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Identification of fipronil metabolites by time-of-flight mass spectrometry for application in a human exposure study

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ABSTRACT

Fipronil is a phenylpyrazole insecticide commonly used in residential and agricultural applications. To understand more about the potential risks for human exposure associated with fipronil, urine and serum from dosed Long Evans adult rats (5 and 10 mg/kg bw) were analyzed to identify metabolites as potential biomarkers for use in human biomonitoring studies. Urine from treated rats was found to contain seven unique metabolites, two of which had not been previously reported—M4 and M7 which were putatively identified as a nitroso compound and an imine, respectively. Fipronil sulfone was confirmed to be the primary metabolite in rat serum. The fipronil metabolites identified in the respective matrices were then evaluated in matched human urine ($n = 84$) and serum ($n = 96$) samples from volunteers with no known pesticide exposures. Although no fipronil or metabolites were detected in human urine, fipronil sulfone was present in the serum of approximately 25% of the individuals at concentrations ranging from 0.1 to 4 ng/mL. These results indicate that many fipronil metabolites are produced following exposures in rats and that fipronil sulfone is a useful biomarker in human serum. Furthermore, human exposure to fipronil may occur regularly and require more extensive characterization.

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1. Introduction

Fipronil (Fig. 1) is a phenylpyrazole broad-spectrum insecticide that is registered for use in residential settings as part of ant and cockroach baits and gels and termite control products; veterinary applications such as spot treatment flea and tick control products for dogs and cats; ornamental turf applications such as fire ant control; and agricultural applications such as pest control on potato crops (Brassard et al., 2011). When initially produced, fipronil was the first insecticide to act by targeting the gamma-aminobutyric acid (GABA) receptor and has favorable selective toxicity towards insects rather than mammals (Hainzl and Casida, 1996; Ikeda et al., 2004; Hainzl et al., 1998).

A 1997 report indicated that 480-metric tons of fipronil was produced per year by Rhône-Poulenc (1997), and a more recent EPA report indicated that between 1998 and 2008 usage averaged 68,039 kg of active ingredient per 607,028 ha (Brassard et al., 2011). Widespread fipronil use has led to contamination of water and soil (1–158 ng/L of parent or environmental degradate) in several states including, but not limited to Alabama, Georgia, California, Louisiana, and Indiana (Ryberg et al., 2010; Gunasekara et al., 2007). Perhaps as a result of this contamination, fipronil has been implicated as one of the chemicals associated with the bee colony collapse (Erickson, 2013).

Because little was found in the peer-reviewed literature about the disposition of fipronil, Cravedi et al. (2013) performed a thorough study on the metabolism, distribution, and elimination of fipronil in rats and showed that fipronil is primarily converted to fipronil sulfone (M1 Fig. 1), a more persistent metabolite (estimated half-life is 208 h in rodents) (Mohamed et al., 2004) which was stored mainly in adipose tissue and adrenals (Cravedi et al., 2013). In addition, fipronil has been associated with thyroid disruption (Tingle et al., 2003), endocrine disruption (Ohi et al., 2004), and neurotoxic effects (Raquel et al., 2011) in rats which has led to concern about the potential for human health effects.

Abbreviations: DI, deionized; ESI, electrospray ionization; GABA, gamma-aminobutyric acid; GSD, geometric standard deviation; HPLC, high performance liquid chromatography; LC, liquid chromatography; LLOQ, lower limit of quantitation; MS, mass spectrometry; NIEHS, National Institute for Environmental Health Sciences; QC, quality control; Q-TOF, quadrupole time-of-flight; %RSD, Percent Relative Standard Deviation; SD, standard deviation; SPE, solid phase extraction; TOF, time-of-flight; UPLC, ultra performance liquid chromatography; US EPA, United States Environmental Protection Agency.

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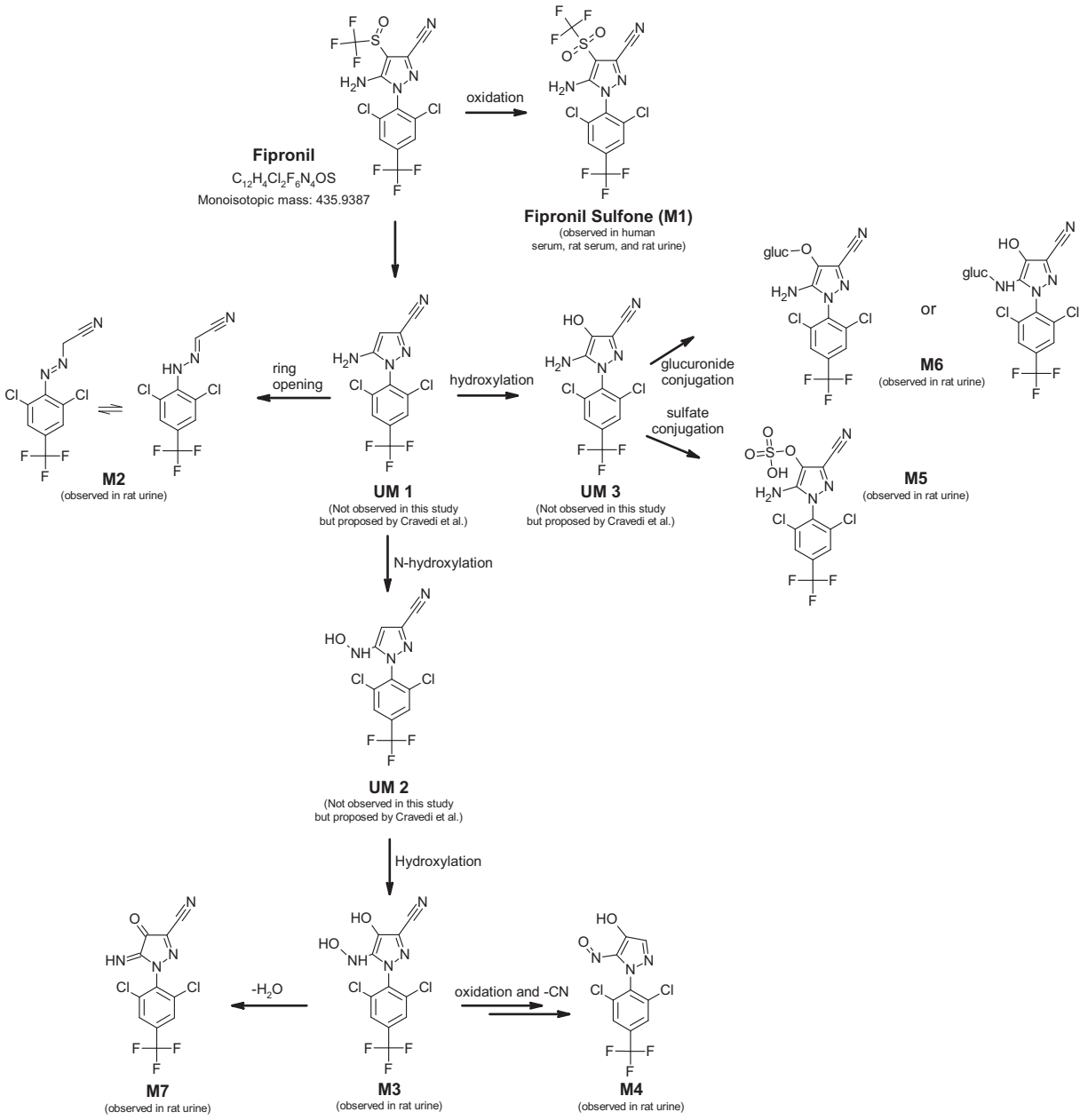

Fig. 1. Proposed metabolic pathway of fipronil in the rat. M4 and M7 are proposed structures based on MS data, isotope distributions, and exact mass. M1, M2, M3, M5, and M6 were identified in rat urine. Unobserved metabolites labeled (UM) were not identified but are likely intermediates.

The effects of acute human exposure to fipronil include headache, dizziness, vomiting, and seizures (Mohamed et al., 2004; Cravedi et al., 2013). Information on the effects of chronic exposure is limited, but the US EPA has classified fipronil as a possible human carcinogen based on data that shows an increase of thyroid follicular cell tumors in both sexes of the rat (Anon, 1996). Vidau et al. (2011) also concluded that fipronil has the potential to cause apoptosis by uncoupling oxidative phosphorylation at relatively low concentrations (5-10 μM) in human cell lines (Vidau et al., 2011), and a case of acute human self-poisoning with fipronil has demonstrated that fipronil levels can remain elevated in serum for days after exposure, and that fipronil sulfone was a primary metabolite (Mohamed et al., 2004). A previous study also showed that fipronil sulfone is the predominant metabolite in human liver microsomes via cytochrome P-450 oxidation (Tang et al., 2004).

Although one occupational exposure study of workers ($n = 159$) at a fipronil production facility reported a mean fipronil sulfone serum level of 7.8 (+/- 7.7 = SD) ng/mL (Herin et al., 2011), very little is known about human exposure to fipronil in the general population (Mohamed et al., 2004; Vidau et al., 2011; Herin et al., 2011). This may be because human samples can be difficult to obtain and analyze due to high concentrations of endogenous chemicals and significant matrix effects which make the identification of metabolites difficult. Literature on the potential routes of human exposure includes one article by Dyk et al. that describes the potential for non-occupational human exposure through contact with pets that have received fipronil applications in the form of flea and tick treatments (Dyk et al., 2012), and a few other studies that have observed fipronil in various environmental media relevant to human exposure (indoor/outdoor dust Mahler et al., 2009, wastewater Stone et al., 2014, surface water Ryberg et al., 2010; Stone et al., 2014 and residential runoff Gan et al., 2012).

The specific objectives of the study were to characterize human exposure by developing a unique workflow where dosed animal samples were used to identify potential serum/urine biomarkers via time-of-flight mass spectrometry. These biomarkers were