DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

Preclinical Transplacental Transfer and Pharmacokinetics of Fipronil in Rats

Ya-Ning Chang, Tung-Hu Tsai*

Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan (Y.N.C., T.H.T.); Graduate Institute of Acupuncture Science, China Medical University, Taichung, Taiwan (T.H.T.); School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan (T.H.T.); Department of Chemical Engineering, National United University, Miaoli, Taiwan (T.H.T.).

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: Transplacental Transfer of Fipronil in Rats

Corresponding author:

Tung-Hu Tsai, Postal Address: Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, No.155, Li-Nong Street Section 2, Taipei, 112 Taiwan, Tel: +886-2 2826 7115, Fax: +886-2 2822 5044, E-mail: thtsai@ym.edu.tw

The number of text pages: 37

number of tables: 2

number of figures: 2

number of references: 47

number of words in the Abstract: 247

number of words in the Introduction: 658

number of words in the Discussion: 1735

ABBREVIATIONS: RT: retention time; $t_{1/2}$, elimination half-life; T_{max}, time to reach maximum plasma concentration following drug administration; C_{max}, maximum plasma drug concentration; AUC_{last}, area under the plasma concentration-time curve from time zero to the time of the last measurable concentration; AUC_{∞}, area under the plasma concentration-time curve from time zero to infinity; Cl, apparent total body clearance of the drug from plasma; MRT_{∞}, mean residence time from time zero to infinity; C_{nom}, nominal concentration; ME, matrix effect.

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

Fipronil, a widely used insecticide and pesticide, with its toxic metabolite fipronil sulfone was detected in fipronil-contaminated eggs due to inappropriate use. However, little was known about whether fipronil and fipronil sulfone transferred into fetus through the blood placenta barrier. Our objectives were to investigate the transplacental transfer and the pharmacokinetics of fipronil and fipronil sulfone in rats. Male and 13-day gestation female Sprague-Dawley rats were used in pharmacokinetics and transplacental transfer experiments, respectively. Biological samples were collected at each time point after fipronil intravenous or oral administration. To monitor fipronil and fipronil sulfone in the plasma, placenta, amniotic fluid, and fetus, a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed. After fipronil administration in male rats, the oral bioavailability decreased while the biotransformation increased as the dose increased, revealed an enhancement of first-pass effect and a fast metabolism in vivo. The results of fipronil transplacental transfer in pregnant rats demonstrated that the concentration of fipronil and fipronil sulfone varied in the following order respectively: placenta > plasma > fetus > amniotic fluid and plasma > placenta > fetus > amniotic fluid. This is the first direct evidence that fipronil and fipronil sulfone cross the blood placental barriers and enter the fetus. The amount of fipronil distributed to the fetus was greater than that of fipronil sulfone in the short term, but by contrast, pharmacokinetic data showed that the latter stayed longer in the body. These findings provide constructive information for public health alarm.

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

SIGNIFICANCE STATEMENT

Fipronil and fipronil sulfone interfere with the GABAergic system. Fipronil can cause thyroid dysfunction, which may affect brain growth and nerve development. Although we knew that fipronil and fipronil sulfone could enter eggs, there was no direct evidence that they would enter fetuses. This research provided evidences on the pharmacokinetics and transplacental transfer of fipronil and fipronil sulfone, confirming our hypothesis.

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Fipronil, a member of the phenyl-pyrazole chemical family, is commonly used as an insecticide and pesticide to eliminate fleas, lice and ticks (Tingle et al., 2003). It is a gamma-aminobutyric acid-gated channel and glutamate-gated chloride channel antagonist (Cole et al., 1993; Horoszok et al., 2001). Due to its low resistance potential, high selective toxicity to arthropods (Cole et al., 1993), and long persistence in the environment (Bobé et al., 1998), fipronil occupies approximately 10% of the global pesticide market. Although fipronil is selectively toxic, it still exhibits adverse effects to multiple target organisms and threatens human health. Human exposure may lead to acute poisoning, including headaches, dizziness, sweating, nausea, vomiting, agitation, and seizures (Mohamed et al., 2004; Lee et al., 2010). U.S. Environmental Protection Agency also reported that fipronil shows acute toxicity, carcinogenicity, neurotoxicity, endocrine disruption reproductive toxicity and developmental toxicity (JMPR, 1997). Fipronil has been classified as a Class II moderately hazardous pesticide by the World Health Organization (WHO). In addition, fipronil sulfone, the major metabolite of fipronil, is more toxic than fipronil itself in the gamma-aminobutyric acid- and glutamate-activated chloride channel systems (Hainzl et al., 1998; Zhao et al., 2005). Previous pharmacokinetic and metabolism studies have indicated that fipronil primarily converted into fipronil sulfone, which persisted for a much longer time and stored mainly in adipose tissue and the adrenal glands within the body (Mohamed et al., 2004; Cravedi et al., 2013).

Although there are only a few studies on this subject, fipronil seems to affect the

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

reproduction and development of the fetus. The developmental toxicity studies of fish demonstrated that fipronil exposure in the embryonic stage results in deforming and sub-lethal effects in Japanese Medaka (Wagner et al., 2017) and impairs the development of spinal locomotor pathways in zebrafish (Stehr et al., 2006). For offspring development in rats, prenatal exposure to fipronil affects the reflex development including negative geotaxis reflex delayed and early loss of palmar grasp, suggesting the interference in the GABAergic system during brain maturation (Udo et al., 2014). In female rats, fipronil interferes with the development of the neonatal female reproductive system, which is evidenced by delay of vaginal opening and estrus cycle alteration (de Barros et al., 2016). In the reproductive system of male rats, perinatal fipronil exposure changes sperm motility by decreasing motile spermatozoa and increasing nonmobile spermatozoa, indicating that the epididymis may be a target organ of fipronil (de Barros et al., 2017). In addition, fipronil can cause thyroid disruption (Leghait et al., 2009; Herin et al., 2011; Roques et al., 2012) and thyroxine is closely related to brain maturation (Bernal, 2007; Anderson, 2008) and nerve development during fetal growth (Cuevas et al., 2005). Functional disorders caused by thyroxine deficiency, such as vision, motor skills, language and memory, varies as the gestation progresses (Zoeller and Rovet, 2004).

In 2017, a company blended fipronil, which was prohibited from using in food-producing animals by either the European Medicines Agency in Europe or the US EPA, into a proprietary natural cleaning product DEGA-16. This illegal product was sold to chicken farms, resulting in repeated exposure of poultry to fipronil (Stafford et al., 2018). This fipronil-contaminated egg event was extended to several European and Asian countries, including Taiwan, and caused wide spread panic. In a previous study, fipronil

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

sulfone was detected in cord blood, which may be due to chronical fipronil exposure (Kim et al., 2019). However, little is known about the transplacental transfer of fipronil and its pharmacokinetic mechanism, so it is important to determine the possible mechanisms of absorption, distribution, metabolism and excretion for the insecticide fipronil. Our hypothesis is that fipronil and its metabolite fipronil sulfone may penetrate the blood placental barrier into the fetus. The aim of this study is to develop a valid method using liquid chromatography tandem mass spectrometry (LC-MS/MS) for the analysis of fipronil and fipronil sulfone in the plasma, placenta, amniotic fluid and fetus to determine the pharmacokinetics, oral bioavailability and transplacental transfer in rats.

Materials and Methods

Chemicals and Reagents. Fipronil (purity higher than 99% by HPLC) and fipronil sulfone (purity higher than 99% by HPLC) were purchased from Toronto Research Chemicals (Toronto, ON. M3J 2J8 Canada) and sorafenib (BAY 43-9006; purity higher than 99% by HPLC) was provided by Bayer® Pharmaceutical Co., Ltd. (Kaiser-Wilhelm-Allee, Leverkusen, Germany). The acetonitrile from J.T. Baker (Phillipsburg, NJ, USA), methanol from Macron (Hamilton, PA, USA) and triply deionized water from Millipore (Bedford, MA, USA) were used in all experiments. Pentobarbital sodium and heparin sodium were obtained from Sigma-Aldrich (St. Louis, MO, USA).

LC-MS/MS. The LC-MS/MS system consisted of a triple-quadrupole mass spectrometer (LCMS-8030; Shimadzu, Kyoto, Japan) equipped with an electrospray

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

ionization interface coupled to an LC system (Shimadzu LC-20AD $_{XR}$). The LC system was equipped with two pumps, a system controller, an autosampler, a column oven, and an online degasser. Chromatographic separation was carried out at 40 °C on a Merck Purospher® STAR RP-18 endcapped (2.1 × 100 mm, 2 μm) with an isocratic elution of acetonitrile/methanol (6:1, v/v) – water (68:32) at a flow rate of 0.3 mL/min. The temperature of the autosampler was 4 °C, and the injection volume was 4 μL. The mass spectrometer was operated in ESI- with multiple reaction monitoring (MRM) scan mode. The interface voltage was 3.5 kV; nebulizing gas (nitrogen) flow was 3.0 L/min; drying gas (nitrogen) flow was 15.0 L/min; dissolution line temperature was 250 °C; heat block temperature was 400 °C; and collision gas (argon) pressure was 230 kPa.

Method Validation. Fipronil and fipronil sulfone were dissolved in acetonitrile at a concentration of 1 mg/mL each and then mixed together to make a standard solution (500 μg/mL). The standard solution was diluted into several individual Eppendorf tubes with a paraffin film wrap as a stock solution (10 μg/mL), which was further diluted to give a series of working standard solutions. The internal standard (1 μg/mL) was prepared by diluting sorafenib, which was dissolved in acetonitrile and stored in several individual Eppendorf tubes wrapped with paraffin. All of the solutions were stored at −20 °C.

Calibration curves were prepared by adding the working standard solutions into blank male rat plasma to give calibration concentrations of 1, 5, 10, 50, 100, and 500 ng/mL; in blank pregnant rat plasma, amniotic fluid, placenta homogenates or fetus homogenates, the calibration concentrations were 2.5, 5, 10, 50, 100, and 500 ng/mL. The calibration curve was constructed from the ratio of the peak areas of fipronil or fipronil sulfone and the internal standard to the nominal concentration of fipronil or fipronil

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

sulfone. Linearity was evaluated by the correlation coefficient (r^2) and a value of at least 0.995 was considered to be acceptable.

Six replications of the calibration curve were performed on the same day (intraday) and over six consecutive days (interday) to evaluate the precision and accuracy. Accuracy describes the closeness of the mean results (observed concentration, C_{obs}) of this method to the true concentration (nominal concentration, C_{nom}). Accuracy, quantified as relative error (RE), was calculated as RE (%) = $[(C_{obs} - C_{nom})/C_{nom}] \times 100\%$. Precision is the proximity of each individual result to the others. Precision, quantified as the correlation of variation (CV), was calculated as follows: CV (%) = [standard deviation (SD)/C_{obs}] × 100%. The relative error and coefficient of variation were maintained within ± 15%, except for the lower limit of quantification (LLOQ), which was not permitted to exceed ± 20%.

The matrix effects and recovery were calculated by three sets of samples. Fipronil and fipronil sulfone were evaluated at 1, 50, and 500 ng/mL in male samples, 2.5, 50, and 500 ng/mL in female samples, and sorafenib was evaluated at 50 ng/mL. For Set 1, working solutions of fipronil, fipronil sulfone and internal standard were diluted with acetonitrile. For Set 2, blank plasma or tissue homogenate was processed as described under sample preparation without the drug to obtain blank matrix followed by the addition of the working solution and internal standard, giving post-extraction spiked fipronil and fipronil sulfone samples. For Set 3, working solution and blank plasma or tissue homogenate were mixed and subjected to sample preparation, obtaining pre-extraction spiked fipronil and fipronil sulfone samples. Then, all samples were injected to the LC-MS/MS system for analysis.

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

The matrix effect was determined by comparing the peak area ratio of the post-extraction spiked samples (Set 2) to that of the standard solution samples (Set 1). The recovery was quantified as the peak area ratio of the pre-extraction spiked samples (Set 3) to that of the post-extraction spiked samples (Set 2).

Experimental Animals and Drug Administration. The animal experimental protocol listed below was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC; approval number 1070525) by the Institutional Animal Experimentation Committee of the National Yang-Ming University and was consistent with the guidelines of the National Research Council, USA. Male Sprague−Dawley rats (230 ± 20 g) and female Sprague-Dawley rats (350 ± 30 g) with 13 days of gestation were used in the bioavailability and transplacental transfer experiments, respectively, and were purchased from the National Yang-Ming University Animal Center, Taipei, Taiwan. Laboratory rodent diet 5001 (PMI Feeds, Richmond, IN, USA) was used as food. Rats were housed with a 12 hr light/dark photoperiod cycle and given *ad libitum* access to water.

Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). A polyethylene tubing filled with heparinized saline (100 units/mL) was inserted into the right jugular vein, emerging from the back of the neck and guided through a protective cap for fixation. After surgery, the rats were allowed to rest and recover in a clean cage overnight before drug administration. After the stabilization period, fipronil was administered (1 mg/kg, i.v.; 3, 10 or 30 mg/kg, p.o.) to the rats (n = 6 for each group). Collection of the blood samples was divided into two sets of time points. One set was 5, 15, 30, 45, 60, 90, 120, 150, 180, 240, and 360 min after intravenous administration of fipronil, and the other was

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16 and 24 hr after oral administration of fipronil. At each time point, 200 μL of blood was drawn into heparin-rinsed Eppendorf tubes and then centrifuged at 13000 rpm for 10 min at 4 °C to obtain plasma. Plasma was stored at −20 °C until analysis.

Pregnant rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and remained anesthetized as needed throughout the experimental period. The left femoral vein was catheterized with polyethylene tubing for drug administration. The laparotomy incision was covered using gauze immersed in warm saline. To investigate the transplacental transfer of fipronil and fipronil sulfone, fipronil was administered intravenously via the femoral vein by bolus injection at a dose of 10 mg/kg (n=6). Biological samples, including maternal blood, placenta, amniotic fluid and fetus from one single uterus of a dam were collected at each time point at 15, 30, 60, 120, 180, 240, 300 and 360 min after drug administration. Collected placenta and fetus samples were weighed immediately, and all samples were stored at −20 °C until further sample preparation.

Sample Preparation. The placenta or fetus was homogenized with a two-fold amount of 0.9% normal saline (w/v) using a Polytron PT 2100 homogenizer (Kinematica, Lucerne, Switzerland). The homogenate was centrifuged at 13000 rpm for 5 min at 4 °C. The supernatant was collected and stored at −20 °C. Biological samples (50 μL) were mixed with 10 μL of internal standard (sorafenib 1 μg/mL in acetonitrile) and 140 μL of acetonitrile for protein precipitation. The mixture was vortexed for 5 min and then centrifuged at 13000 rpm for 10 min at 4 °C. The supernatant was filtered through a 0.22 μm filter. An aliquot (4 μL) of the filtrate was analyzed using LC-MS/MS.

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

Pharmacokinetic Parameters Analysis and Statistics. Pharmacokinetic parameters were calculated using WinNonlin Standard Edition Version 5.3 (Pharsight Corp., Mountain View, CA, USA) with an IV-bolus input and an extravascular input noncompartmental model for the intravenous and oral groups, respectively. In addition, an IV-bolus input noncompartmental model was employed to obtain the pharmacokinetic parameters for the transplacental transfer experiment. All data are presented as the mean ± standard deviation (SD). ANOVA was used to evaluate differences using IBM SPSS Statistics 24.0 (IBM Corp., Armonk, NY, USA), and a value of $P<0.05$ was taken as statistically significant.

Results

Optimization of LC-MS/MS. An LC-MS/MS method was developed to determine the fipronil, fipronil sulfone and internal standard analytes. By optimizing the different collision energies, there was good sensitivity at m/z 434.95 → 329.95 for fipronil with a collision energy of 18 V, m/z 450.95 → 414.95 for fipronil sulfone with a collision energy of 16 V, and m/z 463.10 → 194.10 for the internal standard with a collision energy of 15 V (Supplemental Data Figure S1). After modifying the LC conditions, the experimental results revealed that the sharpest peaks and best retention times (RT) occurred when reversed-phase C18 minibore column was used to separate the analytes from the biological matrix with an isocratic elution system consisting of acetonitrile/methanol (6:1, v/v) and water (68:32, v/v). Under these conditions, the

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

retention times of fipronil, fipronil sulfone and sorafenib were 3.2, 4.2 and 2.9 min, respectively (Supplemental Data Figure S2).

The typical LC–MS/MS chromatograms of the blank biological samples, including male rat plasma, pregnant rat plasma, placenta, amniotic fluid, and fetus, presented no obvious endogenous interference within analyte-free samples (panels A1, B1, C1, and D1 of Supplemental Data Figure S2). As for the chromatograms of blank plasma or organ homogenates spiked with fipronil, fipronil sulfone, and an internal standard, and those depicting samples collected after fipronil administration, the determination of fipronil and fipronil sulfone in biological samples illustrated acceptable selectivity (panels A2-6, B2-3, C2-3, and D2-3 of Supplemental Data Figure S2).

Calibration Curves and Linearity. Linearity was achieved for the calibration curves, which were derived from the peak area ratios of fipronil and the internal standard. The linear range for the calibration curves in male rat plasma was 1-500 ng/mL, and it was 2.5-500 ng/mL in pregnant rat plasma, placenta, amniotic fluid and fetus homogenate. The correlation coefficient (r^2) of all calibration curves was greater than 0.995. The lower limit of quantification (LLOQ) for the male samples were 1 ng/mL, and the LLOQ for the female samples were 2.5 ng/mL.

Matrix Effect and Recovery Evaluation. Matrix effects and recoveries were used to assess ion suppression or enhancement and loss in sample preparation. By using the post-extraction fortification method, the average matrix effects of fipronil in both male

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

and pregnant rat plasma, placenta, amniotic fluid, and fetus samples were 94.55 ± 7.23, 98.71 ± 5.42, 98.73 ± 6.32, 90.96 ± 2.28 and 99.61 ± 3.49%, respectively. Additionally, the average matrix effects of fipronil sulfone in both male and pregnant rat plasma, placenta, amniotic fluid, and fetus samples were 92.12 ± 7.54, 96.93 ± 4.49, 96.20 ± 2.02, 84.94 ± 1.73 and 93.01 ± 3.06%, respectively. Comparing the pre-extraction and post-extraction spiked solutions, the mean extraction recoveries of fipronil in both male and pregnant rat plasma, placenta, amniotic fluid, and fetus samples were 98.57 ± 9.23, 95.03 ± 2.90, 97.95 ± 4.72, 96.47 ± 1.75 and 101.2 ± 2.36%, respectively. The average recoveries of fipronil sulfone in both male and pregnant rat plasma, placenta, amniotic fluid, and fetus samples were 99.03 ± 11.83, 103.0 ± 2.24, 99.47 ± 2.46, 102.2 ± 4.27 and 105.8 ± 1.87%, respectively. (Supplemental Data Table S1)

The results above demonstrated that the amniotic fluid samples had the most severe ion suppression in the analysis of fipronil and fipronil sulfone. However, all the matrix effects and extraction recoveries of the samples were less than ± 20%, meaning that the sample preparation had no significant effect on fipronil, fipronil sulfone or the internal standard in the biological samples.

Intraday and Interday Precision and Accuracy. In intraday assays, the precision ranged from 0.49 to 9.24%, and the accuracy ranged from −10.81 to 15.11% for fipronil analysis (Supplemental Data Table S2). The precision ranged from 0.66 to 9.62%, and the accuracy ranged from -7.24 to 8.31% for fipronil sulfone analysis (Supplemental Data Table S3). In interday assays, the precision was in the range of 0.46 through 10.51% and the accuracy was in the range of −4.11 through 15.44% for fipronil analysis

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

(Supplemental Data Table S2). The precision was in the range of 0.96 through 15.98% and the accuracy was in the range of -12.92 through 18.76% for fipronil sulfone analysis (Supplemental Data Table S3).

The precision and accuracy values were within ± 15% and that the LLOQ values were less than ± 20%, which were considered to be in the acceptable experimental concentration range. This result indicated that the method was considered acceptable and reproducible.

Pharmacokinetic of Fipronil and Fipronil Sulfone. The pharmacokinetics of fipronil and fipronil sulfone were assessed following intravenous administration 1 mg/kg of fipronil and oral administration at low, medium and high doses of fipronil (3, 10 and 30 mg/kg). The maximum plasma concentrations (C_{max}) of fipronil for the three oral doses from low to high were 8.81 ± 1.91, 23.76 ± 9.21 and 47.62 ± 20.16 ng/mL, respectively, and the area under the plasma concentration-time curve from time zero to the time of the last measurable concentration (AUC_{last}) of fipronil for the doses from 3 to 30 mg/kg were 72.26 ± 16.80, 172.4 ± 48.02 and 454.3 ± 186.2 hr ng/mL, respectively. The C_{max} of fipronil sulfone for the doses of 3, 10 and 30 mg/kg were 18.74 ± 11.56, 56.92 ± 18.28 and 152.5 ± 35.62 ng/mL, respectively; and the AUC_{last} of fipronil sulfone for the low, medium and high doses were 286.7 ± 146.4, 1052 ± 414.1 and 3005 ± 671.6 hr ng/mL, respectively. The pharmacokinetic data demonstrated that the C_{max} and AUC were proportional to the administered oral dose of fipronil and fipronil sulfone and had a significant difference ($P<0.001$) (Table 1).

By comparing intravenous administration and oral administration of fipronil using

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

the formula F (%) = (AUC_{oral}/$dose_{oral}$)/(AUC_{iv}/$dose_{iv}$) × 100%, the oral bioavailabilities were 19.28 ± 4.48%, 13.80 ± 3.84% and 12.12 ± 4.97% for the 3, 10 and 30 mg/kg doses of fipronil, respectively, and there was a significant dose-dependent decrease (P=0.035). The low oral bioavailability should be due to the first-pass effect. The biotransformation ratio ($AUC_{fipronil\ sulfone}$/$AUC_{fipronil}$ × 100%) was 104.8 ± 14.88% for fipronil intravenous administration (1 mg/kg). However, the biotransformation ratios for oral administration were 400.8 ± 197.4%, 608.4 ± 197.8% and 730.4 ± 257.7% after fipronil administration (3, 10 and 30 mg/kg, p.o.), respectively. (Table 1) The biotransformation ratio for oral administration was more than four-fold greater than that of the intravenous administration. These results indicated that fipronil metabolism was closely related to the hepatobiliary system and the gastrointestinal tract.

The clearance (CL/F) of fipronil for the three orally administered doses were 25.50 ± 3.46, 50.84 ± 11.29 and 70.36 ± 24.28 L/hr/kg, respectively, which significantly increased from low to high doses of oral administration (P=0.001) (Table 1). Combining the CL/F and the biotransformation results with the pharmacokinetic data of the elimination half-life ($t_{1/2}$) and the mean residence time from time zero to infinity (MRT_{∞}), which both decreased according to the dose, revealed that fipronil was metabolized vary rapidly to the metabolite fipronil sulfone in vivo and that the transforming speed was elevated by the dose. Moreover, the time to reach the maximum plasma concentration following drug administration (T_{max}) of fipronil sulfone was 4.00 ± 1.10, 6.33 ± 3.20 and 14.00 ± 2.19 hr by the oral administration of 3, 10, 30 mg/kg fipronil, respectively, which increased significantly by the dose (P<0.001) (Table 1). The $t_{1/2}$ of fipronil sulfone increased up to the plateau concentration (Figure 1). Both pharmacokinetic parameters

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

suggested that the elimination of fipronil sulfone might be slow.

Transplacental Transfer of Fipronil and Fipronil Sulfone. This validated LC-MS/MS method was used to determine the biodistribution of fipronil and fipronil sulfone in the plasma, placenta, amniotic fluid, and fetus after fipronil administration (10 mg/kg, i.v.) in pregnant rats. The concentration of fipronil gradually decreased after fipronil administration (10 mg/kg, i.v.). Then, the concentration of fipronil sulfone gradually increased to reach an average C_{max} of 60.52 ng/mL at the T_{max} of 4.17 hr in the pregnant rat plasma. A similar phenomenon occurred in the placenta, amniotic fluid and fetus, where the T_{max} of fipronil sulfone was approximately 4 hr. In addition, the T_{max} of fipronil in the placenta, amniotic fluid and fetus were all approximately 2 hr. These results suggest that the biodistributions of fipronil and fipronil sulfone for placental transfer were approximately 2 and 4 hr, respectively (Figure 2 and Table 2).

For fipronil, the C_{max} and the AUC_{last} in the plasma and placenta were both significantly higher than that of amniotic fluid and fetus, respectively ($P<0.001$). The AUC_{∞} of the placenta also significantly exceeded the AUC_{∞} of the amniotic fluid and fetus ($P<0.001$). In terms of fipronil sulfone, the C_{max} and the AUC_{last} in the plasma were significantly higher than that of amniotic fluid and fetus ($P<0.001$). The C_{max} of the placenta was significantly higher than the C_{max} of the amniotic fluid ($P=0.001$) (Table 2).

The AUC_{last} of fipronil in the pregnant rat plasma and placenta were 2859 ± 1438 hr ng/mL and 3411 ± 1097 hr ng/mL, respectively. The AUC_{last} of fipronil sulfone in the pregnant rat plasma and placenta were 287.7 ± 162.2 hr ng/mL and 215.5 ± 87.45 hr ng/mL, respectively (Table 2). Comparing the AUC_{last} of the plasma to the placenta for

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

both fipronil and fipronil sulfone, it can be found that the AUC_{last} of fipronil was lower in the plasma than in the placenta, while the AUC_{last} of fipronil sulfone was higher in the plasma than in the placenta.

The ratio of AUC_{fetus}/AUC_{plasma} was defined as the mother-to-fetus pass transformation ratio of the analyte. The AUC_{last} of the fetus in fipronil and fipronil sulfone were 1085 ± 268.8 hr ng/mL and 84.06 ± 23.83 hr ng/mL, respectively (Table 2). The average ratio of AUC_{fetus}/AUC_{plasma} for fipronil was approximately 38%, while this ratio was approximately 29% for fipronil sulfone, suggesting that both fipronil and fipronil sulfone partially penetrated the blood placental barrier to reach the fetus.

Discussion

The pharmacokinetic data in our study demonstrated that the C_{max} and AUC of both fipronil and fipronil sulfone are proportional to the administered oral dose of fipronil, suggesting a linear pharmacokinetic phenomenon. Previous study mentioned that the first-pass effect of hepatic metabolism in the oral route was very important for fipronil (Roques et al., 2012), which is consistent with our findings. First, the oral bioavailability is only 12 to 19%. Second, the oral biotransformation is about 4 to 7 times higher than intravenous one. These pharmacokinetic phenomena demonstrate that comparing to intravenous administration, the concentration of fipronil dramatically falls in the systemic circulation after oral administration while the concentration of fipronil sulfone is greatly rises. In addition, the average bioavailability of doses 3, 10, and 30 mg/kg are about 19.28%, 13.80%, and 12.12%, respectively, and the average biotransformation are about

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

400.8%, 608.4%, and 730.4%, respectively (Table 1). As the dose increases, the trends of both the above are opposite but the magnification is similar, reflecting the possibility of a first-pass effect enhancement. Fipronil is metabolized through three major mechanisms (Caboni et al., 2003). The first step is the oxidization step, which leads to fipronil sulfone formation via CYP450 (Scharf et al., 2000). In humans, CYP3A4 is the major isoform of P450 that metabolizes fipronil, and its activity toward fipronil is five times higher than that of CYP2C19 (Tang et al., 2004). The second step is the reduction step, which produces fipronil sulfide. The third step is the hydrolysis step, which forms fipronil amide. Considering the interspecies differences, although the main metabolite of fipronil is fipronil sulfone in both humans and rats, and the Michaelis constant (K_m) values of the liver microsomes are similar, there is a 3.8-fold higher rate of fipronil sulfone formation in rat liver microsomes than in human liver microsomes (Tang et al., 2004).

In a previous pharmacokinetic study of rats, after administration of fipronil and fipronil sulfone separately via intravenous and oral administration, the results indicated that fipronil sulfone was substantially converted from fipronil and lasted longer than fipronil (Roques et al., 2012). Compared to the previous study, a dose-dependent (3, 10 and 30 mg/kg) study design is applied in the current study and obtained consistent results. In addition, from the biotransformation and the half-life of fipronil sulfone reaching the plateau concentration, it is confirmed that fipronil is rapidly metabolized in the organisms and the elimination process of fipronil sulfone may be slow. The half-lives of fipronil in this study at 3 and 30 mg/kg were 12.16 hr and 4.44 hr, respectively. A previous report used radiolabeling to detect and measure the elimination half-lives of fipronil at 4 and 40 mg/kg in Charles River CD rats. The former obtained 183 h in males and 245 h in

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

females; the latter got 135 h in males and 171 h in females (JMPR, 1997). The report also proposed that a long half-life reflected the slow release from compartments such as fat, which explained the change in the blood concentration of fipronil sulfone in our study (JMPR, 1997). However, our findings may be inconsistent with previous reports. A potential explanation is that the previous report utilized radiolabels for the detection of fipronil in the analyte, which might not be sensitive enough to distinguish between fipronil and its metabolites. In contrast to our experiment, the fipronil and the metabolite fipronil sulfone were separated by the chromatographic column and detected by tandem mass spectrometer individually. Another explanation for the different pharmacokinetic results could be due to the dissimilar strains, ages and weights of the experimental animals.

The distribution study for fipronil supports the above discourse. The highest levels of fipronil residue were found in abdominal fat, followed by the adrenals. The intermediate levels were observed in the liver, pancreas, thyroid, and ovaries, and the lowest levels were in the muscle, brain, heart, and cardiac blood (JMPR, 1997). Excretion research can also provide some explanation for these phenomena. Feces not only appears to be the main route of excretion, but fipronil itself can also be detected from it, while not found in any other excrement (JMPR, 1997; Cravedi et al., 2013). In urine, the secondary route of excretion, there are several metabolites found inside, including detrifluoromethylsulphinyl fipronil and its derivative (Cravedi et al., 2013).

Among most of the literature on organ distribution of fipronil, the placenta, amniotic fluid, and fetus distribution has not been mentioned. Our research provided important information that in decreasing order, the fipronil concentration is from the placenta,

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

plasma, fetus to amniotic fluid, but the fipronil sulfone concentration is from the plasma, placenta, fetus to amniotic fluid after fipronil administration in the pregnant rat. This result was supported by previous research showing that fipronil sulfone was detected in the serum of mother-neonate pairs (Kim et al., 2019). At the same time, we put forward a different view that long-term exposure to fipronil may not lead to detection of the parent compound in the fetus, which is related to the half-life of fipronil itself (Tang et al., 2004), but in a short period of time, fipronil can still enter the fetus. Considering the developmental and reproductive toxicity of fipronil (JMPR, 1997; Udo et al., 2014; de Barros et al., 2016; de Barros et al., 2017), it still is a potential menace. In addition, the concentration distribution could be explained by the scheme of an in vivo animal model for the transplacental transfer of oseltamivir and oseltamivir carboxylic acid in our previous study (Lin et al., 2012). Fipronil and fipronil sulfone penetrate the placenta from the maternal blood through the blood placental barrier, enter the fetus and finally spread to the amniotic fluid, thus causing a sequential decrease in drug concentration.

Regarding the inconsistency in concentration distribution order of fipronil and fipronil sulfone, the affinity of the drugs and placental tissue may have a certain effect. Some drugs have been found to bind to and accumulate in placental tissue ex vivo, causing a depot phenomenon (Ala-Kokko et al., 2000). This might be due to the uptake of the lipophilic drug by the syncytiotrophoblast in the placenta (Sastry, 1999). If the drug has a high affinity for placental tissue, it cannot be easily released from the placenta into fetal circulation, especially for lipophilic drugs. In our research, the higher concentration of fipronil found in the placenta compared to maternal plasma may have been affected by this phenomenon.

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

Various reasons contributed to the mother-to-fetus transfer ratio of fipronil and fipronil sulfone to be 38% and 29%, respectively, which indicates an incomplete transfer (Griffiths and Campbell, 2014). This can be explained by the physicochemical properties and the mechanism of the drug which passively diffuses through the blood placental barrier (Syme et al., 2004; Griffiths and Campbell, 2014). The molecular weights of fipronil and fipronil sulfone are both less than 500 Da, and both are lipophilic, causing a higher opportunity to cross the blood placental barrier. However, lipophilic drugs with a good protein binding rate usually have more difficulty entering the fetus. According to previous report, fipronil binds to fatty acid site 1 (FA1) of human serum albumin (HSA) in humans (Ascenzi et al., 2018). Moreover, the ATP-binding cassette transporters (ABC transporters) of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) are abundantly expressed in the syncytiotrophoblast layers of placenta and in the fetal brain, liver, spleen and intestine throughout gestation to protect the fetus from drugs in the maternal circulation (Han et al., 2018). In the fipronil-resistant strain of *Plutella xylostella* larvae, *ABCG2* gene is up-regulated but *ABCB1* gene is down-regulated (Qi et al., 2016). It seems that P-gp/ABCB1 and BCRP/ABCG2 could impact fipronil transportation into placenta and fetus. Our data suggest that both fipronil and fipronil sulfone partially penetrate into placenta, amniotic fluid and fetus (Table 2).

On the other hand, the discrepancy in the mother-to-fetus transfer ratio between fipronil and fipronil sulfone is a complicated issue. The main metabolic enzymes of fipronil in the humans are described as follow: CYP3A4 exists in the fetal liver after 9 weeks of gestation and in the placenta, while CYP2C19 presents in the fetal liver after 12 weeks but not in the placenta (Hakkola et al., 1996; Hakkola et al., 1998; Hines, 2008).

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

However, the feto-placental metabolism has little contribution to the overall pharmacokinetics of drugs, due to small organ sizes and low CYPs content of the fetus and placenta. Similarly, studies on the above two enzymes in rat fetus and placenta are also inadequate. It is speculated that the fipronil sulfone in the fetus is mostly transmitted by the maternal blood, thus showing that the lipophilic properties of drugs are the main factors contributing to this inequality. Drugs with high lipophilicity are more likely to cross the blood placental barrier (Dickinson et al., 1989). The octanol–water partition (log P_{ow}) value of fipronil (4.0) is greater than the log P_{ow} value of fipronil sulfone (3.8), inducing a lower mother-to-fetus transfer ratio for fipronil sulfone (Authority, 2006).

There have been many studies on the transplacental transfer of insecticides and pesticides. In animals, ivermectin can be detected in fetal blood after maternal or fetal intravenous administration to sheep (Perez et al., 2008). Through collected carcasses, polychlorinated biphenyls, polybrominated diphenyl ethers, and organochlorine pesticides can be found in the blubber of ringed seal fetuses (Brown et al., 2016). Additionally, pyrethroid insecticides and persistent organic pollutants in dolphin fetal tissue have also been reported (Alonso et al., 2015; Barbosa et al., 2018). In humans, such studies are most common in analytical epidemiology, and most studies are about organochlorine pesticides, which can be found in cord blood (Sala et al., 2001; Covaci et al., 2002; Al-Saleh et al., 2012; Dewan et al., 2013; Ma et al., 2014; Zhang et al., 2018). Although fipronil is not an organochlorine pesticide, according to our study, it can still be transferred through the placenta to the fetus.

In conclusion, we have successfully developed a validated LC-MS/MS method to monitor analytes in various organs and applied this method to assess the

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

pharmacokinetics and transplacental transfer of fipronil and fipronil sulfone. The pharmacokinetic study revealed that after oral fipronil administration, the persistent toxicity of metabolite fipronil sulfone is accelerated by biotransformation in a dose-dependent manner as the dose increases. Besides, since both fipronil and fipronil sulfone incompletely transfer through the blood placental barrier to the fetus, this preclinical study provides conclusive information to suggest that pregnant women should avoid exposure to fipronil under any circumstances.

Supplemental Data

Method validation results, optimization of LC-MS/MS and typical HPLC-MS/MS chromatograms.

Author contributions

Performed the experiments, analyzed the data and prepared the manuscript by Ya-Ning Chang. Designed the experiments, edited the paper and secured the funding by Tung-Hu Tsai.

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

References

Ala-Kokko T, Myllynen P, and Vähäkangas K (2000) Ex vivo perfusion of the human placental cotyledon: implications for anesthetic pharmacology. *Int J Obstet Anesth* **9:**26-38.

Alonso MB, Feo ML, Corcellas C, Gago-Ferrero P, Bertozzi CP, Marigo J, Flach L, Meirelles AC, Carvalho VL, Azevedo AF, Torres JP, Lailson-Brito J, Malm O, Diaz-Cruz MS, Eljarrat E, and Barcelo D (2015) Toxic heritage: Maternal transfer of pyrethroid insecticides and sunscreen agents in dolphins from Brazil. *Environmental pollution (Barking, Essex : 1987)* **207:**391-402.

Anderson GW (2008) Thyroid hormone and cerebellar development. *Cerebellum* **7:**60-74.

Ascenzi P, Leboffe L, Toti D, Polticelli F, and Trezza V (2018) Fipronil recognition by the FA1 site of human serum albumin. *J Mol Recognit* **31:**e2713.

Authority EFS (2006) Conclusion regarding the peer review of the pesticide risk assessment of the active substance fipronil. *EFSA Journal* **4:**65r.

Barbosa APM, Mendez-Fernandez P, Dias PS, Santos MCO, Taniguchi S, and Montone RC (2018) Transplacental transfer of persistent organic pollutants in La Plata dolphins (Pontoporia blainvillei; Cetartiodactyla, Pontoporiidae). *Sci Total Environ* **631-632:**239-245.

Bernal J (2007) Thyroid hormone receptors in brain development and function. *Nature Clinical Practice Endocrinology &Amp; Metabolism* **3:**249.

Bobé A, Meallier P, Cooper J-F, and Coste CM (1998) Kinetics and mechanisms of abiotic degradation of fipronil (hydrolysis and photolysis). *J Agric Food Chem* **46:**2834-2839.

Brown TM, Ross PS, and Reimer KJ (2016) Transplacental Transfer of Polychlorinated Biphenyls, Polybrominated Diphenylethers, and Organochlorine Pesticides in Ringed Seals (Pusa hispida). *Arch Environ Contam Toxicol* **70:**20-27.

Caboni P, Sammelson RE, and Casida JE (2003) Phenylpyrazole insecticide photochemistry, metabolism, and GABAergic action: ethiprole compared with fipronil. *J Agric Food Chem* **51:**7055-7061.

Cole LM, Nicholson RA, and Casida JE (1993) Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pest Biochem Physiol* **46:**47-54.

Cravedi JP, Delous G, Zalko D, Viguie C, and Debrauwer L (2013) Disposition of fipronil

in rats. *Chemosphere* **93:**2276-2283.

Cuevas E, Ausó E, Telefont M, De Escobar GM, Sotelo C, and Berbel P (2005) Transient maternal hypothyroxinemia at onset of corticogenesis alters tangential migration of medial ganglionic eminence-derived neurons. *Eur J Neurosci* **22:**541-551.

de Barros AL, Bae JH, Borges CS, Rosa JL, Cavariani MM, Silva PV, Pinheiro PFF, Anselmo-Franci JA, and Arena AC (2017) Perinatal exposure to insecticide fipronil: effects on the reproductive system in male rats. *Reprod Fertil Dev* **29:**1130-1143.

de Barros AL, Rosa JL, Cavariani MM, Borges CS, Villela e Silva P, Bae JH, Anselmo-Franci JA, and Cristina Arena A (2016) In utero and lactational exposure to fipronil in female rats: Pregnancy outcomes and sexual development. *J Toxicol Environ Health A* **79:**266-273.

Dickinson RG, Fowler DW, and Kluck RM (1989) Maternofetal transfer of phenytoin, p-hydroxy-phenytoin and p-hydroxy-phenytoin-glucuronide in the perfused human placenta. *Clin Exper Pharmacol Physiol* **16:**789-797.

Griffiths SK and Campbell JP (2014) Placental structure, function and drug transfer. *BJA Education* **15:**84-89.

Hainzl D, Cole LM, and Casida JE (1998) Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem Res Toxicol* **11:**1529-1535.

Hakkola J, Pasanen M, Hukkanen J, Pelkonen O, Mäenpää J, Edwards RJ, Boobis AR, and Raunio H (1996) Expression of xenobiotic-metabolizing cytochrome P450 forms in human full-term placenta. *Biochem Pharmacol* **51:**403-411.

Hakkola J, Pelkonen O, Pasanen M, and Raunio H (1998) Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto-placental unit: role in intrauterine toxicity. *Crit Rev Toxicol* **28:**35-72.

Han LW, Gao C, and Mao Q (2018) An update on expression and function of P-gp/ABCB1 and BCRP/ABCG2 in the placenta and fetus. *Expert Opin Drug Metab Toxicol* **14:**817-829.

Herin F, Boutet-Robinet E, Levant A, Dulaurent S, Manika M, Galatry-Bouju F, Caron P, and Soulat J-M (2011) Thyroid Function Tests in Persons with Occupational Exposure to Fipronil. *Thyroid* **21:**701-706.

Hines RN (2008) The ontogeny of drug metabolism enzymes and implications for adverse drug events. *Pharmacol Ther* **118:**250-267.

Horoszok L, Raymond V, Sattelle DB, and Wolstenholme AJ (2001) GLC-3: a novel

fipronil and BIDN- sensitive, but picrotoxinin- insensitive, L- glutamate- gated chloride channel subunit from Caenorhabditis elegans. *Brit J Pharmacol* **132:**1247-1254.

JMPR (1997) *Pesticide Residues in Food, 1997: Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, Lyons, France 22 Sept.-1 Oct. 1997*. Food and Agriculture Organization of the United Nations.

Kim YA, Yoon YS, Kim HS, Jeon SJ, Cole E, Lee J, Kho Y, and Cho YH (2019) Distribution of fipronil in humans, and adverse health outcomes of in utero fipronil sulfone exposure in newborns. *Int J Hyg Environ Health* **222:**524-532.

Lee SJ, Mulay P, Diebolt-Brown B, Lackovic MJ, Mehler LN, Beckman J, Waltz J, Prado JB, Mitchell YA, Higgins SA, Schwartz A, and Calvert GM (2010) Acute illnesses associated with exposure to fipronil--surveillance data from 11 states in the United States, 2001-2007. *Clin Toxicol (Philadelphia, Pa)* **48:**737-744.

Leghait J, Gayrard V, Picard-Hagen N, Camp M, Perdu E, Toutain P-L, and Viguié C (2009) Fipronil-induced disruption of thyroid function in rats is mediated by increased total and free thyroxine clearances concomitantly to increased activity of hepatic enzymes. *Toxicology* **255:**38-44.

Lin CC, Yen JC, Wu YT, Lin LC, and Tsai TH (2012) Chemical analysis and transplacental transfer of oseltamivir and oseltamivir carboxylic acid in pregnant rats. *PLoS One* **7:**e46062.

Ma WL, Gao C, Bell EM, Druschel CM, Caggana M, Aldous KM, Louis GM, and Kannan K (2014) Analysis of polychlorinated biphenyls and organochlorine pesticides in archived dried blood spots and its application to track temporal trends of environmental chemicals in newborns. *Environ Res* **133:**204-210.

Mohamed F, Senarathna L, Percy A, Abeyewardene M, Eaglesham G, Cheng R, Azher S, Hittarage A, Dissanayake W, Sheriff MH, Davies W, Buckley NA, and Eddleston M (2004) Acute human self-poisoning with the N-phenylpyrazole insecticide fipronil--a GABAA-gated chloride channel blocker. *J Toxicol Clin Ttoxicol* **42:**955-963.

Perez R, Palma C, Nunez MJ, and Cox J (2008) Pharmacokinetics of ivermectin after maternal or fetal intravenous administration in sheep. *J Vet Pharmacol Ther* **31:**406-414.

Qi W, Ma X, He W, Chen W, Zou M, Gurr GM, Vasseur L, and You M (2016)

Characterization and expression profiling of ATP-binding cassette transporter genes in the diamondback moth, Plutella xylostella (L.). *BMC Genomics* **17:**760.

Roques BB, Lacroix MZ, Puel S, Gayrard V, Picard-Hagen N, Jouanin I, Perdu E, Martin PG, and Viguie C (2012) CYP450-dependent biotransformation of the insecticide fipronil into fipronil sulfone can mediate fipronil-induced thyroid disruption in rats. *Toxicol Sci* **127:**29-41.

Sala M, Ribas-Fito N, Cardo E, de Muga ME, Marco E, Mazon C, Verdu A, Grimalt JO, and Sunyer J (2001) Levels of hexachlorobenzene and other organochlorine compounds in cord blood: exposure across placenta. *Chemosphere* **43:**895-901.

Sastry BR (1999) Techniques to study human placental transport. *Adv Drug Deliv Rev* **38:**17-39.

Scharf ME, Siegfried BD, Meinke LJ, and Chandler LD (2000) Fipronil metabolism, oxidative sulfone formation and toxicity among organophosphate- and carbamate-resistant and susceptible western corn rootworm populations. *Pest Manag Sci* **56:**757-766.

Stafford EG, Tell LA, Lin Z, Davis JL, Vickroy TW, Riviere JE, and Baynes RE (2018) Consequences of fipronil exposure in egg-laying hens. *J Am Vet Med Assoc* **253:**57-60.

Stehr CM, Linbo TL, Incardona JP, and Scholz NL (2006) The Developmental Neurotoxicity of Fipronil: Notochord Degeneration and Locomotor Defects in Zebrafish Embryos and Larvae. *Toxicol Sci* **92:**270-278.

Syme MR, Paxton JW, and Keelan JA (2004) Drug transfer and metabolism by the human placenta. *Clin Pharmacokinet* **43:**487-514.

Tang J, Amin Usmani K, Hodgson E, and Rose RL (2004) In vitro metabolism of fipronil by human and rat cytochrome P450 and its interactions with testosterone and diazepam. *Chem Biol Interact* **147:**319-329.

Tingle CC, Rother JA, Dewhurst CF, Lauer S, and King WJ (2003) Fipronil: environmental fate, ecotoxicology, and human health concerns. *Rev Environ Contam Toxicol* **176:**1-66.

Udo MS, Sandini TM, Reis TM, Bernardi MM, and Spinosa HS (2014) Prenatal exposure to a low fipronil dose disturbs maternal behavior and reflex development in rats. *Neurotoxicol Teratol* **45:**27-33.

Wagner SD, Kurobe T, Hammock BG, Lam CH, Wu G, Vasylieva N, Gee SJ, Hammock BD, and Teh SJ (2017) Developmental effects of fipronil on Japanese Medaka (Oryzias latipes) embryos. *Chemosphere* **166:**511-520.

Zhang X, Wu X, Lei B, Jing Y, Jiang Z, Zhang X, Fang X, and Yu Y (2018) Transplacental transfer characteristics of organochlorine pesticides in paired maternal and cord sera, and placentas and possible influencing factors. *Environ Pollut (Barking, Essex : 1987)* **233:**446-454.

Zhao X, Yeh JZ, Salgado VL, and Narahashi T (2005) Sulfone metabolite of fipronil blocks gamma-aminobutyric acid- and glutamate-activated chloride channels in mammalian and insect neurons. *J Pharmacol Exp Ther* **314:**363-373.

Zoeller RT and Rovet J (2004) Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *J Neuroendocrinol* **16:**809-818.

Footnotes.

This research was supported in part by the research grants from the Ministry of Science and Technology of Taiwan [MOST 107-2113-M-010-005, MOST 109-2113-M-010-007]; the Yin Yen-Liang Foundation Development and Construction Plan of the School of Medicine, National Yang-Ming University [107F-M01]; and the NYMU-FEMH Joint Research Program [108DN31].

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.

Figure captions

Figure 1. Concentration-time curves of (A) fipronil and (B) fipronil sulfone in male rat plasma after fipronil administration at the doses of 1 mg/kg, i.v. (●), 3 mg/kg, p.o. (○), 10 mg/kg, p.o. (▼), and 30 mg/kg, p.o. (△). Data are expressed as mean ± SD (n = 6).

Figure 2. Concentration-time curves of (A) fipronil and (B) fipronil sulfone in pregnant rat plasma (●), placenta (○), amniotic fluid (▼), and fetus (△) after 10 mg/kg fipronil intravenous administration. Data are expressed as mean ± SD (n = 6).

Table 1. Pharmacokinetic data from plasma for (A) fipronil and (B) fipronil sulfone in male rats

Pharmacokinetic parameter	Fipronil 1 mg/kg, i.v.	Fipronil 3 mg/kg, p.o.	Fipronil 10 mg/kg, p.o.	Fipronil 30 mg/kg, p.o.	P value
Fipronil					
$t_{1/2}$ (hr)	2.66 ± 0.56	12.16 ± 5.21	9.16 ± 11.28	4.44± 1.19	0.207
T_{max} (hr)	0.83 ± 0.00	1.75 ± 0.88	2.17 ± 0.75	3.17 ± 1.47	0.098
C_{max} (ng/mL)	100.5 ± 39.65	8.81 ± 1.91[c]	23.76 ± 9.21[b]	47.62 ± 20.16	<0.001*
AUC_{last} (hr ng/mL)	125.0 ± 20.66	72.26 ± 16.80[c]	172.4 ± 48.02[b]	454.3 ± 186.2	<0.001*
AUC_{∞}(hr ng/mL)	153.8 ± 17.90	119.4 ± 16.19[c]	204.6 ± 42.84[b]	481.3 ± 197.1	<0.001*
CL/F (L/hr/kg)	6.58± 0.82	25.50 ± 3.46[ac]	50.84 ± 11.29	70.36 ± 24.28	0.001*
MRT_{∞} (hr)	3.34 ± 0.88	17.32 ± 6.63	12.98 ± 11.94	8.30 ± 1.39	0.177
Bioavailability (%)		19.28 ± 4.48[c]	13.80 ± 3.84	12.12 ± 4.97	0.035*
Fipronil sulfone					
T_{max} (hr)	1.75 ± 0.42	4.00 ± 1.10[c]	6.33 ± 3.20[b]	14.00 ± 2.19	<0.001*
C_{max} (ng/mL)	27.66 ± 6.65	18.74 ± 11.56[ac]	56.92 ± 18.28[b]	152.5 ± 35.62	<0.001*
AUC_{last} (hr ng/mL)	130.35 ± 25.92	286.7 ± 146.4[ac]	1052 ± 414.1[b]	3005 ± 671.6	<0.001*

Biotransformation (%)	104.8 ± 14.88	400.8 ± 197.4	608.4 ± 197.8	730.4 ± 257.7	0.058

Data are expressed as mean ± SD (n=6).

[*] $P < 0.05$ compared among the 3 mg/kg, 10 mg/kg, and 30 mg/kg, p.o. fipronil groups

[a] $P < 0.05$ compared with the 3 mg/kg and 10 mg/kg p.o. fipronil group

[b] $P < 0.05$ compared with the 10 mg/kg and 30 mg/kg p.o. fipronil group

[c] $P < 0.05$ compared with the 3 mg/kg and 30 mg/kg p.o. fipronil group

Table 2. Pharmacokinetic data for the plasma, placenta, amniotic fluid and fetus of fipronil (10 mg/kg, i.v.) in pregnant rats

Pharmacokinetic parameter	Plasma	Placenta	Amniotic fluid	Fetus	P value
Fipronil					
T_{max} (hr)	0.25 ± 0.00	2.25 ± 1.60	2.00 ± 1.26	2.29 ± 2.52	0.125
C_{max} (ng/mL)	1060 ± 443.3[a, b]	820.1 ± 282.6[c, d]	182.7 ± 57.79	286.4 ± 92.49	<0.001*
AUC_{last} (hr ng/mL)	2859 ± 1438[a, b]	3411 ± 1097[c, d]	787.4 ± 242.9	1085 ± 268.3	<0.001*
AUC_{∞} (hr μg/mL)	4468 ± 1933	6642 ± 3319[c, d]	1253 ± 386.0	2204 ± 1086	0.002*
Fipronil sulfone					
T_{max} (hr)	4.17 ± 1.60	4.16 ± 1.69	4.83 ± 0.23	4.83 ± 1.11	0.619
C_{max} (ng/mL)	60.52 ± 32.24[a, b]	52.80 ± 17.78[c]	8.84 ± 3.97	22.43 ± 5.79	<0.001*
AUC_{last} (hr ng/mL)	287.7 ± 162.2[a, b]	215.5 ± 87.45	54.72 ± 39.96	84.06 ± 23.83	0.001*

Data are expressed as mean ± SD (n=6).

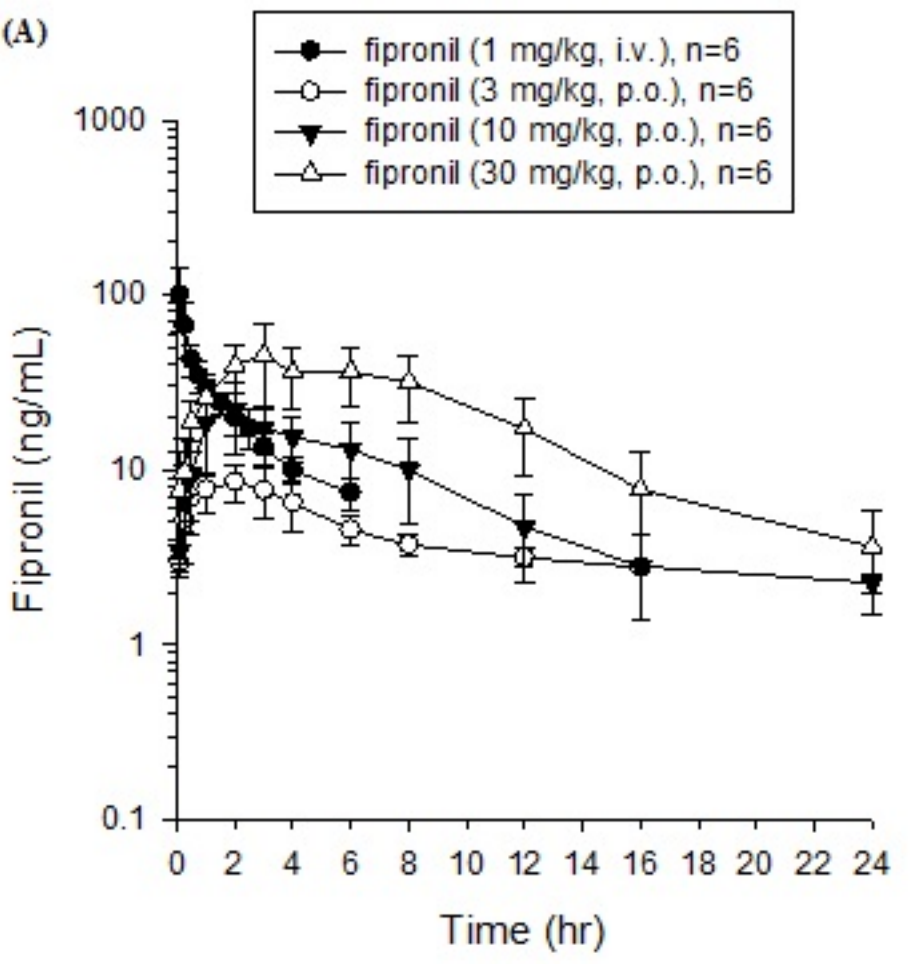
[a] $P < 0.05$ compared with the plasma and amniotic fluid group.

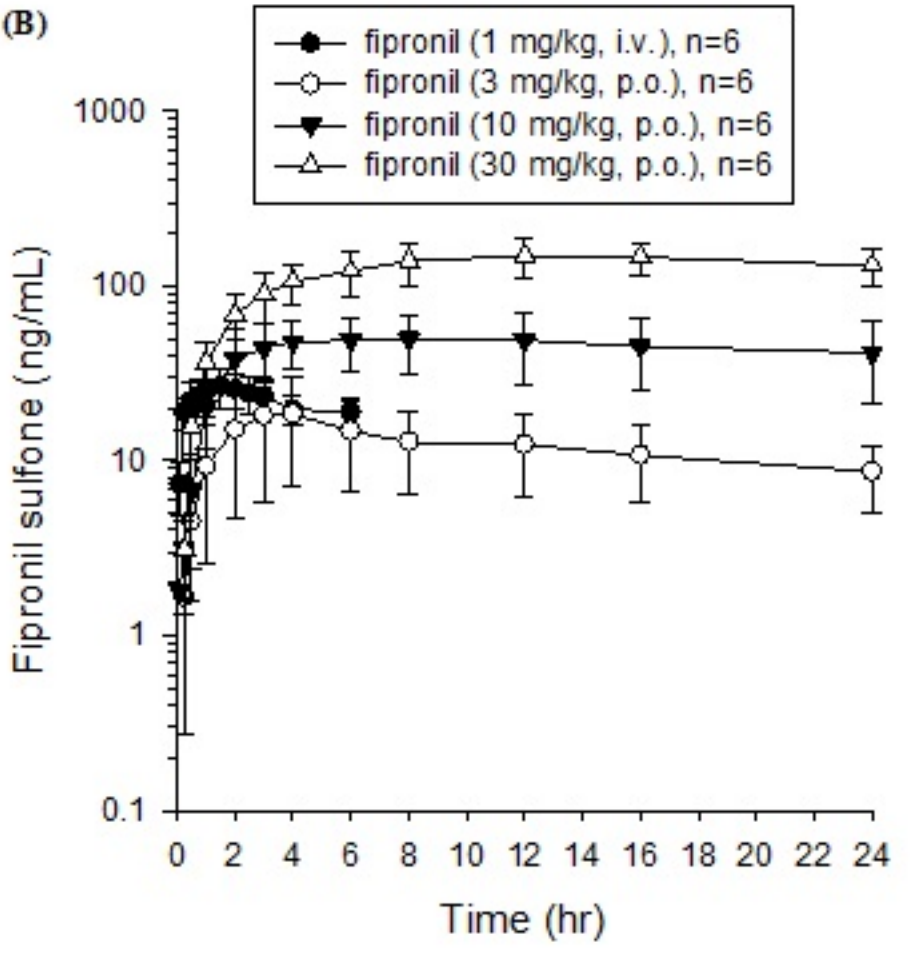
[b] $P < 0.05$ compared with the plasma and fetus group.

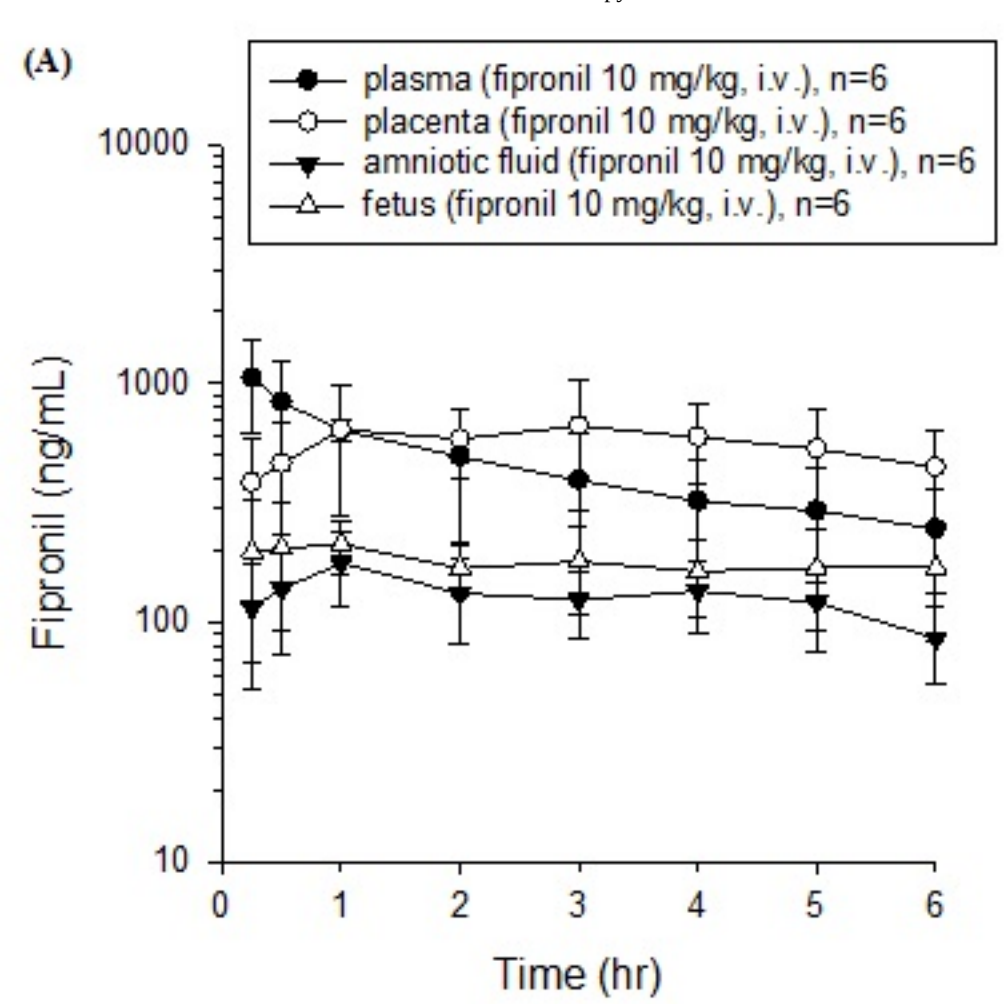
[c] $P < 0.05$ compared with the placenta and amniotic fluid group.

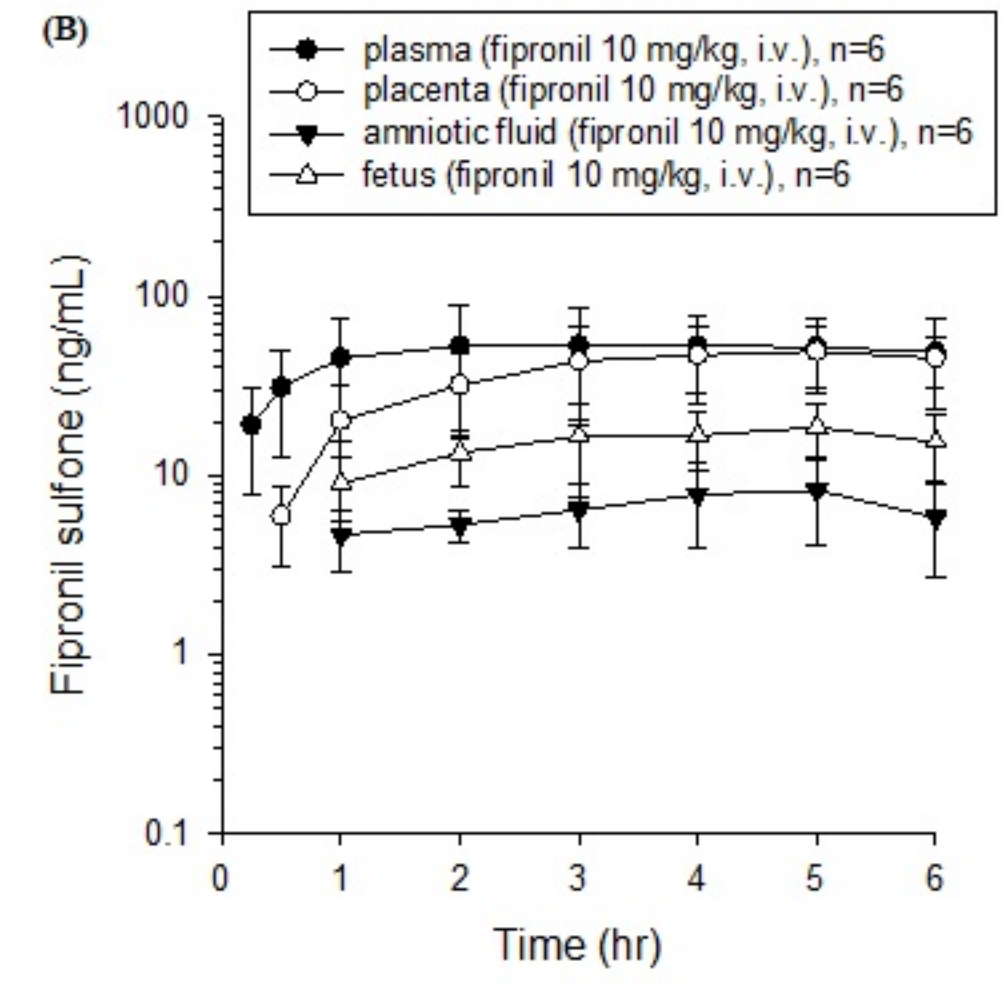
[d] $P < 0.05$ compared with the placenta and fetus groups.

DMD Fast Forward. Published on July 28, 2020 as DOI: 10.1124/dmd.120.000088
This article has not been copyedited and formatted. The final version may differ from this version.


(A)
fipronil (1 mg/kg, i.v.), n=6
fipronil (3 mg/kg, p.o.), n=6
fipronil (10 mg/kg, p.o.), n=6
fipronil (30 mg/kg, p.o.), n=6
Fipronil (ng/mL)
1000
100
10
1
0.1
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (hr)


(B)
fipronil (1 mg/kg, i.v.), n=6
fipronil (3 mg/kg, p.o.), n=6
fipronil (10 mg/kg, p.o.), n=6
fipronil (30 mg/kg, p.o.), n=6
Fipronil sulfone (ng/mL)
1000
100
10
1
0.1
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (hr)


(A)
plasma (fipronil 10 mg/kg, i.v.), n=6
placenta (fipronil 10 mg/kg, i.v.), n=6
amniotic fluid (fipronil 10 mg/kg, i.v.), n=6
fetus (fipronil 10 mg/kg, i.v.), n=6
Fipronil (ng/mL)
10000
1000
100
10
0
1
2
3
4
5
6
Time (hr)


(B)
plasma (fipronil 10 mg/kg, i.v.), n=6
placenta (fipronil 10 mg/kg, i.v.), n=6
amniotic fluid (fipronil 10 mg/kg, i.v.), n=6
fetus (fipronil 10 mg/kg, i.v.), n=6
Fipronil sulfone (ng/mL)
1000
100
10
1
0.1
0
1
2
3
4
5
6
Time (hr)