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Development of PBPK model of molinate and molinate sulfoxide in rats and humans

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ABSTRACT

Molinate has been widely used as a pre-emergent herbicide in the rice fields of California's Central Valley. In rat studies, the metabolite molinate sulfoxide is suspected of causing testicular toxicity after exposure to molinate. The sulfoxide is generated in the liver and can circulate in the blood, eventually reaching the testis. Man qualitatively produces the same molinate metabolites as the rat. To extrapolate the reproductive risk to man, the present study outlines the development of a preliminary PBPK (physiologically-based pharmacokinetic) model, validation in the rat and extrapolation to man.

The preliminary seven-compartment PBPK model for molinate was constructed for the adult, male Sprague–Dawley rat that employed both flow-limited (blood, kidney, liver, rapid-perfused tissues and slowly perfused tissues) and diffusion-limited (fat) rate equations. The systemic circulation connects the various compartments. The simulations predict the molinate blood concentrations of the rat blood and testes compartment favorably with the profiles obtained from 10 and 100 mg/kg po or 1.5 and 15 mg/kg iv doses. Human physiological parameters were substituted into the oral dosed model and the simulations closely predicted the molinate blood concentration obtained from 5.06 mg oral dose. A sensitivity analysis determined for an oral dose that peak blood molinate concentrations were most responsive to the blood flows to kidney and fat compartments while testicular molinate sulfoxide concentrations depended on molinate sulfoxide partition coefficients for the testes compartment and the K_m for glutathione conjugation of molinate sulfoxide in the liver compartment.

1. Introduction

s-Ethyl hexahydro-1H-azepine-1-1carbothioate (MOLINATE) is pre-emergent herbicide used in the rice growing industry around the world. Primary occupational exposure routes include both dermal and inhalation for field workers exposed to the herbicide as well as the oral route from drinking water contaminated from agricultural runoff (Cochran et al., 1997; Tomenson et al., 1999). Molinate induces numerous biochemical, morphological, and toxicological responses including reproductive toxicities such as a distinctive sperm lesion and delayed release of the late spermatids to the seminiferous tubular lumen (Cochran et al., 1997; Ellis et al., 1998; Jewell et al., 1998; Berger and Miller, 2000; Kavlock and Cummings, 2005). Moreover in rat studies, high doses of molinate were shown to cause testicular toxicity and lower dose levels were associated with sperm abnormalities. Further, the testicular toxicity was related to formation of the molinate metabolite, molinate sulfoxide (Fig. 1).

Current understanding of the mechanism of molinate's reproductive toxicity implicates a sulfoxide metabolite generated in the liver that can circulate in the blood to reach the testis (see Fig. 1). The sulfoxide can be further metabolized to the sulfone, and theoretically both are reactive and capable of covalent protein binding and conjugation with glutathione ultimately appearing after further metabolism (Jewell and Miller, 1998) as mercapturates in the urine. The major urinary metabolites are the mercapturate, the hydroxy-molinate(s) and their respective glucuronides (DeBaun et al., 1978a,b). The covalent protein binding can impact both toxicity (a) by covalently modifying cysteine residues at the active site of enzymes and altering function (Zimmerman et al., 2002; Jewell and Miller, 1998) as well as (b) the kinetics and disposition of the sulfoxide (Campbell et al., 2008).

Presently, understanding of the molecular mechanisms which trigger reproductive effects of molinate is quite limited. One possible hypothesis suggests that once molinate is bioactivated to molinate sulfoxide, the sulfoxide is free to bind proteins that may be important to the production and activation of the testosterone or other biologically important molecules such as retinoic acid receptor which initiates a cascade of events leading to the effects. In sum, the mechanism of toxicity of molinate sulfoxide remains controversial.

Ellis et al. (1998) described a variety of toxic effects on the male rat reproductive system induced by molinate. Briefly, they found administration of molinate to rats (40 mg/kg/day for 7 days) caused a distinctive sperm lesion. Higher doses of molinate

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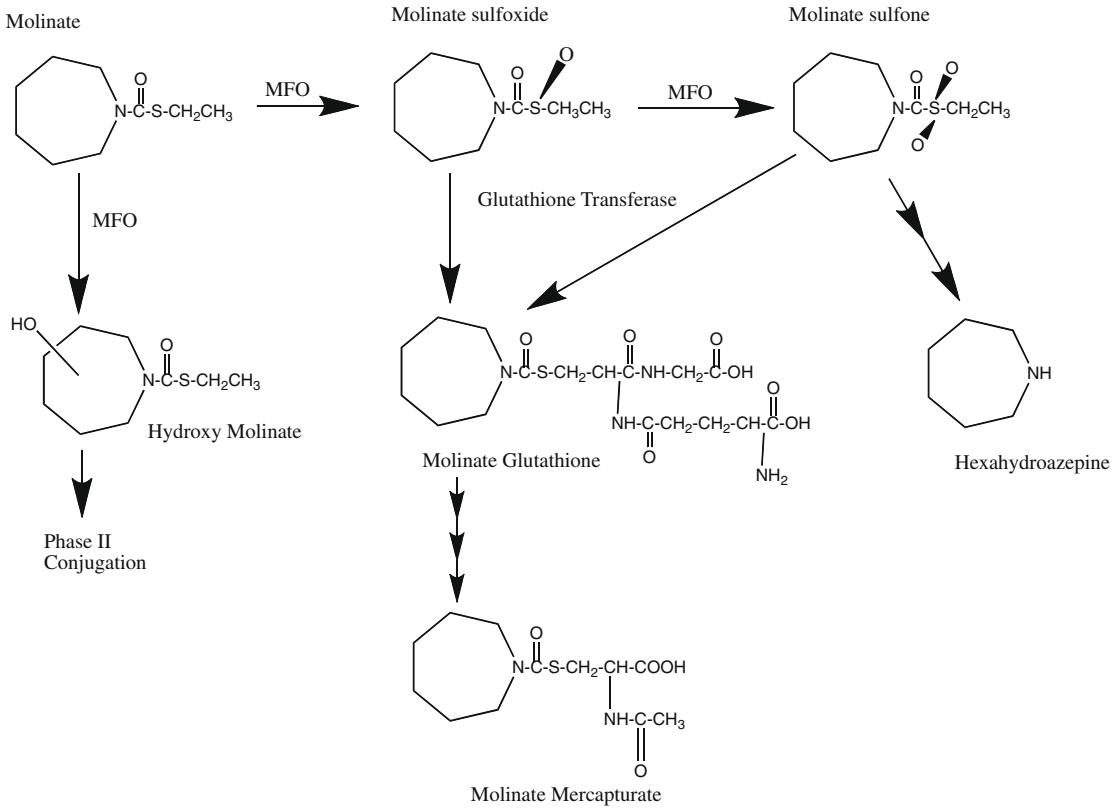

Fig. 1. Proposed pathway for molinate metabolism in rat and human.

(140 mg/kg for 7 days) produced morphological changes to the testis that included a delayed release of the late spermatids to the seminiferous tubular lumen. This is a process controlled by the release of testosterone, and by using [^{3}H]molinate, the primary target site appears to be the Leydig cells of the testis. Another important observation in rats dosed with molinate ($\geqslant$40 mg/kg) and molinate sulfoxide (>10 mg/kg) was the substantial decrease in both circulating and testicular testosterone concentration.

The morphological changes can be explained by an inhibition of Leydig cell function, including testosterone production, which is required for the maintenance of spermatogenesis. Since molinate sulfoxide inhibits general ester hydrolysis including neutral cholesterol ester hydrolase (nCEH) within the Leydig cells of the rat testis, cholesterol release would be prevented from its storage ester within this cell type. Therefore one explanation for the rodent's increased susceptibility to molinate testicular toxicity compared to man lies in the difference in major source of cholesterol; rodents utilize high-density lipoproteins (HDLs) in plasma that are hydrolyzed within the cell cytosol by nCEH (Gwynne et al., 1976) while conversely human obtain the majority of their cholesterol from low-density lipoproteins (LDLs) (Payne et al., 1985).

Several studies have examined the pharmacokinetics of molinate in rat, rabbit, monkey and man (Jewell et al., 1998; Dean, 1977; Lythgoe et al., 1992; Krieger et al., 1992; Batten et al., 1992). In this study we add to the preliminary pharmacokinetic studies plus several metabolism experiments are utilized to develop a Physiologically-based Pharmacokinetic model (PBPK), a tool that can be used to predict the distribution of molinate, determine target tissue molinate concentrations, and provide simultaneous tissue concentration versus time profiles for the various compartments in the model. Since certain chemicals (arsenic, benomyl, dioxin, dibromochloropropane) can cause toxicity at minute concentrations in experimental animals; linking the temporal relationship between dose, exposure, and response would be an important step towards accurately estimating the potential adverse risk to human health.

A PBPK model is a body composed of compartments, and each compartment contains mathematical descriptions of a chemical's absorption, distribution, metabolism, and elimination (ADME). Similar to conventional allometery, PBPK models provide a quantitative means of extrapolating ADME properties across species. The difference lies in the PBPK models ability to substitute species-specific physiological and biochemical parameters into the model. Thus by developing a PBPK model that can predict molinate concentrations in adult rat compartments the aim of this work was to extrapolate molinate blood and testes concentration predictions to humans.

In order for a pharmacokinetic model to successfully extrapolate between species the differences in (a) metabolism and kinetics and (b) physiological changes between species must be incorporated. Since rat and man qualitatively share the same pathway for molinate detoxification and bioactivation, an attempt was made to incorporate the appropriate metabolic and physiologic parameters that would optimize the interspecies prediction. Finally, since documented molinate administration to humans is rare, the opportunity to validate this model in humans is limited; therefore the utility of this model to predict human tissue concentrations is restricted to oral exposures.

2. Materials and methods

2.1. Chemicals

Molinate (s-ethyl hexahydro-1H-azepine-1-1carbothioate) was obtained from ChemService (Westchester, PA). It was 99% pure. Molinate sulfoxide was a gift from Dr William Helke, Syngenta, Greensboro, NC. Metabolite standards for molinate were generously provided by Zeneca Ag Products (Richmond, CA). Glycerol