blood flow such as fat. However, studies on tissue residues (see section C of this review) indicate that approximately 2.5% of the administered dose was associated with red blood cells, apparently due to binding to hemoglobin. The long half-life (approx. 180 hours) determined for the slow phase of excretion correlates with the half-life of red blood cell turnover in the rat. This fact led the investigators to speculate that the erythrocyte was the slow phase compartment. Repeated doses of acetochlor had little effect on the excretion kinetics as can be seen by comparisons of groups B and D. The half-lives for both the rapid and slow phases were about 50% longer for animals given the single high dose (group C) than for either of the low dose groups. This effect is consistent with saturation of metabolic enzymes of excretory mechanims. Kinetic data are presented in table 2.

Table 2. Kinetic Constants for Excretion of (14-C)-Acetochlor[a]

Group		t-1/2 (rapid)	t-1/2 (slow)
B	Male	7.1 hours	161.9 hours
	Female	5.8	182.4
C	Male	10.4	249.3
	Female	9.3	286.4
D	Male	7.1	128.6
	Female	5.4	186.3

[a]data excerpted from submitted study.

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B. Metabolic Pathway- Acetochlor was extensively metabolized, with less than 1% of the administered dose excreted unchanged into the feces and no unmetabolized acetochlor detectable in the urine. Approximately 20 different metabolites were characterized from urine and feces. The most common metabolites excreted at early time points (<24 hours) were mercapturates. At later time points the relative proportion of mercapturates decreased as the proportion of other sulfur-containing metabolites (sulfoxides, sulfones, sulfates) increased. Based on these data it is apparent that an early step in the metabolism of acetochlor is conjugation with glutathione. The proposed metabolic pathway for acetochlor and identified structures of metabolites (photocopied from the submitted study) are depicted in figures 68-70, appended.

C. Tissue Residues- The only tissue which retained significant amounts of radiolabeled acetochlor was the red blood cell. Acetochlor apparently bound covalently to hemoglobin, as determined by gel electrophoresis. The amount of label retained by other tissues was proportional to tissue mass and/or degree of perfusion. Relatively little label was retained in body fat, suggesting that bioaccumulation due to fat storage is not a factor with this compound. These data are depicted in table 3.

Table 3. Tissue Residues of 14-C Acetochlor[a]

	Group B		Group C		Group D	
Tissue	Male	Female	Male	Female	Male	Female
Brain	2743[b] (0.005)[c]	3268 (0.007)	15316 (0.006)	15429 (0.007)	2471 (0.003)	2809 (0.006)
Heart	20885 (0.024)	24000 (0.026)	107407 (0.026)	89873 (0.022)	29677 (0.027)	27209 (0.026)
Kidney	12466 (0.028)	13067 (0.025)	62500 (0.033)	65658 (0.032)	12491 (0.025)	[illegible]3 (0.022)
Liver	12342 (0.157)	13900 (0.134)	46094 (0.148)	50414 (0.135)	10881 (0.125)	10919 (0.104)
Lung	25035 (0.036)	24112 (0.033)	112409 (0.035)	113514 (0.040)	20839 (0.023)	18692 (0.027)
Spleen	27246 (0.019)	23256 (0.013)	131612 (0.018)	146087 (0.021)	25000 (0.012)	26800 (0.015)
GI tract	3209 (0.032)	2558 (0.026)	12440 (0.029)	13356 (0.032)	3467 (0.027)	2208 (0.020)
GI contents	1252 (0.061)	623 (0.025)	10420 (0.093)	5286 (0.052)	4710 (0.132)	812 (0.020)

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Table 3. Tissue Residues of 14-C Acetochlor (con't.)

	Group B		Group C		Group D	
Tissue	Male	Female	Male	Female	Male	Female
Eyes	ND -	ND -	8845 (0.0005)	8395 (0.0007)	ND -	ND -
Gonads	1602 (0.004)	ND -	6603 (0.005)	50480 (0.002)	1154 (0.002)	ND -
Fat	1314 (0.051)	1788 (0.069)	9744 (0.092)	11580 (0.110)	1125 (0.044)	1218 (0.047)
Muscle	2517 (0.273)	2110 (0.229)	9277 (0.245)	10640 (0.284)	1948 (0.212)	2072 (0.225)
Femur	2700 **	3314 **	14770 **	20670 **	3338 **	3566 **
Sternum	4872 **	5393 **	28960 **	29600 **	4298 **	4480 **
Whole blood	173000 (2.54)	166000 (2.45)	766000 (2.77)	811000 (2.95)	131000 (1.95)	142000 (2.10)
Plasma	684 **	660 **	3671 **	4684 **	1016* **	1012* **
Total % Retained	3.23	3.04	3.50	3.69	2.58	2.79

[a]data excerpted from submitted study.

[b]dpm/g tissue, calculated by reviewer from average organ weig.

[c]percent of administered dose.

ND = Not detectable

*data not included in Group D summary table, obtained by reviewer from raw data.

**total body mass of this tissue has not been estimated for the rat.

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Discussion and Conclusion

Acetochlor was rapidly eliminated from the rat, with >70% of the administered dose excreted within 48 hours for most of the groups. Animals given a single high dose (Group C, 400 mg/kg) or repeated low doses (Group D, 10 mg/kg for 15 consecutive days) appeared to preferentially eliminate radiolabel via the urine, with little difference between sexes. Group B males (single dose of 10 mg/kg) appeared to excrete more label in the feces than in urine, whereas females excreted approximately equal amounts in urine or feces. The kinetics of excretion were biphasic, with a rapid phase ($t_{1/2}$ = 5.4-10.4 hours) and a slow phase ($t_{1/2}$ = 128.6-286.4 hours). Animals given the high dose (400 mg/kg) had somewhat larger half-lives for both phases, consistent with saturation of metabolic enzymes and/or excretion mechanisms.

Acetochlor was extensively metabolized, with <1% of the administered compound excreted unchanged in the feces. An early step in the proposed metabolic pathway is conjugation with glutathione, and the majority of the excreted metabolites were mercapturic acid derivatives. The remainder of excreted metabolites were other sulfur-containing derivatives of acetochlor such as sulfates, sulfoxides and sulfones.

The only tissue which accumulated significant amounts of acetochlor was the red blood cell, which retained about 2.5% of an administered dose. This percentage was not dose-dependent (although the absolute amount retained obviously was) since similar percentages were retained by all three dosage groups. A slightly smaller percentage was retained by group D animals as compared to group B animals. This effect is consistent with competition for target receptor sites between labeled and unlabeled chemical, and induction of metabolic and/or excretory mechanisms. This conclusion is supported by the findings that group D rats, compared to group B animals, had slightly higher levels of radioactivity retained in the plasma in conjunction with a higher percentage of administered dose excreted in the urine at 2 days and a slightly higher percentage excreted overall at 2 or 7 days (Tables 1 and 3). The radioactivity was determined to be covalently bound to the hemoglobin fraction of the erythrocyte. Since a significant amount of label was bound even after 14 consecutive doses of unlabeled chemical (group D), these data suggest that a cumulative effect of acetochlor on red blood cell function is possible.

Classification: Core-Guideline