Project No. RP-7

Annual Report
Comprehensive Research on Rice
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Project Title: Molinate: A Metabolic Explanation for Species Differences in Susceptibility to Male Reproductive Toxicity.

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Objectives and Experiments Conducted to Accomplish Objectives:

Since rational assessment of risk and the ability to extrapolate between species is greatly aided by understanding the events underlying toxicity, this research has investigated the possibility that species differences in metabolism play a key role in determining species sensitivity to molinate induced male reproductive toxicity. The overall hypothesis is that a molinate metabolite (putatively molinate sulfoxide) is responsible for molinate toxicity and that rats readily form this toxic metabolite whereas human do not. As indicated in Figure 1, the hypothesis centers on the ability of a species to metabolize and clear a compound via non toxic metabolic pathways (hydroxy molinate) vs the ability to form a toxic metabolite (molinate sulfoxide). If humans are poor metabolizers via the toxic pathway and readily form nontoxic metabolites, then it is unlikely that they would be susceptible to molinate toxicity.

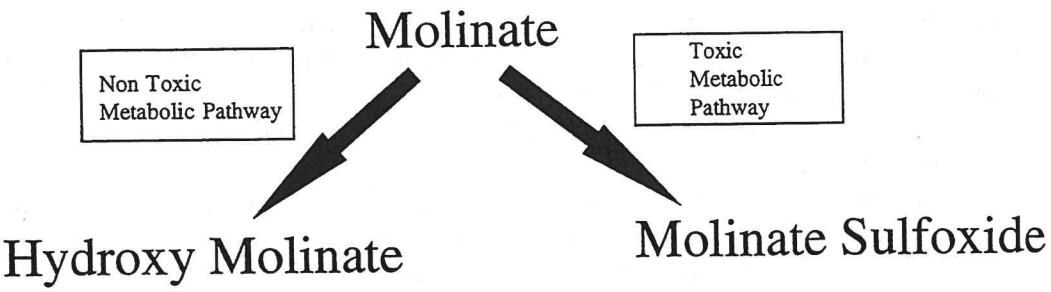


Figure 1. Proposed Toxic and Nontoxic Pathways of Molinate Metabolism

The **OBJECTIVES** of the research were :
1. To establish the rat as an animal model for molinate-induced testicular toxicity by describing testicular histopathology at various times after administration of molinate.
2. To determine whether molinate or a molinate metabolite is responsible for testicular toxicity by conducting in vivo metabolism and toxicity studies.
3. To compare molinate metabolism in human and rat liver preparations and, based on species differences in metabolism, predict species susceptibility to toxicity.

Summary of 1995 Research by Objective:

OBJECTIVE 1. Rat Model for Molinate-Induced Testicular Toxicity: Dose-Response and Time Course

In studies where a role for metabolism in toxicity is proposed, the simplest study design is to administer a single dose of toxicant allowing direct relationships to be drawn between metabolite formation and toxicity. However, single dose exposure usually requires administration of relatively high doses in order to give clear cut toxicity. These high doses are not meant to represent the situation found after environmental exposure, but the single dose model allows studies to be designed which can implicate a particular metabolic pathway in toxicity.

A first experiment to establish the time course of lesion development was carried out at a dose level of 400 mg/kg. Molinate (99% purity) was obtained from ChemService Inc.(West Chester, PA) and administered by the intraperitoneal route in 0.9% saline. Testes were evaluted histopathologically 2 days, 1, 2 and 3 weeks after molinate administration. At this high dose level, testicular toxicity was apparent even at the 2 day time point. The lesion was characterized by disorganization of developing germ cells and vacuolization of the seminiferous epithelium. However, some seminiferous tubules were unaffected. 1 week after toxicant administration, the disruption of the germ cell layer was extensive and damage was present in all tubules. The number of germ cells present was substantially decreased. The lesion progressed and was of equivalent severity 2 and 3 weeks after administration of molinate showing a Sertoli cell only tubule with very few germ cells. Further studies were designed to establish dose-response relationships and time course of the lesion for additional 100 and 200 mg/kg dose levels. With these lower doses, the onset of the lesion was delayed and the lesion was less severe.

OBJECTIVE 2. Molinate or a Molinate Metabolite as Testicular Toxicants.

Molinate can be oxidatively metabolized via cytochrome P450 enzymes to form either molinate sulfoxide or hydroxy molinate (De Baun et al., 1978). Further oxidative metabolism of the sulfoxide would form a sulfone. Both the sulfoxide and sulfone have chemical reactivity and could be involved in testicular toxicity. For this objective, molinate sulfoxide was viewed as the prime candidate for a toxic metabolite. Both molinate sulfoxide and sulfone could be detoxified by glutathione conjugation.

Molinate (100-400 mg/kg, ip) or molinate sulfoxide (50-200 mg/kg, ip) were administered to rats. Both compounds induced a qualitatively similar testicular lesion. However, the sulfoxide metabolite was 2-3 times more potent than the parent compound suggesting that the sulfoxidation pathway was involved in testicular toxicity. The lesion differed only in severity for the two compounds and was quite unique in character. No other testicular toxicant has been shown to produce a lesion of similar appearance. Intratesticular injection of the sulfoxide (220 μg/testis) produced only a minimal lesion, suggesting that the sulfoxide is not directly resonsible for toxicity. Interestingly, the degree of damage in the contralateral uninjected testis was similar to that seen in the testis which was directly injected with the sulfoxide. Overall, the data strongly implicates the sulfoxidation pathway as playing a role in molinate induced testicular toxicity. However, the sulfoxide itself does not seem to be the ultimate toxic metabolite. *In vivo* metabolism studies are, as yet, preliminary and have indicated that, in the rat, the sulfoxide is the major metabolite found in the blood after administration of molinate.

OBJECTIVE 3. Molinate Metabolism in Rat and Human Liver Preparations.

The objective here was to utilize *in vitro* preparations to compare the metabolic capabilities of rat vs. human liver preparations. The goal was to establish whether human metabolize molinate via the sulfoxidation pathway or via the nontoxic hydroxy molinate pathway. Liver microsomes contain high levels of the different cytochrome P450 enzymes which are likely to be responsible for formation of molinate sulfoxide and hydroxy molinate (Madan et al., 1995). Human liver microsomes were obtained from the archived collection of Dr Carol Green (SRI, Palo Alto, CA) and rat liver microsomes were prepared using routine centrifugation techniques and stored at -80°C. Microsomes were incubated with ^{14}C-molinate at 37 °C and metabolites were separated and quantitated by HPLC (Tjeerdema and Crosby (1987)).

Enzyme kinetic analysis of the data indicated classical Km.Vmax relationships for metabolite formation at different susbstrate concentrations. Lineweaver-Burk plots revealed excellent straight line patterns for formation of both metabolites in both species (Figs. 2 & 3). The intercepts of these lines reflect the reciprocals for Km (x-axis) and Vmax (y-axis) for the enzyme catalyzed formation of metabolites. These kinetic data are presented in Table 1. The Km represents the affinity of the enzyme for molinate and is defined as the substrate concentration where the velocity of the reaction proceeds at a half maximal rate. The lower the Km value the more readily the substrate is metabolized at low concentrations. The Vmax value indicates the maximal capacity of the enzyme to form a particular metabolite. The data indicates some very interesting differences between the human and rat liver microsomal preparations. While the Km values are not strikingly different, the differences in the Vmax values for the human and the rat would predict a substantially greater proportion of an administered dose of molinate proceeding down the sulfoxidation pathway in the rat. This is indicated by the high Vmax for sulfoxidation in the rat (1230 pmol/min/mg microsomal protein in the rat vs 632 pmol/min/mg microsomal protein in the human). Similarly, the human liver preparation had a three-fold greater capacity for formation of the nontoxic hydroxylated metabolite.

Figure 2

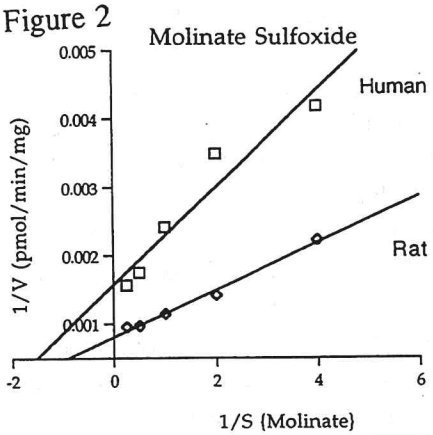
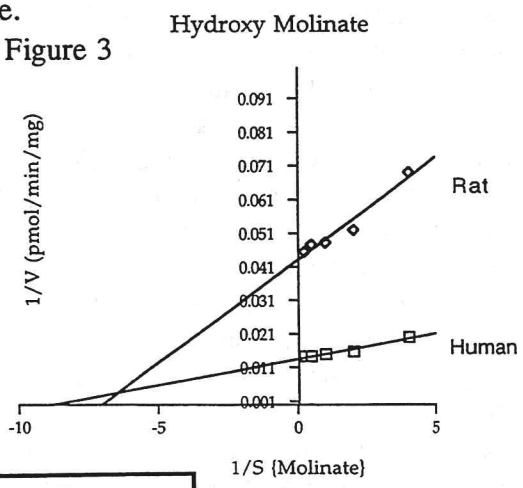


Figure 3



	Human		Rat	
	Km (um)	Vmax (pmol/min/mg)	Km (um)	Vmax (pmol/min/mg)
Sulfoxidation	450	632	422	1230
Hydroxylation	114	74.1	142	23.3

Table 1: Human vs Rat Km and Vmax comparisons

These data are encouraging but, due to the difficulty of establishing appropriate substrate concentrations for a kinetic study, results have been obtained for only one microsomal preparation in both species. However, the data points reflect triplicate incubations and are so reproducible that error bars are not discernible on the graphs. When this work progresses to liver slices, the kinetic analysis will be extended to include measurement of the ability of rat vs human liver to detoxify the sulfoxide and sulfone metabolites via formation of glutathione conjugates. Since glutathione transferase is primarily located in the cell cytosol, such studies cannot be carried out with microsomal preparations.

References

DeBaun, J.R., D. L. Bova, C.K. Tseng, and J.J. Menn. Metabolism of ^{14}C-Ordram (Molinate) in the rat. 2. Urinary metabolite identification. J. Agric. Food Chem., (26) 5, 1096-1104 (1978).

Tjeerdema, R., and D.G. Crosby. The biotransformation of molinate(Ordram) in the striped bass (*Morone saxatilis*). Aquatic Toxicol. 305-317 (1987).

Madan, A., A. Parkinson, and M.F. Faiman. Identification of the human and rat P450 enzymes responsible for the sulfoxidation of S-methyl N,N-diethylthiolcarbamate (DETC-ME). Drug Metab. Dispos. 23, 1153-1162 (1995).

Summary of 1995 Research (Major Accomplishments) by Objective:

The overall goal of this research is first to determine whether the rice herbicide, molinate causes testicular toxicity after metabolic activation to a toxic metabolite. Once this is determined, the relative ability of the rat animal model to form this toxic metabolite will be compared with the human in order to predict species differences in susceptibility to toxicity.

OBJECTIVE 1. Rat Model for Molinate-Induced Testicular Toxicity: Dose-Response and Time Course. A single dose animal model for the male reproductive toxicity has been developed. Time course for onset of the testicular lesion and dose-response relationships have been established. Although the single dose exposure requires administration of relatively high doses in order to give clear cut toxicity, this study design is necessary in order to draw direct relationships between metabolism and toxicity.

OBJECTIVE 2. Molinate or a Molinate Metabolite as Testicular Toxicants. The possibility that not molinate but molinate sulfoxide is responsible for testicular toxicity has been addressed. The data indicate that molinate is not the chemical species responsible for testicular damage. Molinate sulfoxide also does not appear to be the direct testicular toxicant, although the ultimate metabolite responsible for toxicity does seem to be generated through the sulfoxidation pathway.

OBJECTIVE 3. Molinate Metabolism in Rat and Human Liver Preparations. *In vitro* studies were designed to compare the metabolic capabilities of rat vs. human liver microsomal preparations, with the goal of establishing whether humans metabolize molinate via the toxic sulfoxidation pathway or via the nontoxic hydroxy molinate pathway. The data would predict that sulfoxidation capacity in the rat is substantially greater than in the human. Similarly, the human liver preparation had a three-fold greater capacity than the rat to form the nontoxic hydroxy molinate. These preliminary data predict the rat would be a species which is susceptibility to molinate induced male reproductive toxicity. Further work is necessary to make a more quantitative assessment of the relative susceptibility of the human to male reproductive damage.

Publications or Reports

Abstracts:

1. W.T. Jewell and M.G. Miller. Testicular toxicity in rats after administration of molinate: role of metabolism in toxicity. International Congress of Toxicology - VII, Seattle, WA. July 2-6, 1995.
2. W.T. Jewell and M.G. Miller. Role of metabolism in testicular toxicity of molinate (Ordram) in male rats. Society of Toxicology Annual Meeting, Anaheim, CA, March 10-14, 1996.

Concise General Summary of Current Years Results:

The overall hypothesis is that a metabolite of the rice herbicide, molinate, is responsible for the male reproductive toxicity previously reported after administration of molinate to rats. In the present study, it is proposed that rats readily form the metabolite responsible for toxicity whereas humans do not. A single dose animal model for molinate induced testicular toxicity has been established in rats with the goal of using this model to draw direct relationships between metabolism and toxicity. A molinate metabolite, formed through the sulfoxidation metabolic pathway, has been implicated as the chemical agent responsible for testicular damage. Preliminary *in vitro* studies using rat and human liver preparations indicate that, compared to the rat, the human has a greater capacity to form nontoxic metabolites of molinate and a lesser ability to form the toxic metabolite. Overall, these data support the hypothesis that the rat would be a susceptible species and that the human will be relatively insensitive to toxicity. Further work is necessary to make a more quantitative assessment of the relative susceptibility of the human to male reproductive damage.