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RESEARCH ARTICLE

In vivo and *in vitro* metabolism of fipronil by larvae of the European corn borer *Ostrinia nubilalis*

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Abstract: *In vivo* and *in vitro* metabolism of [¹⁴C]fipronil was examined in a susceptible European corn borer (*Ostrinia nubilalis*, Hübner) laboratory strain. [¹⁴C]Fipronil penetrated the larval integument slowly, with 71.5% of the applied radioactivity recovered from surface rinses 24 h after topical application. Despite this slow penetration, radioactivity was detected in both the excrement and internal organo-soluble fractions. Radioactivity in the internal aqueous fraction and tissue pellet accounted for less than 0.8% of total radioactivity. The *in vivo* studies suggest that fipronil oxidation to its sulfone metabolite is the major route of metabolic conversion. *In vitro* studies were performed using subcellular microsomal fractions isolated from European corn borer larval midguts. Cytochrome P450-dependent monooxygenase activity (methoxyresorfin *O*-demethylase) was consistently observed in midgut preparations, and formation and detection of the sulfone metabolite in the same midgut preparations was also NADPH-dependent and inhibited by piperonyl butoxide. *In vitro* metabolism results indicate microsomal monooxygenases are responsible for the conversion of fipronil to its sulfone form in the European corn borer.

Keywords: fipronil, phenylpyrazole, cytochrome P450, *Ostrinia nubilalis*, European corn borer, metabolism

1 INTRODUCTION

Fipronil [(±)-5-amino-1-(2,6-dichloro- α,α,α -trifluoro-*p*-tolyl)-4-trifluoromethylsulfanylpyrazole-3-carbonitrile] is the first member of the phenylpyrazole insecticide class, and possesses a trifluoromethylsulfanyl functional group on the heterocyclic ring. Fipronil's mode of action involves disruption of chloride ion flow by interacting at the γ -aminobutyric acid (GABA)-gated chloride ionophore of the central nervous system, in a way similar to the cyclo-diene class of insecticides.^{1–3} Fipronil has been developed for delivery by soil, foliar, bait or seed-treatment applications,^{2,4} and has shown activity across a broad spectrum of insect orders, including Blattaria,^{5–7} Diptera,^{8–10} Hymenoptera,¹¹ Orthoptera^{12–14} and Lepidoptera.^{15,16} A fipronil formulation (Regent®) was recently introduced for control of corn insect pests such as the European corn borer, *Ostrinia nubilalis* Hübner and western corn rootworms, *Diabrotica virgifera virgifera* LeConte, in many Midwest states, including Nebraska.

In vivo and *in vitro* metabolism studies have shown that fipronil is converted by microsomal monooxygenases to its oxidative sulfone metabolite, which is the predomi-

nant metabolite formed in both insects^{3,15,17} and in mammals.^{3,4,18,19} The sulfone metabolite is similar in toxicity to the parent compound,^{17,18} and competitive binding assays using 1-[4-ethynylphenyl]-4-*n*-propyl-2,6,7-trioxabicyclo [2.2.2]octane (EBOB), a ligand specific for the GABA receptor ionophore, suggest the binding site for fipronil and fipronil-sulfone are similar if not the same.^{1–3} Studies using the cytochrome P450 inhibitor, piperonyl butoxide (PBO), which should inhibit sulfone formation, have provided variable results. In German cockroaches (*Blattella germanica* L), PBO is antagonistic to fipronil toxicity,⁶ or has no effect,⁵ and in house flies (*Musca domestica* L), PBO is synergistic.^{1,5} In western corn rootworms, fipronil toxicity was unaffected by pretreatment with PBO.²⁰

Understanding the metabolism of an insecticide, including its potential for bioactivation, is an important component of understanding a molecule's full potential as a toxicant. Much of the work involving fipronil metabolism has been performed in mice and rats, except for recent studies involving *M. domestica*³ and *D. v. virgifera*.¹⁷ Fipronil metabolism in *O. nubilalis*, an important target for this new compound, has not been investigated. The following

in vivo and *in vitro* experiments were performed to identify the metabolic system(s) by which fipronil is converted to fipronil-sulfone in *O. nubilalis*. *In vivo* metabolism experiments were performed to identify the tissue distribution of both the parent molecule and its metabolites. *In vitro* assays were performed to determine the rate of *O. nubilalis* cytochrome P450-dependent oxidation of fipronil to form the sulfone metabolite.

2 METHODS AND MATERIALS

2.1 Insects

An *O. nubilalis*-susceptible laboratory strain was used in all experiments. This laboratory colony was initiated from field-collected adults obtained from field corn in Saunders Co, Nebraska during June 1994 and June 1995.²¹ *O. nubilalis* rearing methods were derived from procedures developed by the USDA-ARS Corn Insects Research Unit, Ames, IA.²² Larvae were reared on wheat germ-based diet at 27 (± 0.7) °C with a photoperiod of 24:0 h light : dark and 80% relative humidity.²³ At pupation, insects were transferred to mating cages where emergent adults were maintained with an 8-h scotophase at 18 (± 0.7) °C and a 16-h photophase at 27 (± 0.7) °C with 80% relative humidity. The mating cages were misted twice a day with water and supplied with adult diet for maximum egg production.²⁴ The egg masses were collected and incubated within polystyrene Petri dishes on moistened filter paper until hatching. Newly hatched *O. nubilalis* larvae were transferred onto 1 ml of solidified artificial diet in 128-well bioassay trays (each well 16 mm diameter \times 16 mm height; C-D International, Pitman, NJ). The larvae were placed in a growth chamber at 27 °C in 24 h light and 80% RH for 8 days. On the eighth day, larvae (fifth instar) were randomly selected and placed in polystyrene Petri dishes (100 mm \times 15 mm) for treatment. Late (fifth) instar larvae were used for both *in vivo* and *in vitro* experiments.

2.2 Chemicals

[*Phenyl*-¹⁴C]Fipronil (26.5 mCi mmol⁻¹) and the non-radio-labeled standards (with% AI): fipronil (97.1%) and fipronil-sulfone (99.9%) were supplied by Rhône-Poulenc Ag Company (Research Triangle Park, NC). [¹⁴C]Fipronil was purified by thin layer chromatography (TLC) using 0.25-mm thick Whatman LK6DF silica gel plates (Clifton, NJ). The labeled compound was identified by co-chromatography with cold standards visualized under UV light. The radioactive band was scraped from the plate and eluted from the silica with repeated rinses in acetone. The microsomal cofactor, β -nicotinamide adenine dinucleotide phosphate (NADPH) reduced form, and the cytochrome P450 substrate, methoxyresorufin, were purchased from Sigma Chemical Company (St Louis, MO). Piperonyl butoxide (PBO) was purchased from Crescent Chemical Company (Hauppauge, NY). All other chemicals were of reagent grade or better.

2.3 *In vivo* metabolism

Groups of five *O. nubilalis* larvae were anesthetized by refrigeration at 4 °C and topically treated with a sub-lethal dose (5897 dpm per larva) of [¹⁴C]fipronil in acetone (0.5 μ l) using a Hamilton syringe with a repeating dispenser. Five individuals were treated and held in 20-ml glass scintillation vials for varying time intervals (0.5, 1, 3, 6, 12 and 24 h), and the experiment was independently replicated three times. At each time point, the five larvae were transferred to 20-ml vials and acetone (4 ml) was added. The larvae were gently swirled for 2 s, the acetone decanted and transferred to a separate vial. The acetone was removed by evaporation and scintillation cocktail (5 ml) was added to each vial for quantification of external radioactivity determined by liquid scintillation counting (LKB 1209 Rack-Beta; Wallac Inc, Gaithersburg, MD; 95.0% counting efficiency). The original holding vials were also scintillation counted in a similar fashion to estimate excreted levels of radioactivity.

After rinsing with acetone as described above, the five larvae were homogenized in acetone (4 ml) using a rotor/stator-type biohomogenizer (Biospec Products, Bartlettville, OK). The vial and homogenizer were rinsed twice with acetone (2 + 1 ml). The homogenate and rinses were combined and centrifuged for 6 min at 2600 g and the supernatant decanted. The pellet was re-suspended in acetone (2 ml), vortexed, centrifuged and the second supernatant combined with the first. The remaining pellet (*i.e.* unextracted internal radioactivity) was solubilized for 72 h in 1 ml of Solvable® (NEN Research Products, Boston, MA), and the radioactive content determined by scintillation counting 100 μ l of the solubilized residue.

Acetone supernatants were evaporated to dryness under nitrogen. Water (double-distilled; 1 ml) was added and subsequently extracted in ethyl acetate (3 \times 2 ml). The combined ethyl acetate extracts were dried by adding anhydrous sodium sulfate (*c* 10 mg). Aqueous and organic radioactive content were determined by scintillation counting 100 μ l samples of each fraction.

The ethyl acetate fraction was evaporated to dryness under nitrogen in a water bath. The remaining residue was dissolved in methanol (100 μ l) and transferred to a clean tube. The solution was evaporated to dryness and redissolved in methanol (20 μ l). The entire volume of methanol solution was applied to pre-scored Whatman LK6DF thin layer chromatograph (TLC) plates and developed for 40 min with dichloromethane + acetone + ethyl acetate (95 + 3 + 2 by volume). The samples applied to the TLC plate were co-developed with technical standards (fipronil, sulfone, and sulfide) to identify radioactive bands. The TLC plates were dried and exposed to X-ray film (X-OMAT AR; Eastman Kodak, Rochester, NY) for 7 days at -20 °C. The TLC lanes corresponding to the technical standards and areas of exposure from the X-ray film were each scraped and

placed in 20-ml scintillation vials with scintillation cocktail (5 ml) for radioactivity determination. Results were combined and transformed to percentage of total radioactivity for each radioactive band per lane.

2.4 Enzyme preparation and P450 model substrate assay

Midguts from fifth-instar larvae were obtained by removal of head and thorax and the last two abdominal segments. The gut tissue was pulled from the body and a small glass test tube was gently rolled over the length of the gut to expel gut contents. The dissected gut tissue was held at ice temperature until homogenization.

Fresh gut tissue from 50 fifth-instar *O. nubilalis* larvae was homogenized with a Teflon glass Potter-Elvehjem type homogenizer in sodium phosphate buffer (0.1 M, pH 7.6; 1 ml) containing glycerol (150 g liter⁻¹).^{25,26} Gut homogenates were centrifuged at 10,000 g for 12 min at 4 °C in a microcentrifuge. The resulting supernatant was filtered through glass wool, and ultracentrifuged at 100,000 g for 60 min at 4 °C. The resulting microsomal pellet was resuspended in homogenization buffer (0.5 ml). Protein concentrations for all preparations were determined with a commercially available bicinchoninic acid protein assay kit (Pierce, Rockford, IL) according to the manufacturer's instructions using bovine serum albumin as the standard. Cytochrome P450-dependent monooxygenase activity was measured for each preparation by determining the rate of methoxyresorufin *O*-demethylation. The reaction mixture contained protein from the 100,000 g microsomal fraction (0.017–0.030 mg), methoxyresorufin (2.5 μM), with or without NADPH (1.5 mM), and with or without PBO (0.2 mM) to a total volume of 200 μl in sodium phosphate buffer (0.1 M, minus glycerol). Activity was measured in a 96-well fluorescent plate reader (Fluoroskan Ascent FL Series, Labsystems; Helsinki, Finland) with wavelength settings of 544 nm for excitation and 590 nm for emission. A standard curve of resorufin formation was used to determine the specific enzyme activity for the *O*-demethylation of methoxyresorufin.²⁷

2.5 *In vitro* metabolism

Metabolism of [¹⁴C]fipronil was studied *in vitro* using microsomal protein fractions prepared as described above from dissected *O. nubilalis* gut tissue. Individual reactions (0.5 ml) contained protein (0.34–0.44 mg), NADPH (1.5 mM), and [¹⁴C]fipronil (13 158 dpm) in sodium phosphate buffer (0.1 M, pH 7.6). Duplicate reactions were performed without NADPH (controls). An additional experiment was performed to determine the effect of PBO (0.2 mM), a specific inhibitor of P450 activity on sulfone formation. Reactions were initiated by the addition of NADPH and continued for 1 h at 31 °C with shaking. The reactions were terminated by partitioning the incubation mixture with ethyl acetate (3 × 1 ml).

The entire aqueous phase and a subsample (0.05 ml) of the combined ethyl acetate extracts were scintillation-counted to determine the proportion of radioactivity partitioning between the aqueous and organic phases. The ethyl acetate extracts were dried over anhydrous sodium sulfate and evaporated to dryness under nitrogen. The remaining residue was dissolved in methanol (75 μl), transferred to a new vial and evaporated under nitrogen. The residue was redissolved in methanol (20 μl).

Samples were analyzed by HPLC (Shimadzu Series SCL-10A, Kyoto, Japan), using a methanol + water gradient (78 + 22 to 72 + 28 ml over a 12-min period) with a reverse-phase C₁₈ column (Luna 5 μm, 25 mm × 4.6 mm ID; Phenomenex, Torrance, CA) to separate fipronil from the sulfone metabolite.⁴ Quantification of the parent molecule and metabolite was achieved with an in-line flow scintillation analyzer (Radiomatic 500TR Series, Packard Instrument Co, Downers Grove, IL). Known quantities of non-radioactive and radioactive fipronil and fipronil-sulfone were injected before each experiment and radioactive peaks were identified by co-chromatography with cold standards. Known quantities of [¹⁴C]fipronil were injected into the HPLC to produce a standard curve, which was used in combination with analytical software (FLO-ONE for Windows Analysis–Version 3.61, Packard Instrument Co, Meriden, CT) accompanying the flow scintillation analyzer, to determine the correlation between the area reported by the scintillation detector and the radioactivity injected.

3 RESULTS

3.1 *In vivo* metabolism

Over the 24-h sampling period, the majority of radioactivity was recovered in the external rinse (71%), and 11.1% of the total radioactivity applied was recovered from the internal fractions (Table 1). Approximately 17% of the recovered radioactivity was present in the holding vials and is likely to represent a combination of excreted radioactivity and transfer from the insect integument to the holding vial. The majority of internalized radioactivity was present in the organo-soluble fraction, with 1.1% or less of total radioactivity comprising the aqueous and unextracted internal fractions. Although movement through the integument was slow, the percentage of internalized radioactivity increased over the 24-h sampling period. Percentage recovery was close to 100% for each time interval.

Thin layer chromatography of the internal organic fraction produced four regions of radioactivity identified by autoradiography. Two bands migrated with cold standards of fipronil ($R_f = 0.56$) and fipronil-sulfone ($R_f = 0.69$). Additional radioactivity was identified on the autoradiogram at the origin ($R_f = 0.00$) and as a smear ($R_f = 0.10$) just above the loading zone of the TLC plate. The smear is likely composed of dehalogenated and/or conjugated fipronil and fipronil-sulfone.¹⁷ The majority of radioactivity in the organo-sol-

Table 1. Percentage distribution and recovery of radioactivity following topical treatment of fifth-instar *Ostrinia nubilalis* larvae with [¹⁴C]fipronil

| Fraction | Radioactivity recovered (%) (\pm SEM) ^a at | | | | | |
|---|--|--------------------|--------------------|-------------------|--------------------|--------------------|
| | 0.5h | 1h | 3h | 6h | 12h | 24h |
| External radioactivity ^b | 91.6 (\pm 1.5) | 88.7 (\pm 0.7) | 84.4 (\pm 0.6) | 79.1 (\pm 1.8) | 73.8 (\pm 1.5) | 71.5 (\pm 2.2) |
| Holding vial radioactivity ^c | 4.2 (\pm 0.9) | 7.3 (\pm 0.5) | 9.4 (\pm 0.8) | 14.2 (\pm 1.2) | 15.8 (\pm 1.5) | 17.3 (\pm 3.6) |
| Internal organosoluble radioactivity ^d | 3.4 (\pm 1.3) | 3.1 (\pm 1.0) | 4.9 (\pm 0.2) | 5.6 (\pm 1.0) | 9.0 (\pm 0.6) | 10.0 (\pm 1.3) |
| Internal aqueous radioactivity ^e | 0.2 (\pm 0.1) | 0.4 (\pm 0.2) | 0.6 (\pm 0.3) | 0.4 (\pm 0.1) | 0.8 (\pm 0.2) | 0.5 (\pm 0.2) |
| Unextracted internal radioactivity ^f | 0.5 (\pm 0.2) | 0.4 (\pm 0.2) | 0.6 (\pm 0.3) | 0.5 (\pm 0.3) | 0.6 (\pm 0.1) | 0.6 (\pm 0.2) |
| Percentage recovery of radioactivity ^g | 117.9 (\pm 2.5) | 101.5 (\pm 1.9) | 100.5 (\pm 5.4) | 97.3 (\pm 1.9) | 109.0 (\pm 5.4) | 106.5 (\pm 2.8) |

^a 29 485 dpm [¹⁴C]fipronil applied to each group of five larvae; $n = 3$.

^b Radioactivity present in larval acetone rinses.

^c Excreted radioactivity and radioactivity transferred directly from insect integument.

^d Organo-soluble radioactivity present in ethyl acetate phase after partitioning between ethyl acetate and water.

^e Aqueous radioactivity after partitioning between ethyl acetate and water.

^f Radioactivity remaining in tissue pellets.

^g Percentage recovery of radioactivity based on total radioactivity (29 485 dpm) applied per replicate per time period.

uble fraction was unmetabolized fipronil, which increased as a percentage of total radioactivity over time (Figure 1). The sulfone metabolite of fipronil was the major metabolite identified, and the concentration generally increased during the first 6 h. The unidentified metabolite, directly above the origin, decreased over time, while the amount of radioactivity at the origin remained as a small percentage (less than 3%) of the total radioactivity recovered.

3.2 *In vitro* metabolism

Microsomal preparations of *O. nubilalis* midgut homogenates contained highly active NADPH-dependent microsomal monooxygenase activity toward the model substrate, methoxyresorufin. The cytochrome P450 specific activity ($0.31 \mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$) was NADPH-dependent (1.5 mM) and was inhibited by piperonyl butoxide (0.2 mM) (data not shown).

Fipronil and fipronil-sulfone standards and experimental samples were separated by HPLC using a methanol: water gradient. Fipronil and the sulfone metabolite eluted from the column at $7.7 (\pm 0.2)$ and $9.3 (\pm 0.2)$ min after injection, re-

spectively (Fig 2). The interval between UV and scintillation detection coincided with the expected delay in retention time based on the physical separation of the two detectors.

The formation and detection of radiolabeled sulfone was NADPH-dependent (Fig 3). The distribution of radioactivity recovered after HPLC separation and liquid flow scintillation counting is reported in Table 2. The sulfone metabolite could not be detected when NADPH was absent from the reaction mixture, and sulfone formation was completely inhibited by the cytochrome P450 monooxygenase inhibitor, PBO (Table 2).

4 DISCUSSION AND CONCLUSIONS

The rate of [¹⁴C]fipronil penetration into *O. nubilalis* larvae was relatively slow, with 71% of the applied radioactivity remaining in surface rinses 24 h after application. Additionally, fipronil does not appear to be metabolized rapidly, although fipronil-sulfone was the major metabolite identified by *in vivo* metabolism experiments. The microsomal monooxygenase detoxification system appears to be intricately involved in the metabolism of fipronil, as for-

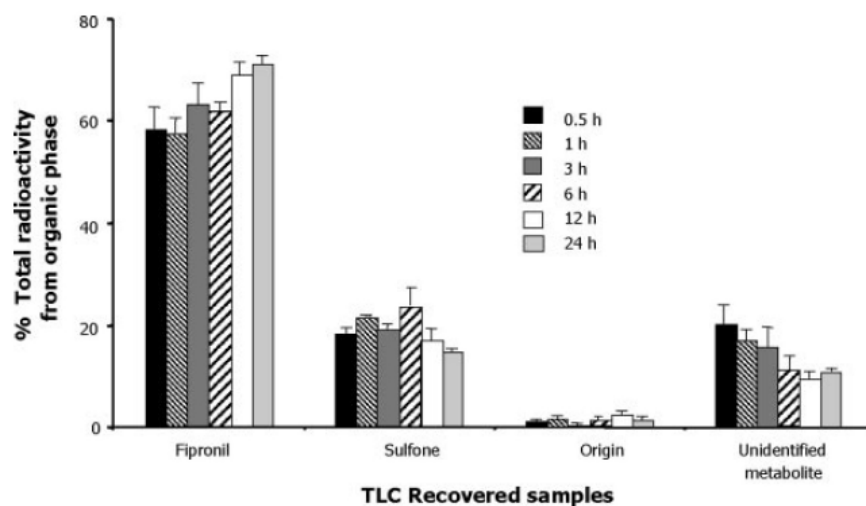


Figure 1. Percentage distribution of radioactivity among four TLC zones (fipronil, fipronil-sulfone, origin and unidentified metabolite) isolated from the organo-soluble fraction following *in vivo* treatment with [¹⁴C]fipronil ($n = 3$).

mation of the sulfone metabolite is dependent on this enzyme system. Previous studies have indicated the involvement of this system in the metabolism of fipronil by the use of cytochrome P450 inhibitors such as PBO and *N*-benzylimidazole (NBI).^{3,17,19} Fipronil toxicity was enhanced by the pretreatment with PBO in house flies,^{1,5} but was slightly antagonized⁶ or had no effect⁵ in German cockroaches, *Blattella germanica* (L). These results suggest that blocking the metabolic conversion of fipronil to the sulfone metabolite has a negligible effect on toxicity because both molecules are effective toxins. In addition, these results suggest that there are slight differences in sensitivity between insect groups for the two compounds. House flies appear to be more affected by the parent molecule whereas German cockroaches are equally or slightly more affected by fipronil-sulfone.

Brookhart and Bushey¹⁵ reported fipronil-sulfone was the predominant metabolite formed from [¹⁴C]fipronil when orally administered to the southern armyworm, *Spodoptera eridania* (Cram). The EPA new pesticide fact sheet,¹⁸ reported that the major oxidation product, MB 46136 (fipronil-sulfone) was present in all fractions (urine, feces and tissues) tested from rats orally dosed with [¹⁴C]fipronil. These studies also reported trace amounts of another minor oxidative product, MB 45950 (sulfide),^{15,18} which was not observed in the present study. In a study examining mice treated with [¹⁴C]fipronil, fipronil-sulfone was reported to be the exclusive metabolite formed and distributed in tissues (brain, liver, kidney, fat) and the feces, and no polar metabolites were detected in the aqueous extracts.⁴

Dupuy *et al*¹⁹ reported conclusive evidence that fipronil metabolism was facilitated by microsomal monooxygenases in rats. These authors reported fipronil oxidation was

inhibited (100%) by clotrimazole, a P450-3A subfamily inhibitor, and sulfone formation was enhanced (149%) by the P450-3A6 inducer in rabbits. Other P450 inhibitors (proadifen, metyrapone) also reduced the formation of fipronil-sulfone but to a lesser extent. In a separate study, *in vivo* and *in vitro* experiments utilizing a susceptible *D v virgifer* population showed that fipronil-sulfone was formed exclusively by the cytochrome P450 enzyme system.¹⁷

In house flies, Hainzl *et al*³ reported that 90% of topically applied fipronil was converted to the sulfone within 24 h, and this conversion was completely blocked by PBO. It was also observed that PBO increased the metabolic stability of both fipronil and fipronil-sulfone, and increased the level of inhibition of [³H]EBOB binding, a ligand theorized to bind at the same region as fipronil and fipronil-sulfone. The conversion of fipronil to fipronil-sulfone is totally blocked for at least 2 h in the liver and brain tissue of mice treated intraperitoneally with PBO and then with radiolabeled fipronil. These four studies provide strong evidence supporting the present observations that fipronil conversion to fipronil-sulfone is catalyzed by cytochrome P450s.

Table 2. Percentage distribution of organo-soluble metabolites identified by HPLC injection following *in vitro* treatment of microsomes with [¹⁴C]fipronil from a susceptible *Ostrinia nubilalis* population^a

| Treatment | Radioactivity recovered (%) (\pm SEM) ^a | |
|-----------|--|-------------------------------|
| | Fipronil ^b | Fipronil-sulfone ^c |
| (-) NADPH | 99.6 (\pm 0.4) | 0.4 (\pm 0.4) |
| (-) PBO | | |
| (+) NADPH | 85.9 (\pm 0.9) | 14.0 (\pm 0.9) |
| (-) PBO | | |
| (+) NADPH | 100.0 (\pm 0.0) | 0.0 (\pm 0.0) |
| (+) PBO | | |

^a $n = 3$ for [(-) NADPH (-) PBO] and [(+) NADPH (+) PBO]; $n = 9$ for [(+) NADPH (-) PBO] \pm standard error of the mean.

^b Percentage of averaged total radioactivity detected 0.3 min after fipronil eluted from the column, 8.0 (\pm 0.2) min.

^c Percentage of averaged total radioactivity detected 0.3 min after fipronil-sulfone eluted from column, 9.5 (\pm 0.2) min.

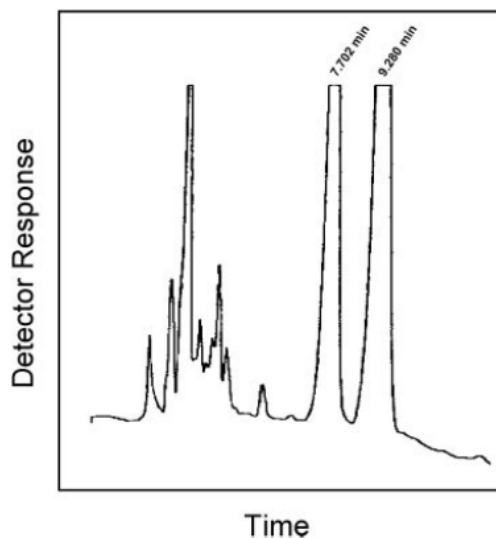


Figure 2. UV detection of 10 $\mu\text{g ml}^{-1}$ solutions of fipronil and fipronil-sulfone standards (7.702 min and 9.280 min retention times, respectively) separated by HPLC.

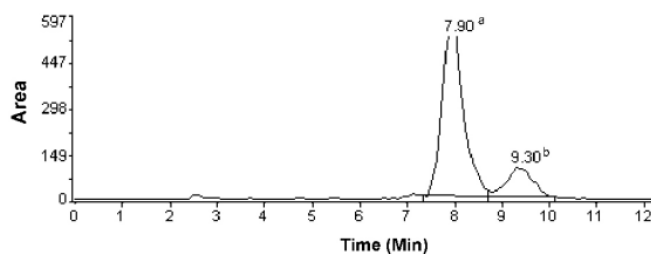


Figure 3. Radiometric detection of [¹⁴C]fipronil (7.90 min; a) and its sulfone metabolite (9.30 min; b) formed during *in vitro* metabolism experiments containing NADPH (1.5 mM) and separated by HPLC.

The results from the present experiments conclusively show that late-instar *O. nubilalis* larvae metabolize fipronil via cytochrome P450 microsomal monooxygenases to form the sulfone metabolite. Fipronil-sulfone was the major metabolite identified from *in vivo* metabolism of [¹⁴C]fipronil, and sulfone formation by microsomal preparations from midgut homogenates was NADPH-dependent and inhibited when the P450 inhibitor, PBO, was added to the reaction mixture containing both the substrate and cofactor. There are likely to be penetration, pharmacokinetic, and kinetic differences between different species in the rate of oxidative sulfone formation, although experimental conclusions strongly suggest that sulfone formation in *O. nubilalis* larvae is dependent on cytochrome P450 activity. Because both the metabolite and parent molecule are highly toxic to *O. nubilalis* larvae, this metabolic pathway is critical to understanding the overall toxic response to fipronil.

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