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Development and application of a physiologically based pharmacokinetic model for triadimefon and its metabolite triadimenol in rats and humans☆

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ABSTRACT

A physiologically based pharmacokinetic (PBPK) model was developed for the conazole fungicide triadimefon and its primary metabolite, triadimenol. Rat tissue:blood partition coefficients and metabolic constants were measured *in vitro* for both compounds. Pharmacokinetic data for parent and metabolite were collected from several tissues after intravenous administration of triadimefon to male Sprague-Dawley rats. The model adequately simulated peak blood and tissue concentrations but predicted more rapid clearance of both triadimefon and triadimenol from blood and tissues. Reverse metabolism of triadimenol to triadimefon in the liver was explored as a possible explanation of this slow clearance, with significant improvement in model prediction. The amended model was extrapolated to humans using *in vitro* metabolic constants measured in human hepatic microsomes. Human equivalent doses (HEDs) were calculated for a rat no observable adverse effect level (NOAEL) dose of 3.4 mg/kg/day using area under the concentration curve (AUC) in brain and blood for triadimefon and triadimenol as dosimetrics. All dosimetric-based HEDs were 25–30 fold above the human oral reference dose of 0.03 mg triadimefon/kg/day, but did not account for intra-human variability or pharmacodynamic differences. Ultimately, derivations of this model will be able to better predict the exposure profile of these and other conazole fungicides in humans.

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1. Introduction

Conazoles (1,2,4 triazole and imidazole) constitute approximately 40 broad-spectrum fungicides with agricultural and pharmaceutical applications. The mechanism of fungicidal action is through the inhibition of 14α-lanosterol demethylase (Buchenauer, 1977), halting ergosterol synthesis and compromising the integrity and fluidity of fungal cell walls. This specific biosynthetic pathway is irrelevant in vertebrates; rather, lanosterol demethylase is involved in cholesterol synthesis. Thus, it is not surprising that conazoles have been implicated in the disruption of steroid biosynthesis in mammals (Zarn et al., 2003). Conazoles also modulate the expression and activity of enzymes, including many from the cytochrome P450 (CYP) family (Allen et al., 2006; Barton et al., 2006; Goetz et al., 2006; Ronis et al., 1994), and have been observed to elicit thyroid and liver tumors in rodents (Hurley et al., 1998).

Triadimefon is a lipophilic, non-volatile conazole fungicide with agricultural and ornamental applications. The U.S. Environmental Protection Agency (U.S. EPA) estimates an application rate of approximately 135,000 lbs/year with an upper-end estimate of 266,000 lbs/year (U.S. EPA, 2006). In vertebrates, triadimefon undergoes carbonyl reduction to its primary metabolite, triadimenol, a reaction catalyzed by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (Kenneke et al., 2008), which together with 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) is responsible for the regulation of the availability of active glucocorticoids to local steroid receptors (Seckl and Walker, 2001). Triadimenol retains the fungicidal activity of its parent, and is itself applied as a fungicide at a rate of 24,000 lbs/year (U.S. EPA, 2006). Exposures to triadimefon and its metabolite triadimenol occur most frequently via inhalation and dermal absorption for occupational and residential handlers, as well as through oral ingestion of contaminated food products or drinking water (U.S. EPA, 2006).

☆ The work presented here has been reviewed in accordance with the U.S. Environmental Protection Agency (U.S. EPA) peer and administrative review policies and approved for publication. Mention of trade names or commercial products does not constitute endorsement of recommendation for use.

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A range of toxicities have been observed subsequent to triadimefon and triadimenol exposure. Observed reproductive and developmental toxicity, as well as tumorigenicity, are held in common with other members of the conazole class. Teratogenic effects have been observed in both *in vivo* studies in mice at non-maternotoxic doses (300 mg/kg) of both parent and metabolite (Menegola et al., 2005a, 2005b) and *in vitro* studies in Sprague-Dawley rats; the latter support the hypothesized teratogenic mechanism involving triadimefon and triadimenol interaction with retinoic acid levels in the developing embryo (Di Renzo et al., 2009). Delayed reproductive development in Wistar rats born subsequent to maternal dietary exposure to 100 ppm, 500 ppm, or 1800 ppm triadimefon during gestation, parturition, and lactation has been attributed to the perturbation of steroid homeostasis (Goetz et al., 2007), as has reduced male/female sex ratio and female fertility index (FAO/WHO, 1985; Zarn et al., 2003). In chronic feeding studies (2 years), at doses of 50 ppm, 300 ppm, and 1800 ppm dietary triadimefon exposure in CF_1/W and NMRI mice caused a dose-dependent increase in liver abnormalities, with high dose groups showing an increase in liver adenomas (FAO/WHO, 2004). Unique among conazoles, triadimefon and its metabolite cause neurotoxicity, including hyperactivity and stereotyped (repetitive) behavior in rodents exposed to 50–400 mg/kg via oral gavage (Crofton, 1996). Regulation of environmental exposure to triadimefon relies on neurotoxicity as the sensitive endpoint, with an oral reference dose (RfD) of 0.034 mg/kg/day. This RfD is based on a sub-chronic feeding study performed by the manufacturer with a no observed adverse effects level (NOAEL) dose of 3.4 mg/kg/day in Wistar rats, with an applied uncertainty factor of 100. Hyperactivity was observed at higher doses (U.S. EPA, 2006). Neurotoxicity consisting of stereotyped behavior and hyperactivity has also been observed in Sprague-Dawley rats following single administration oral gavage of 50–200 mg/kg triadimefon. Analysis of the central nervous system in these animals suggested that the mechanism of triadimefon-induced neurotoxicity may involve alteration of monoamine (*e.g.*, dopamine and/or serotonin) metabolism (Walker et al., 1990; Crofton, 1996), likely as a monoamine oxidase inhibitor (Gagnaire and Micillino, 2006).

Chemical risk assessment is increasingly performed with the aid of physiologically based pharmacokinetic (PBPK) models, which facilitate interspecies extrapolations and the estimation of internal dose metrics (i.e. internal exposure to relevant tissues). Understanding the relationship between exposure and internal dose provides critical context to chemical risk assessment, allowing defensible comparisons between experimental data, such as that obtained in rodents or *in vitro* systems, with human data and exposure scenarios. Internal dose is an important surrogate for external exposure, which, because of myriad differences in physiology, may not be equivalent between organisms. These models provide a quantitative alternative to default uncertainty factors. Additionally, PBPK models often elucidate biochemical or physiological complexities, based on the adequacy or inadequacy of the information available to create the model and its subsequent predictive ability, and thus provide an excellent means for generating hypothesis-driven research (Clewell and Clewell, 2008).

Currently, there are no published PBPK models for the conazoles. Additionally, no kinetic time course data or partition coefficient values are available for triadimefon or triadimenol, and information on metabolism of these pesticides is very limited. The purpose of this research was to conduct *in vitro* and *in vivo* experiments to develop a PBPK model for triadimefon and triadimenol in Sprague-Dawley rats, and then to extrapolate the model to humans to estimate dosimetry-based oral human equivalent dose (HEDs) using a rat NOAEL value.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (280–320 g, approximately 8–9 weeks of age) were obtained from Charles River Breeding Laboratory (Raleigh, NC). Animals ($n = 66$) were housed in isolator cages in rooms maintained at 21 ± 2 °C and 50 ± 10% relative humidity with a 12-h light/dark cycle. Rats were given a minimum acclimation period of 7 days before experiments began. Lab Diet Certified Rodent Chow and water were provided *ad libitum*, except during exposure. The facility is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Georgia and studies were performed in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

2.2. Chemicals

Triadimefon, triadimenol, and myclobutanil (for use as an internal standard) were obtained from the EPA National Pesticide Standard Repository (Fort Meade, MD). Phosphate buffer was purchased from Sigma Chemical Co. (St. Louis, MO). Methyl-*tert*-butyl ether (MTBE) and acetonitrile purchased from Fisher Chemicals (Fair Lawn, NJ) were of analytical grade. Chemicals were dissolved in acetonitrile to create concentrated stock solutions.

2.3. Analytical

For measurement of both tissue and blood partition coefficient and *in vivo* pharmacokinetic data, quantification of triadimefon, triadimenol, and myclobutanil was accomplished using an HP 6890 Series Gas Chromatograph equipped with a 5973 Mass Selective Detector and an HP 6890 Series Injector (Hewlett–Packard, Palo Alto, CA). The injector and capillary transfer lines were set to 275 °C, the source to 230 °C, and the quadrupole to 150 °C. Splitless autosampler injection volume was 1 μl using an Agilent DB-5MS column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Santa Clara, CA), with helium carrier gas at a constant pressure (16 psi) and variable flow rate. The gas chromatograph oven temperature program began at an initial temperature of 50 °C (1 min initial hold), ramped to 175 °C at 27°/min, then to 250 °C at 5°/min, then 10°/min to 300 °C (10 min hold). The mass spectrometer was operated with the electron ionization source in selected ion monitoring (SIM) mode at m/z values of 128, 181, 208, and 210 (triadimefon, retention time 14.0 min); 112, 128, 168, and 208 (triadimenol diastereomers, retention time 15.4 and 15.6 min); and 179, 206, 245, and 288 (myclobutanil, retention time 17.1 min).

2.4. Experimental

Human *in vitro* metabolic parameters governing triadimefon reduction to triadimenol were measured as described previously, using hepatic microsomes (Crowell et al., 2010).

Partition coefficients were measured using methods adapted from Jepson et al. (1994). Rats were sacrificed by CO_2 asphyxiation followed by exsanguination. Blood was collected via the inferior vena cava and deposited in heparinized tubes, and liver, brain, kidneys, and peri-renal fat were also excised. Using a Tissue Tearor®, each tissue with the exception of blood was homogenized for 10 min with two volumes of phosphate buffered saline (PBS) to create stock tissue slurries. Blood was vortexed with one volume of PBS to create a blood slurry. These stock slurries, as well as all original tissues, were stored at −80 °C until use.

Partition coefficients were then determined by analyzing the relative concentrations of chemical in the saline fraction and the tissue fraction of a sample of slurry, comprised of a known quantity of saline and tissue (1 mL total volume). For liver, kidney, brain, and blood, 150 μL of the appropriate tissue slurry was added to 850 μL PBS. For fat, 50 μL slurry was added to 950 μL PBS. Each sample was vortexed and then spiked to the appropriate final concentration of triadimefon or triadimenol with concentrated stock solutions (total organic solvent, less than 1% v/v). High and low final concentrations were used (25 and 5 μM) to ensure there was no dependency of partitioning on concentration. Tissue-free controls were prepared identically to samples. The samples were then placed on a shaking incubator at 37 °C for three (blood, liver, kidney, brain) or six (fat) hours. A time course of samples incubated for various lengths of time (30 min–6 h) was performed to ensure equilibration (data not shown). Samples were incubated for the shortest time necessary to reach equilibrium to prevent tissue degeneration. Each sample was then centrifuged at 1500 × g for 30 min, and 700 μL (blood, liver, kidney, brain) or 850 μL (fat) of the saline supernatant drawn off to a new tube. Myclobutanil was added to both the tissue and saline fractions to a final concentration of 10 μM to act as an internal standard. Fractions were vortexed for 10 min to allow the internal standard to mix thoroughly.

MTBE was used to extract the chemicals from each fraction of each sample. For blood, liver, kidney, and brain, two volumes of MTBE were used to extract each fraction of each sample. For fat, two volumes of MTBE were used to extract the saline fraction, and four volumes of MTBE were used to extract the tissue fraction. The appropriate volume of MTBE was added to each sub-sample, and then vortexed for 30 min. Each sub-sample was then centrifuged at 1500 × g for 30 min, and the