Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supporting Information for

Metabolization and Degradation Kinetics of the Urban-use Pesticide Fipronil by White Rot Fungus *Trametes versicolor*

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I. Supporting Method Details

A. LC/MS MRM Parameters

Table S.1. LC-MS parameters for fipronil and fipronil metabolites

MRM Mass Transition	Q1 Mass (Da)	Q3 Mass (Da)	Declustering Potential (V)	Focusing Potential (V)	Entrance Potential (V)	Collision Energy (V)	Collision Cell Exit Potential (V)
Fipronil-1	434.971	330.000	-66	-270	-10	-24	-11
Fipronil-2	434.971	250.000	-66	-270	-10	-36	-13
Fipronil Sulfone-1	450.898	415.000	-81	-250	-10	-24	-15
Fipronil Sulfone-2	450.898	282.000	-81	-250	-10	-40	-19
Fipronil desulfinyl-1	387.947	282.978	-91	-280	-10	-42	-5
Fipronil desulfinyl-2	387.947	325.962	-91	-280	-10	-42	-9
M468-1	466.921	430.933	-30	-200	-10	-30	-15
M468-2	466.921	132.957	-30	-200	-10	-30	-15
M600-1	598.963	281.994	-30	-200	-10	-30	-15
M600-2	598.963	316.958	-30	-200	-10	-30	-15
M600-3	598.963	430.943	-30	-200	-10	-30	-15
M600-4	598.963	449.918	-30	-200	-10	-30	-15
M600-5	598.963	414.951	-30	-200	-10	-30	-15

B. Metabolomics Method Details: XCMS and R Code

Agilent MassHunter data files were converted to mzXML format using Trapper (Seattle Proteome Center). mzXML files were analyzed by XCMS; procedural details are provided in the relevant references.[1] Further data processing of the resultant comparisons was described in the manuscript body in the Methods section. A sample R code for a comparison between treatment and control groups is as follows:

```
library(xcms)
xset<-xcmsSet()
xset<-group(xset)
xset2<-retcor(xset,family="s",plottype="m")
xset2<-group(xset2)
xset3<-retcor(xset2,family="s",plottype="m")
xset3<-group(xset3)
xset4<-retcor(xset3,family="s",plottype="m")
xset4<-group(xset4,bw=10)
xset5<-fillPeaks(xset4)
reporttab<-
diffreport(xset5,"Treatment","Control","Treatment_vs_Control",500)
```

C. Purity of purchased standards

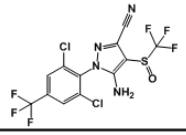
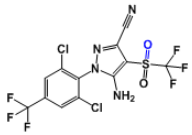
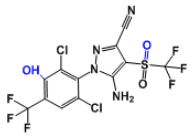
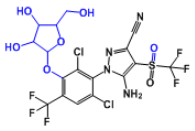
Table S.2. Purity of purchased standards. All standards were PESTANAL® analytical standards purchased from Sigma-Aldrich.

Standard	CAS #	Purity (%)
Fipronil	120068-37-3	99.6
Fipronil sulfone	120068-36-2	99.9
Fipronil desulfinyl	205650-65-3	> 94
Fipronil sulfide	120067-83-6	99.5

II. Supplementary Results

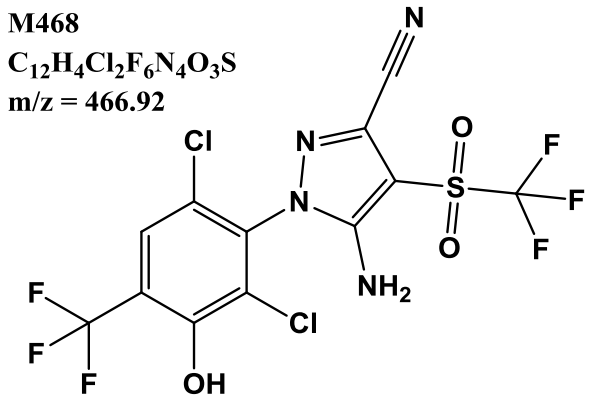
A. Fipronil metabolite data with full MS/MS fragment ion data

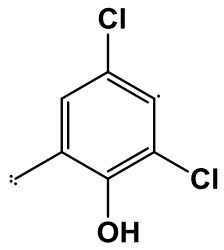
Table S.3. Fipronil and metabolite data with full MS/MS fragment ion data. This table supplements Table 1 in the manuscript body.

Fipronil fungi metabolites							Fragment ions		
Metabolite		**Proposed Structure**	**Proposed Formula**	**Confidence Level***	**Retention Time (min)**	**Accurate Mass (m/z)**	**Nominal mass (m/z)**	**Accurate mass (m/z)**	**Proposed molecular formula**
Fipronil			$C_{12}H_4Cl_2F_6N_4OS$	Parent compound	14.3	434.93143	399	398.95	$C_{12}H_2ClF_6N_4OS$
							330	329.95	$C_{11}H_2ClF_3N_4OS$
							250	249.959	$C_{10}H_4Cl_2N_4$
Fipronil sulfone			$C_{12}H_4Cl_2F_6N_4O_2S$	Level 1; Standard confirmation, HR-MS, MS-MS	15.9	450.92691	431	430.9393	$C_{12}H_2ClF_6N_4O_3S$
							282	281.9963	$C_{11}H_2ClF_3N_4$
							415	414.9403	$C_{12}H_2ClF_6N_4O_2S$
							244	243.989	$C_{10}H_4N_4O_2S$
							133	132.9578	CF_3O_2S
M468	Hydroxylated fipronil sulfone		$C_{12}H_4Cl_2F_6N_4O_3S$	Level 3; HR-MS, MS/MS	14.9	466.92136	415	414.9403	$C_{12}H_2ClF_6N_4O_2S$
							173	172.9529	$C_7H_3Cl_2O$
							133	132.9578	CF_3O_2S
							220	219.9903	$C_5H_2F_2N_4O_2S$
							431	430.9393	$C_{12}H_2ClF_6N_4O_3S$
M600	Glycosylated fipronil sulfone		$C_{17}H_{12}Cl_2F_6N_4O_7S$	Level 3; HR-MS, MS/MS	12.1	598.96256	415	414.9403	$C_{12}H_2ClF_6N_4O_2S$
							431	430.9393	$C_{12}H_2ClF_6N_4O_3S$
							213	212.949	$C_7H_2Cl_2F_3$
							220	219.9903	$C_5H_2F_2N_4O_2S$
							282	281.994	$C_{11}H_2ClF_3N_4$
M584	Unknown	Unknown	Ambiguous	Level 5; HR-MS, exact mass	19.3	582.87523	Unknown	n/a	n/a
M770	Unknown	Unknown	Ambiguous	Level 5; HR-MS, exact mass	22.0	768.87632	Unknown	n/a	n/a

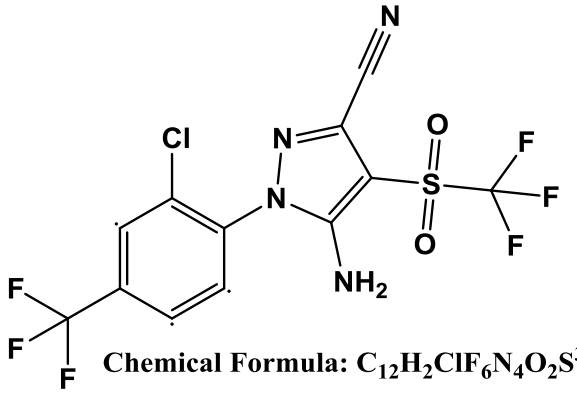
B. Visual representation of MS/MS fragment analysis

M468
$C_{12}H_4Cl_2F_6N_4O_3S$
m/z = 466.92

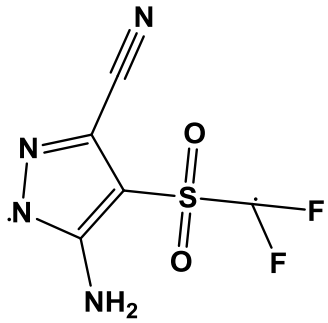




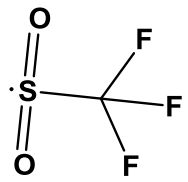
Chemical Formula: $C_7H_3Cl_2O^{3\bullet}$
Exact Mass: 172.96



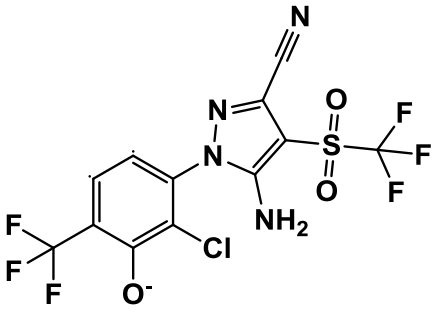
Chemical Formula: $C_{12}H_2ClF_6N_4O_2S^{3\bullet}$
Exact Mass: 414.95



Chemical Formula: $C_5H_2F_2N_4O_2S^{2\bullet}$
Exact Mass: 219.99



Chemical Formula: $CF_3O_2S^{\bullet}$
Exact Mass: 132.96

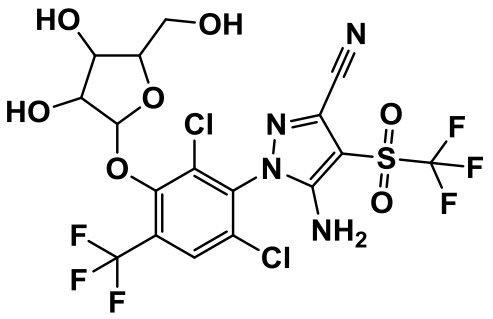


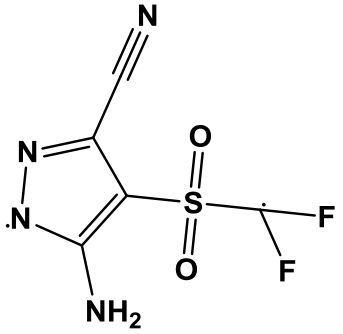
Chemical Formula: $C_{12}H_2ClF_6N_4O_3S^{2\bullet-}$
Exact Mass: 430.94

M600

$C_{17}H_{12}Cl_2F_6N_4O_7S$

m/z = 598.96



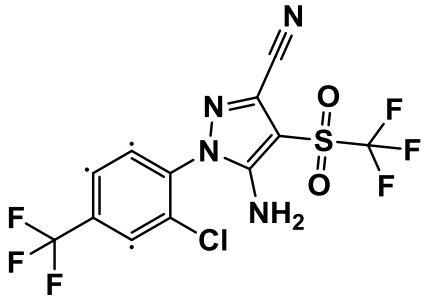


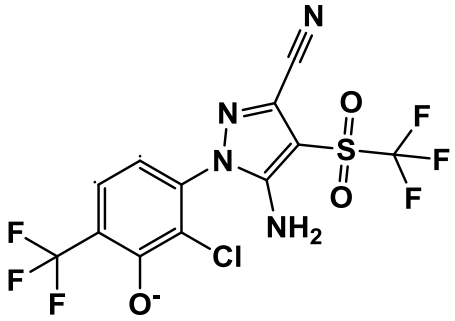
Chemical Formula: $C_5H_2F_2N_4O_2S^{2\bullet}$

Exact Mass: 219.99

Chemical Formula: $C_{12}H_2ClF_6N_4O_2S^{3\bullet}$

Exact Mass: 414.95



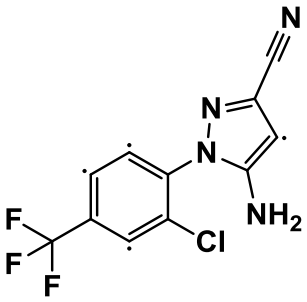


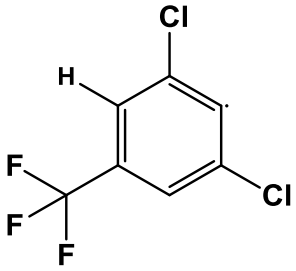
Chemical Formula: $C_{12}H_2ClF_6N_4O_3S^{2\bullet-}$

Exact Mass: 430.94

Chemical Formula: $C_{11}H_2ClF_3N_4^{4\bullet}$

Exact Mass: 281.99





Chemical Formula: $C_7H_2Cl_2F_3^{\bullet}$

Exact Mass: 212.95

C. Statistics for comparison tests

Table S.4. Results of Tukey comparison for all treatment groups and measured fipronil derivatives. The alpha for the post-test was set at 0.05 to generate the reported adjusted p-values.

log (concentration fipronil [µg/L])	Mean Diff.	95% CI of diff.	Significant?	Adjusted P Value
Treatment vs. Negative control	-0.58	-1.1 to -0.078	Yes	0.0199
Enzyme-inhibited treatment vs. Negative control	-0.053	-0.55 to 0.44	No	0.9903
Sorption control vs. Negative control	-0.14	-0.63 to 0.36	No	0.8688
Enzyme-inhibited treatment vs. Treatment	0.52	0.024 to 1.0	Yes	0.0377
Sorption control vs. Treatment	0.44	-0.058 to 0.94	No	0.0958
Sorption control vs. Enzyme-inhibited treatment	-0.083	-0.58 to 0.41	No	0.9658

log (concentration fipronil sulfone [µg/L])	Mean Diff.	95% CI of diff.	Significant?	Adjusted P Value
Treatment vs. Negative Control	1.5	0.55 to 2.5	Yes	0.0015
Enzyme-inhibited treatment vs. Negative Control	0.94	-0.040 to 1.9	No	0.0629
Sorption control vs. Negative Control	0.80	-0.18 to 1.8	No	0.1368
Enzyme-inhibited treatment vs. Treatment	-0.59	-1.6 to 0.39	No	0.3547
Sorption control vs. Treatment	-0.74	-1.7 to 0.25	No	0.1883
Sorption control vs. Enzyme-inhibited treatment	-0.14	-1.1 to 0.84	No	0.9767

log (peak area of M468)	Mean Diff.	95% CI of diff.	Significant?	Adjusted P Value
Treatment vs. Negative Control	1.4	0.46 to 2.4	Yes	0.0027
Enzyme-inhibited treatment vs. Negative Control	0.048	-0.91 to 1.0	No	0.999
Sorption control vs. Negative Control	0.033	-0.93 to 1.0	No	0.9997
Enzyme-inhibited treatment vs. Treatment	-1.4	-2.3 to -0.41	Yes	0.0037
Sorption control vs. Treatment	-1.4	-2.4 to -0.43	Yes	0.0033
Sorption control vs. Enzyme-inhibited treatment	-0.015	-0.98 to 0.95	No	> 0.9999

log (peak area of M600)	Mean Diff.	95% CI of diff.	Significant?	Adjusted P Value
Treatment vs. Negative Control	0.83	0.21 to 1.5	Yes	0.0063
Enzyme-inhibited treatment vs. Negative Control	-0.099	-0.72 to 0.52	No	0.9697
Sorption control vs. Negative Control	-0.061	-0.68 to 0.56	No	0.9926
Enzyme-inhibited treatment vs. Treatment	-0.93	-1.5 to -0.31	Yes	0.0023
Sorption control vs. Treatment	-0.89	-1.5 to -0.27	Yes	0.0034
Sorption control vs. Enzyme-inhibited treatment	0.038	-0.58 to 0.66	No	0.9981

Biomass (mg)	Mean Diff.	95% CI of diff.	Significant?	Adjusted P Value
Enzyme-inhibited treatment vs. Treatment	-0.80	-4.6 to 3.0	No	0.8446
Sorption control vs. Treatment	-2.05	-5.9 to 1.8	No	0.3568
Sorption control vs. Enzyme-inhibited treatment	-1.3	-5.1 to 2.6	No	0.6664