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Estimation of placental and lactational transfer and tissue distribution of atrazine and its main metabolites in rodent dams, fetuses, and neonates with physiologically based pharmacokinetic modeling

Zhoumeng Lin [a,b], Jeffrey W. Fisher [c], Ran Wang [d,e], Matthew K. Ross [d], Nikolay M. Filipov [a,b,*]

[a] Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA
[b] Interdisciplinary Toxicology Program, University of Georgia, Athens, GA 30602, USA
[c] Division of Biochemical Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079, USA
[d] Center for Environmental Health Sciences, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, USA
[e] Institute of Food Safety, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

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ABSTRACT

Atrazine (ATR) is a widely used chlorotriazine herbicide, a ubiquitous environmental contaminant, and a potential developmental toxicant. To quantitatively evaluate placental/lactational transfer and fetal/neonatal tissue dosimetry of ATR and its major metabolites, physiologically based pharmacokinetic models were developed for rat dams, fetuses and neonates. These models were calibrated using pharmacokinetic data from rat dams repeatedly exposed (oral gavage; 5 mg/kg) to ATR followed by model evaluation against other available rat data. Model simulations corresponded well to the majority of available experimental data and suggest that: (1) the fetus is exposed to both ATR and its major metabolite didealkylatrazine (DACT) at levels similar to maternal plasma levels, (2) the neonate is exposed mostly to DACT at levels two-thirds lower than maternal plasma or fetal levels, while lactational exposure to ATR is minimal, and (3) gestational carryover of DACT greatly affects its neonatal dosimetry up until mid-lactation. To test the model's cross-species extrapolation capability, a pharmacokinetic study was conducted with pregnant C57BL/6 mice exposed (oral gavage; 5 mg/kg) to ATR from gestational day 12 to 18. By using mouse-specific parameters, the model predictions fitted well with the measured data, including placental ATR/DACT levels. However, fetal concentrations of DACT were overestimated by the model (10-fold). This overestimation suggests that only around 10% of the DACT that reaches the fetus is tissue-bound. These rodent models could be used in fetal/neonatal tissue dosimetry predictions to help design/interpret early life toxicity/pharmacokinetic studies with ATR and as a foundation for scaling to humans.

Introduction

Atrazine [ATR; 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine, CAS# 1912-24-9] is a chlorotriazine herbicide used extensively on crops to control broadleaf weeds (EPA, 2003). Due to its widespread use, relative persistence in water (ATSDR, 2003) and extreme persistence in soil (Jablonowski et al., 2009), ATR is ubiquitous in the environment (Battaglin et al., 2009).

Possible exposure sources for ATR include contaminated air (dust), food, and drinking water (García et al., 2012; Lozier et al., 2012; Mosquin et al., 2012). Surface and drinking water ATR concentrations (up to 224 and 34 μg/L, respectively) in places with heavy ATR use, such as the Midwestern U.S., substantially exceed current maximum contaminant levels (MCL), i.e., 3 and 0.1 μg/L in U.S. and Europe, respectively (ATSDR, 2003; Mosquin et al., 2012). According to the EPA guidelines for acute exposure risk assessment of ATR, the lowest observed adverse effect level (LOAEL), no observed adverse effect level (NOAEL), reference dose (RfD) and population adjusted dose (PAD) are 70,000, 10,000, 100 and 10 μg/kg/day, respectively (EPA, 2003). For the general population in the U.S., the estimated acute and chronic dietary exposures to ATR are 0.234–0.857 and 0.046–0.286 μg/kg/day, respectively, which is relatively low (Gammon et al., 2005). On the other hand, the exposure levels could reach up 151,000 μg per work shift for ATR manufacturing workers, indicating a much higher occupational overexposure risk (Catenacci et al., 1993).

ATR and/or its metabolites have been frequently detected in spot urine samples from pesticide applicators (ATR equivalents [ATR and

Abbreviations: ABC, ATP-binding cassette; ATR, atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine; AUC, area under the curve; BW, body weight; ^{14}C-ATR, ^{14}C-atrazine; DACT, didealkylatrazine, 2-chloro-4,6-diamino-1,3,5-triazine; DE, desethylatrazine, 2-chloro-4-amino-6-isopropylamino-s-triazine; DIP, desisopropylatrazine, 2-amino-4-chloro-6-ethylamino-s-triazine; GD, gestational day; LOAEL, lowest observed adverse effect level; MCL, maximum contaminant level; NOAEL, no observed adverse effect level; NSC, normalized sensitivity coefficient; PAD, population adjusted dose; PBPK, physiologically based pharmacokinetic; PC, tissue:blood partition/distribution coefficient; PND, postnatal day; RBC, red blood cell; RfD, reference dose; SRM, selected reaction monitoring.

* Corresponding author at: Department of Physiology and Pharmacology, University of Georgia, 501 D. W. Brooks Dr., Athens, GA 30602, USA. Fax: +1 706 542 3015.
E-mail address: filipov@uga.edu (N.M. Filipov).

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up to 8 identifiable metabolites, of which DE, DIP and DACT, abbreviations defined below, account for >85%]: 100–510 μg/L; detected in every sample; Barr et al., 2007), their families (ATR mercapturate: 0.024–4.9 μg/L, 27% positive samples) and the general population (ATR mercapturate: ≤3.8 μg/L [creatinine normalized]; 14% positive samples, Curwin et al., 2007), including pregnant women and young children (Chevrier et al., 2011; Curwin et al., 2007). Quantifiable levels of ATR (>0.05 μg/L) or one of its metabolites (ATR mercapturate: >0.02 μg/L) in pregnant women's first-morning-void urine have been associated with adverse birth outcomes, such as fetal growth restriction (Chevrier et al., 2011).

ATR and its metabolites have also been detected in plasma, urine and multiple tissues (including the brain, liver and kidney) of ATR-treated rodents (Fraites et al., 2011; Ross et al., 2009), in fetuses and in the milk of orally exposed rat dams (Fraites et al., 2011), in human umbilical cord plasma samples from residentially exposed, low risk, urban population (Whyatt et al., 2003), and in breast milk samples collected from a general population in France (Balduini et al., 2003). Thus, in utero and lactational exposures may be important routes for ATR to reach the developing fetus or neonate.

In the body, ATR is metabolized by several hepatic P450s (e.g., CYP2B1, CYP2D1, and CYP2E1; Hanioka et al., 1998a) fairly rapidly to desethylatrazine (DE; 2-chloro-4-amino-6-isopropylamino-s-triazine, CAS# 6190-65-4) and desisopropylatrazine (DIP; 2-amino-4-chloro-6-ethylamino-s-triazine, CAS# 1007-28-9), which, in turn, are metabolized to didealkylatrazine (DACT; 2-chloro-4,6-diamino-1,3,5-triazine, CAS# 3397-62-4), the major in vivo metabolite of ATR in mice (Ross and Filipov, 2006; Ross et al., 2009), rats (Brzezicki et al., 2003), and, apparently, humans (Barr et al., 2007; Fig. 1). During gestational and/or lactational stages, ATR is also extensively metabolized following a similar pattern (Fraites et al., 2011). Emerging evidence suggests that the metabolism of ATR is auto-inducing and is physiological stage-independent, i.e., short-term ATR exposure increases its own metabolism and/or the expression of ATR-metabolizing P450 isoforms in peripubertal (Pogrmic-Majkic et al., 2012), adult (Hanioka et al., 1998b), and pregnant and/or lactating rats (Fraites et al., 2011). However, there is still much unknown about the pharmacokinetic behavior of ATR in dams, fetuses, and neonates. Of note, while some such pharmacokinetic data are available in the rat (Fraites et al., 2011), there is no gestational of lactational pharmacokinetic study in the mouse, which is important for species comparison and extrapolation.

Developmental exposure of laboratory animals to higher levels of ATR (35–200 mg/kg) results in various adverse effects ranging from suppression of postnatal development to full-litter resorption (Narotsky et al., 2001; Rayner et al., 2005; Rooney et al., 2003). Of note, perinatal exposure of rodents to environmentally-relevant low doses of ATR causes neurobehavioral deficits (≥1 μg/kg) and structural brain changes (100 μg/kg) in the offspring (Belloni et al., 2011; Giusi et al., 2006), suggesting that the developing brain might be particularly sensitive to ATR. In terms of the effects of ATR's main metabolite DACT on the developing nervous system, in vivo studies do not exist at this point. However, our recent in vitro study suggested that DACT is less potent than ATR. Nevertheless, high concentrations of DACT disrupt dopaminergic neuron morphological differentiation (Lin et al., 2013). The studies described above highlight the potential of adverse effects of ATR overexposure on the developing fetus and neonate, the brain in particular. However, these studies do not correlate adverse effects with estimations of fetal or neonatal target tissue concentrations of ATR or its metabolites.

Risk assessment of ATR in sensitive subpopulations, including fetuses and infants, is limited by the scarcity of human pharmacokinetic data. Physiologically based pharmacokinetic (PBPK) models in rodents are useful tools that can aid the process because they can perform route-to-route, species, and dose extrapolations, as well as dose–response analysis. Fetal and/or neonatal rodent PBPK models have been developed for several other xenobiotics (Corley et al., 2003; Lu et al., 2012); these

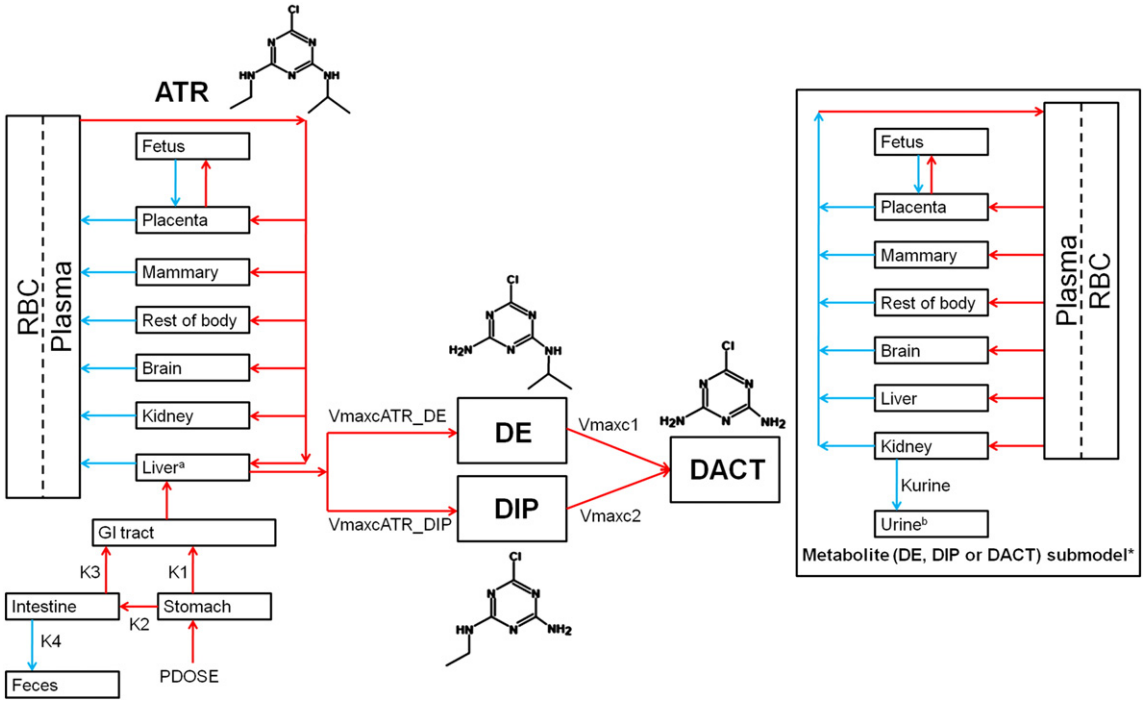

Fig. 1. A schematic diagram for a gestational PBPK model of ATR (atrazine) in the pregnant rat. ATR's major metabolites are: DE (desethylatrazine), DIP (desisopropylatrazine), and DACT (didealkylatrazine). The fetus is modeled as a single compartment. *The inset on the right shows the submodel for DACT; the submodels for DE and DIP are identical to DACT's submodel except that they do not include RBC or plasma protein binding in the blood compartment. The chemical structures of ATR, DE, DIP, and DACT are also included in the figure. [a] and [b] highlight representative novel modeling algorithms used in the present model: ([a]) exposure dependent P450-mediated autoinduction metabolism of ATR, DE and DIP in the liver compartment and ([b]) gestational stage-dependent urine elimination of metabolites. VmaxcATR_DE, VmaxcATR_DIP, Vmaxc1, and Vmaxc2 represent maximal metabolic rates from ATR to DE, from ATR to DIP, from DE to DACT, and from DIP to DACT, respectively, in naïve rats.