Supporting information for:

In vitro biotransformation of pharmaceuticals and pesticides by trout liver S9 in the presence and absence of carbamazepine

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Table S1. Physico-chemical properties of target compounds

Target compounds	***Specific classification***	***CAS #***	***Formula***	***Exact mass***	***logK_{ow}****	***ISTD****
Carbamazepine (CMZ)	Antiepileptic	298-46-4	$C_{15}H_{12}N_2O$	236.0944	2.8	Carbamazepin-C13,D2
Azoxystrobin (AZX)	Fungicide	131860-33-8	$C_{22}H_{17}N_3O_5$	403.1163	4.2	Azoxystrobin-d4
Diazinon (DIZ)	Insecticide	333-41-5	$C_{12}H_{21}N_2O_3PS$	304.1005	4.2	Diazinon-D10
Fipronil (FPN)	Insecticide	120068-37-3	$C_{12}H_4Cl_2F_6N_4OS$	435.9387	4.5	Diclofenac-D4
Propranolol (PRN)	Beta-blocker	525-66-6	$C_{16}H_{21}NO_2$	259.1567	2.6	Propranolol-D7

* estimated by MarvinSketch Ver. 5.10.3, **Isotope-labelled internal standard for quantification of parent compounds and their metabolites

55 Table S2. Information on suppliers and purity/grade for used chemicals
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Compound	Supplier	Remarks
Carbamazepine (CMZ)	Toronto Research Chemicals Inc.	98.4 %
Azoxystrobin (AZX)	Toronto Research Chemicals Inc.	> 98 %
Diazinon (DIZ)	Dr. Ehrenstorfer GmbH	> 99 %
Fipronil (FPN)	Sigma-Aldrich Co. LLC	98 %
Propranolol (PRN)	Toronto Research Chemicals Inc.	98.7 %
Carbamazepin-10,11-epoxid (MCM)	Toronto Research Chemicals Inc.	> 99 %
Azoxystrobin acid (MAZ1)	HPC Standards GmbH	99 %
Diazoxon (MDI1)	Dr. Ehrenstorfer GmbH	> 98 %
Pyrimidinol (MDI2)	Sigma-Aldrich Co. LLC	99 %
Fipronil-sulfide (MFP1)	Dr. Ehrenstorfer GmbH	> 99%
Fipronil-sulfon (MFP2)	Dr. Ehrenstorfer GmbH	> 98 %
Carbamazepin-C13,D2	Dr. Ehrenstorfer GmbH	> 98 %
Azoxystrobin-d4	Sigma-Aldrich Co. LLC	98 %
Diazinon-D10	Dr. Ehrenstorfer GmbH	> 98 %
Diclofenac-D4	Toronto Research Chemicals Inc.	> 98 %
Propranolol-D7	Toronto Research Chemicals Inc.	> 98 %
Ammonium acetate	Merck	Analytical grade
Calcium chloride	Sigma-Aldrich Co. LLC	Analytical grade
Formic acid	Acros Organics	HPLC grade
β-Nicotinamide adenine dinucleotide phosphate (NADPH), reduced tetra salt	Sigma-Aldrich GmbH	> 95 %
Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA)	Sigma-Aldrich GmbH	> 99%
Trout Liver S9 Homogenate	Life Technology (Basel, CH)	20 mg protein/ml
Bovine Serum Albumin	Sigma	> 96%
Water	Acros Organics	HPLC grade
Methanol	Acros Organics	HPLC grade
Acetonitrile	Acros Organics	HPLC grade

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Table S3. Predicted biotransformation reactions and the resulting changes in molecular formula and exact mass occurring at parent compounds (Carbamazepine (CMZ), Azoxystrobin (AZX), Diazinon (DIZ), Fipronil (FPN), and Propranolol (PRN))

	reactions	loss	gain	Δmass	Parent compound
Universal	Hydroxylation, N/S-Oxidation, or Epoxidation		O	+15.9949	
	Demethylation	CH2		-14.0157	
	Dehydrogenation	H2		-2.0157	
	Hydrogenation		H2	+2.0157	
Structure specific	Oxidative displacement of amine	NH2	OH	+0.9840	CMZ,
	N-Dealkylation1	CH2NO	H	-43.0058	CMZ
	O-Dealkylation1	C7H4N	H	-101.0266	AZX
	O-Dealkylation2	C11H6N3O	H	-195.0433	AZX
	O-Dealkylation3	C2H4		-28.0313	DIZ
	O-Dealkylation4	C4H8		-56.0626	DIZ
	O-Dealkylation5	C4H10O2PS	H	-152.0061	DIZ
	S-Dealkylation1	CF3	H	-67.9874	FPN
	N-Dealkylation2	C7H2F3Cl2	H	-211.9407	FPN
	O-Dealkylation6	C6H13NO		-115.0997	PRN
	Deisopropylation	C3H6		-42.0470	PRN

Table S4. Time program of HPLC gradient for mobile and loading phase.

Separation	Time (min)	H_2O (µL/min)	MeOH (µL/min)
Mobile phase	0.00	40	260
	4.00	40	260
	14.00	280	20
	26.00	280	20
	26.20	40	260
	32.30	40	260
Enrichment	Time (min)	2mM ammonium acetate* (µL/min)	Acetonitrile** (µL/min)
Loading phase	0.00	200	0
	0.10	0	2000
	0.60	0	2000
	0.65	2000	0
	5.60	2000	0
	5.65	400	0
	6.20	400	0
	6.30	0	400
	9.90	0	400
	10.00	400	0
	20.60	400	0
	20.70	2000	0
	32.00	2000	0
	32.10	200	0

*ammonium acetate solution was used for loading online sample, **acetonitrile was used for cleaning line, loop, and SPE cartridge where the sample passes through.

Table S5. Adjusted conversion factors calculated by two regression models to estimate log IE based on physic-chemical properties (pKa and log (molecular volume)) and chromatographic retention time.

Molecules	*Conversion factor **	*Adjusting factor***	*Adjusted conversion factor****
Propranol	1.00	1.00	1.00
MPR218	0.32	0.94	0.30
MPR276A	0.53	0.83	0.44
MPR276B	1.12	1.12	1.25

*Relative IE of BTP to the parent calculated by the multivariable regression model (pka/MV vs IE, equation (1)-(3) in SI of Jeon et al. (Jeon et al., 2013)), ** Relative IE of BTP to the parent calculated by the linear regression curves (retention time vs IE, shown in Figure S4 in SI of Jeon et al. (Jeon et al., 2013)) , *** Adjusted conversion factor = conversion factor x adjusting factor

Table S6. Parameters used in SIEVE 2.1 for comparative analysis over two different raw data sets to identify non-targeted metabolites.

Alignment parameters		**Frame parameters**		**Identification parameters**	
AlignmentBypass	False	FrameFromMS2	False	Adduct	M+H
AlignmentMinIntensity	1000	MaximumFrames	20000	AccumedCharge	1
CorrelationBinWidth	1	MZStart	150	ChemSpiderDBs	KEGG
RTLimitsForAlignment	True	MZStop	600	FrameIDCriteria	Order by P value
TileIncrement	150	MZWidth	0.02	MaximumIDs	1000
TileMaximum	300	PRMaxCharge	5	MZTolerence	0.02
TileSize	300	RTStart	10-14	RandomSeed	12345
TileThreshold	0.6	RTStop	18-20	SearchSource	SEQUEST
		RTWith	2.5	UserPercolator	False
		Threshold	5000		

SI A. Setting parameters for ionization and data acquisition in QExactive

Detection of analytes was performed with the QExactive using electrospray ionization (ESI) interface in positive and negative mode (Q Exactive Orbitrap LC-ESI-MS/MS, Thermo Fisher Scientific Inc.). The spray voltage was +4000 V (positive mode) or -3000 V (negative mode), the capillary temperature was held at 350 °C, S-lens-level was set at 50 (default value, numerical factor from 0-100), the auxiliary gas flow rate was 15 L/min, and the sheath gas flow rate was 40 L/min for both ionization modes. A mass range from 100 - 1000 m/z was analyzed in full scan with a maximal ion count of 1,000,000. External mass calibration was used to ensure a mass accuracy of < 3 ppm over the mass range. The QExactive operation method was designed to perform data-dependent MS/MS for the five most intense ions among the mass list of parents and BTPs for each scan event. During the MS/MS acquisition, 'dynamic exclusion' function was simultaneously applied to prevent an ion from triggering a subsequent data-dependent MS/MS scan for a pre-selected length of time (we chose 3 sec) after it has already triggered a data-dependent MS/MS scan. If no mass from the list of predicted BTPs was found, the most intense ions were fragmented. Optimum collision energy for MS/MS fragmentation in the higher energy collision dissociation (HCD) was estimated by an empirical linear equation which is a function of exact mass. High-resolution mass spectra with a resolution of 70,000 and a mass accuracy of <3 ppm were recorded. Data-dependent MS/MS acquisition at a resolution of 35,000 was triggered at each scan event. Data were analyzed with Xcalibur (Thermo Scientific, USA) in the Qual Browser for identification.

Table S7. Recovery, limit of detection (LOD), and limit of quantification (LOQ) of parent compounds and metabolites with S9 matrix for online-SPE-Orbitrap method

Target compounds	Recovery* (%)	LOQ** (pg/mL)
Carbamazepine (CMZ)	118	0.1
Azoxystrobin (AZX)	100	0.5
Diazinon (DIZ)	105	0.5
Fipronil (FPN)	85	5.0
Propranolol (PRN)	128	0.1
Carbamazepin-10,11-epoxid (MCM)	75	0.5
Azoxystrobin acid (MAZ1)	88	0.5
Diazoxon (MDI1)	60	1.0
Pyrimidinol (MDI2)	80	0.1
Fipronil-sulfide (MFP1)	75	1.0
Fipronil-sulfon (MFP2)	70	5.0

* Recovery of whole sample treatment procedure for S9 matrix (injection amount : 2 ng for the parents, 0.1 for the metabolites), **Limit of quantification for online SPE-Orbitrap method, defined as concentration yielding 10 times greater than signal to noise

Figure S1. Variation of propranolol concentrations in active S9 fraction and heat-treated control. (Error bars indicate standard deviation between replicates, n=3)

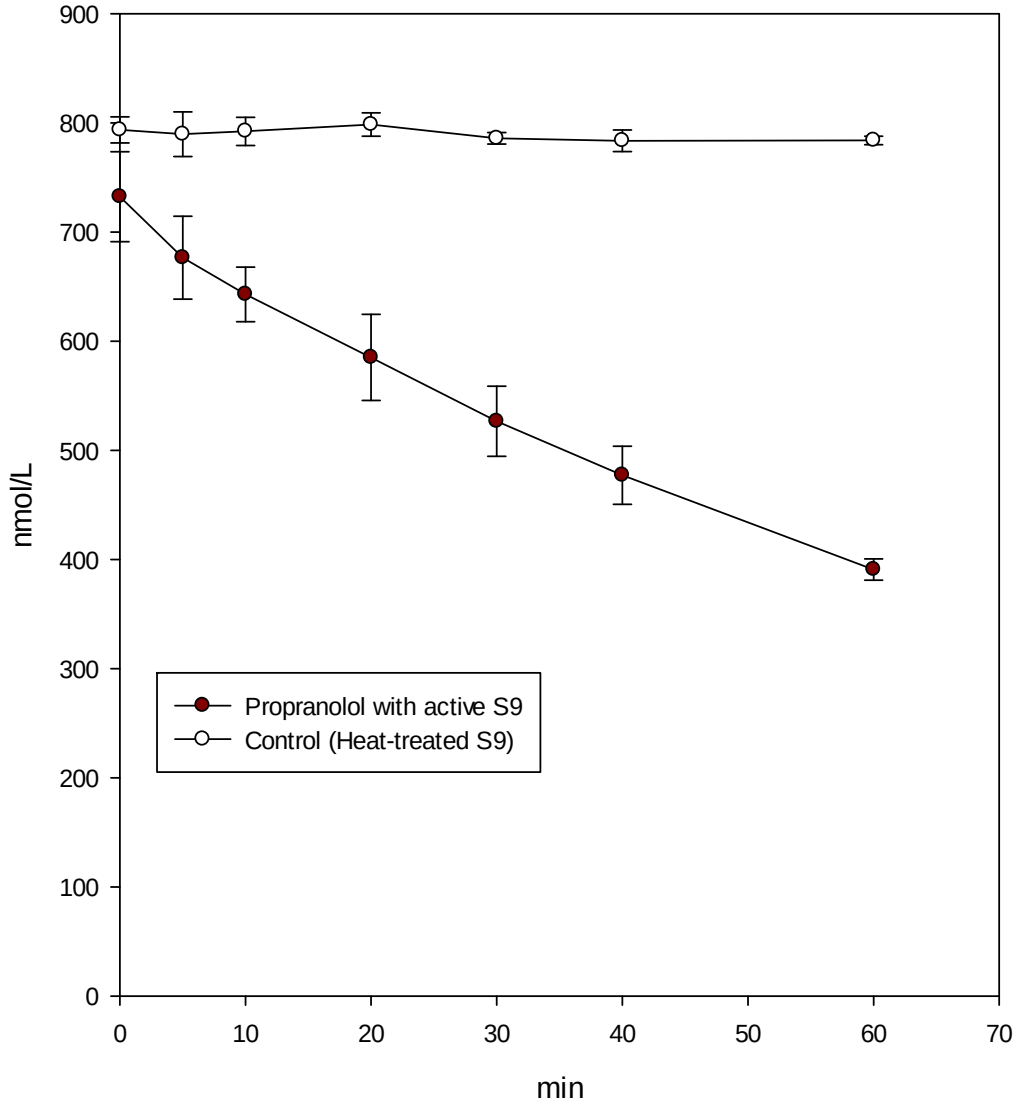


Figure S2. Extracted ion chromatograms of propranolol and its metabolites with retention time and normalized largest peak intensity (NL)

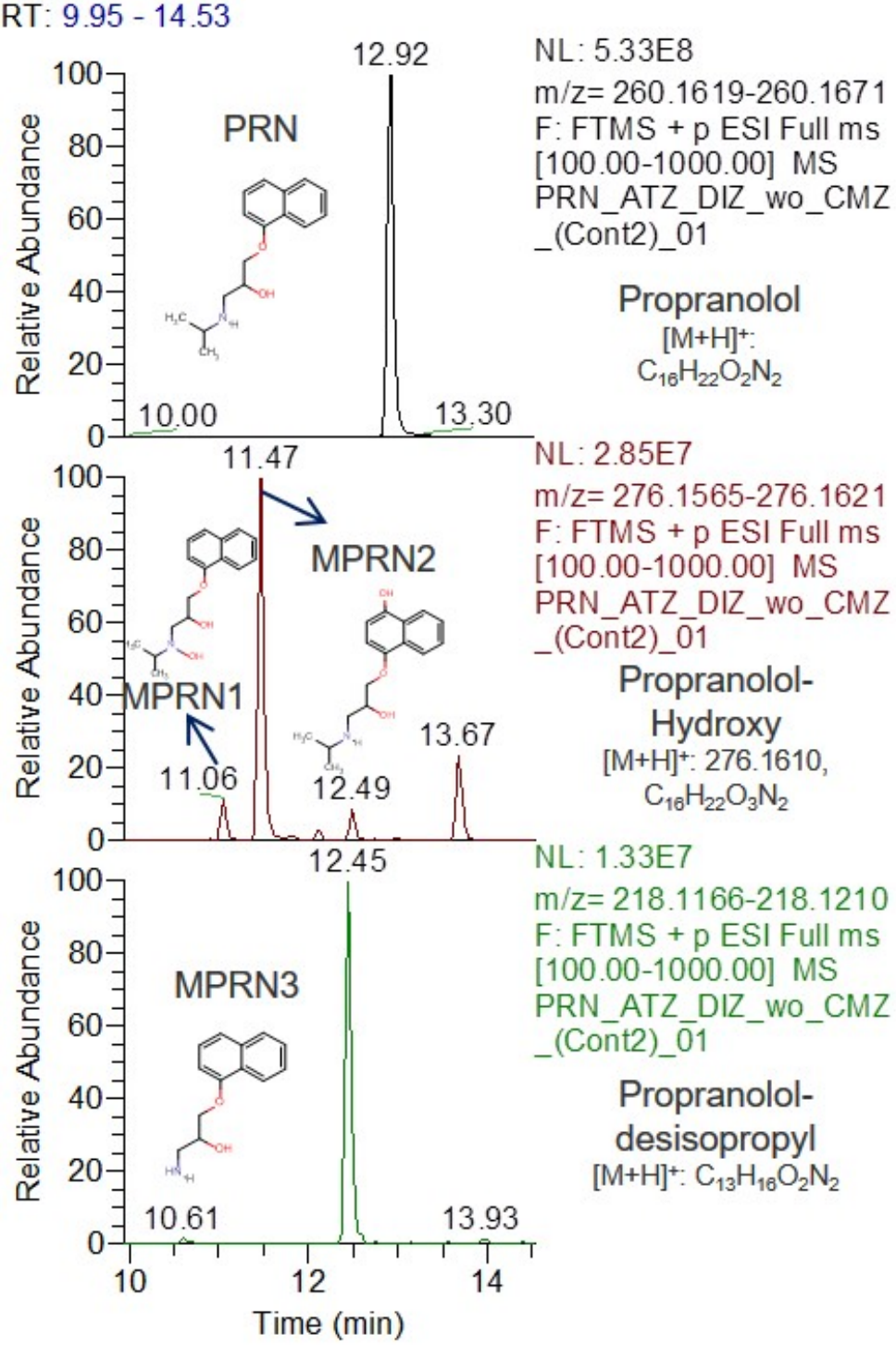
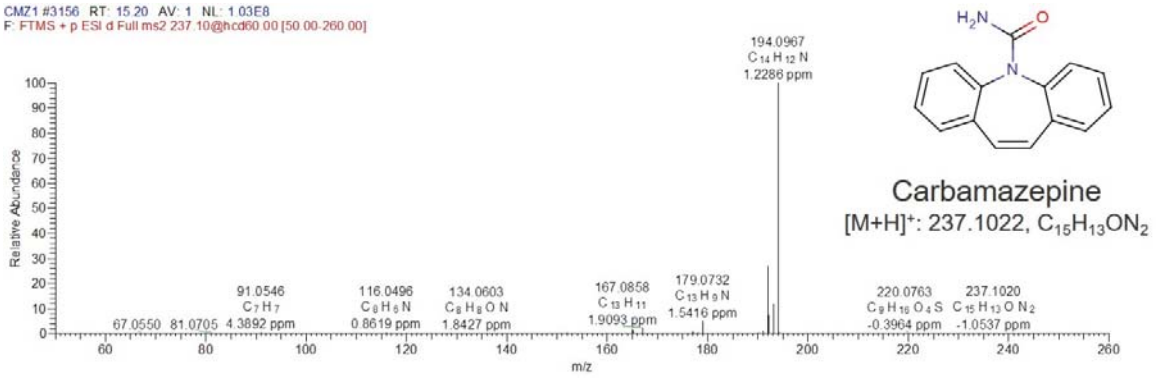


Figure S3. MS/MS spectra for carbamazepine (a) and its metabolites (b).

(a)



(b)

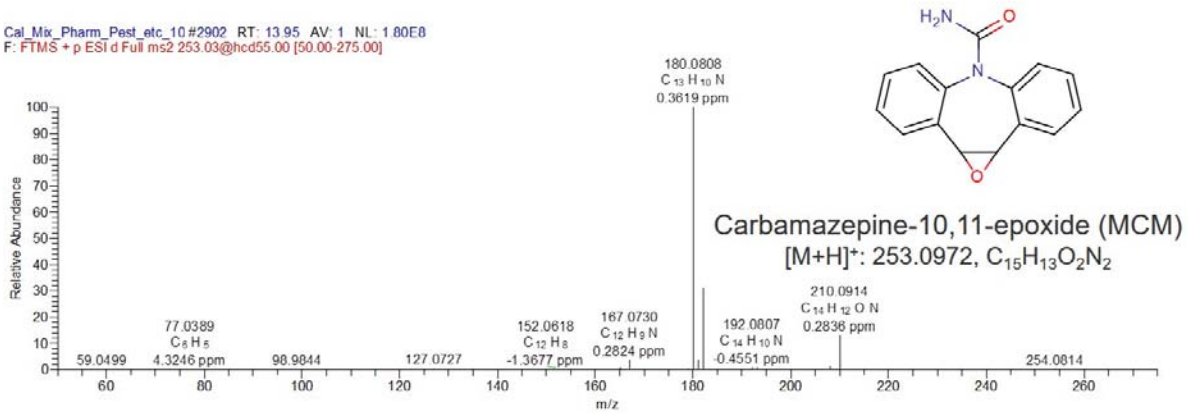
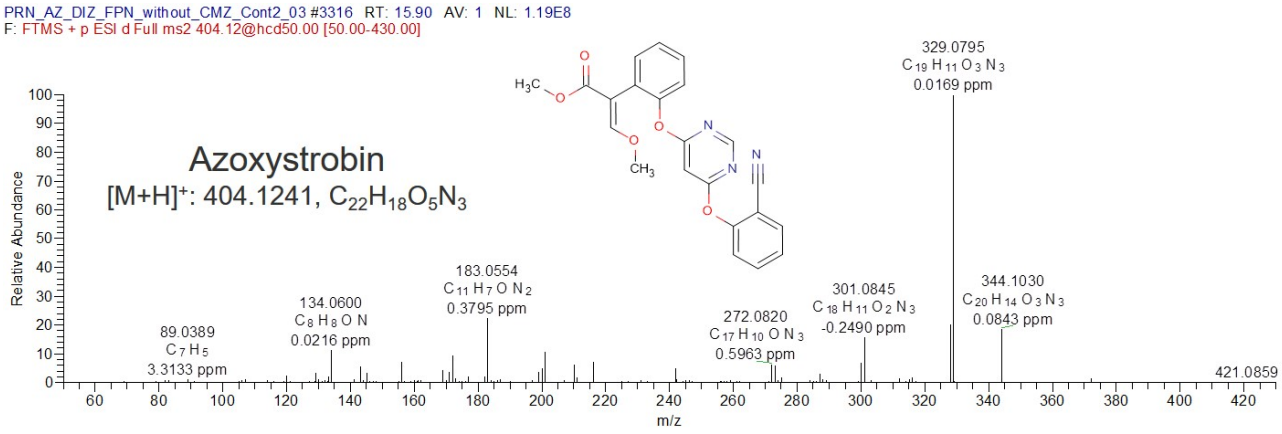
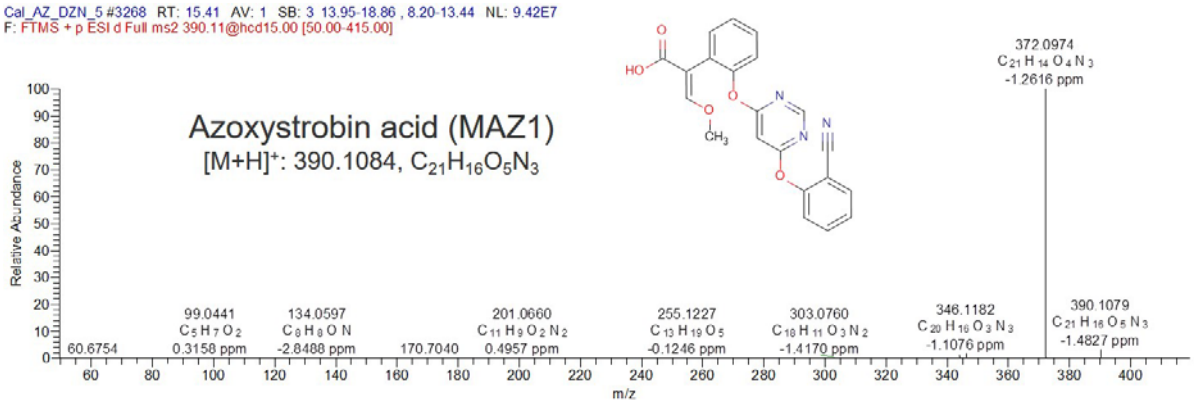


Figure S4. MS/MS spectra for azoxystrobin (a) and its metabolites (b)~(c).

(a)



(b)



(c)

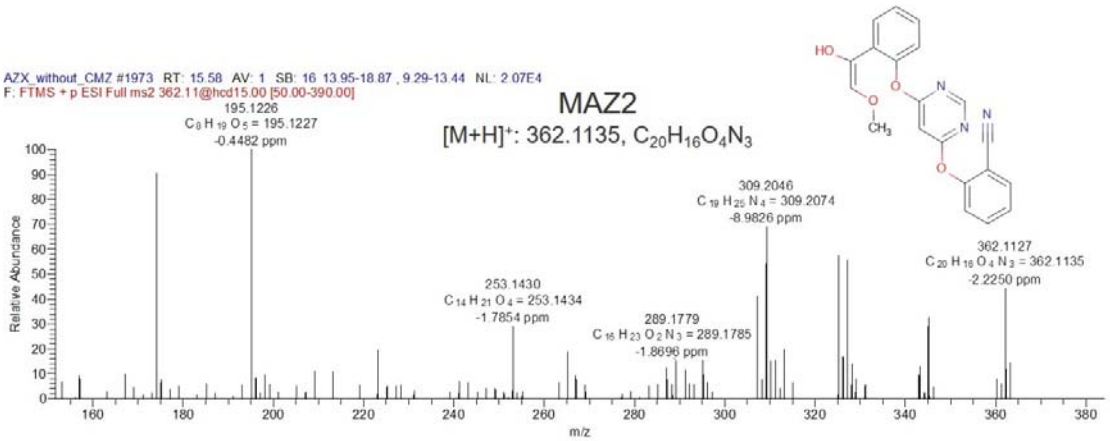
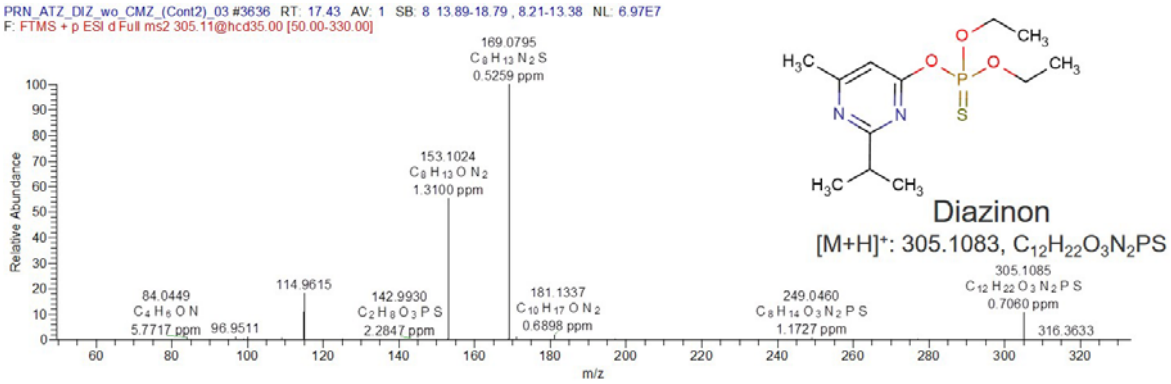
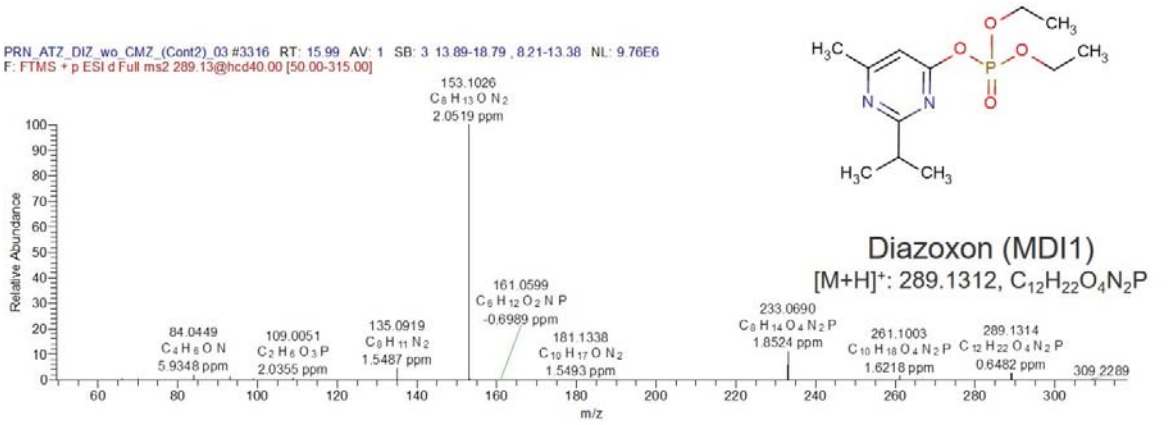


Figure S5. MS/MS spectra for diazinon (a) and its metabolites (b)~(c).

(a)



(b)



(c)

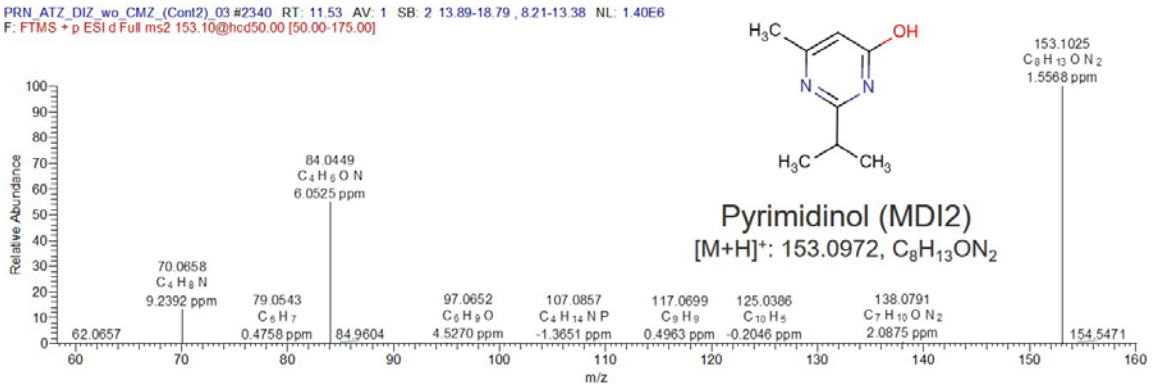
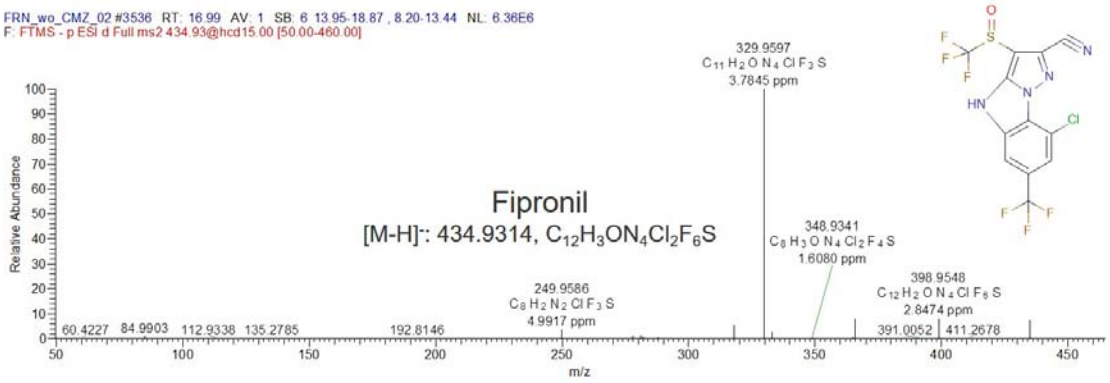
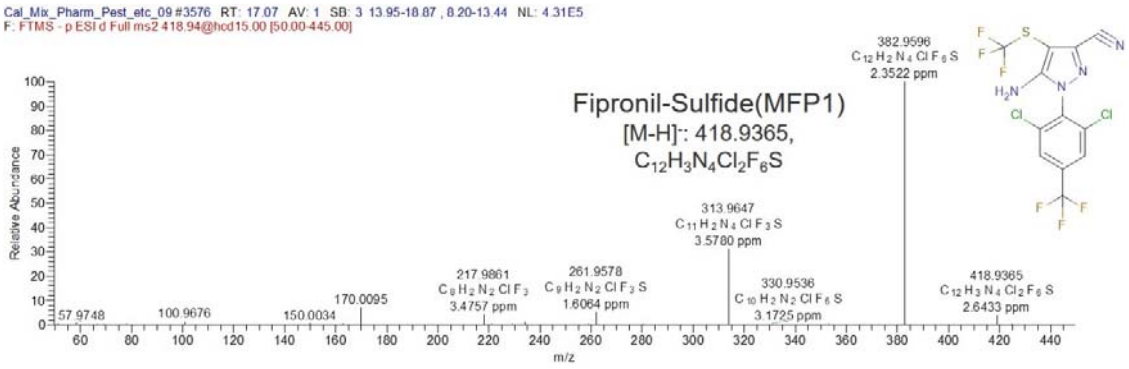


Figure S6. MS/MS spectra for fipronil (a) and its metabolites (b)~(c).

(a)



(b)



(c)

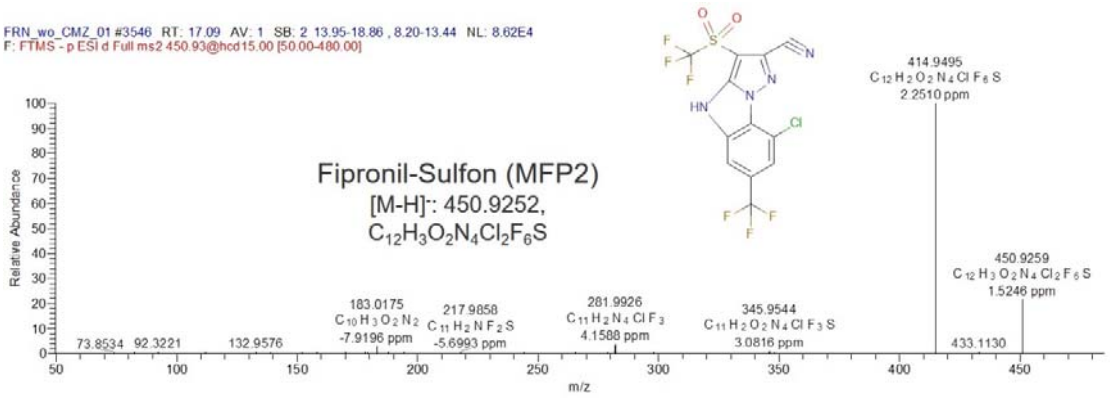


Figure S7. Linear increase in formation of metabolites over incubation time up to 60 min (Error bars indicate standard deviation between replicates, n=3)

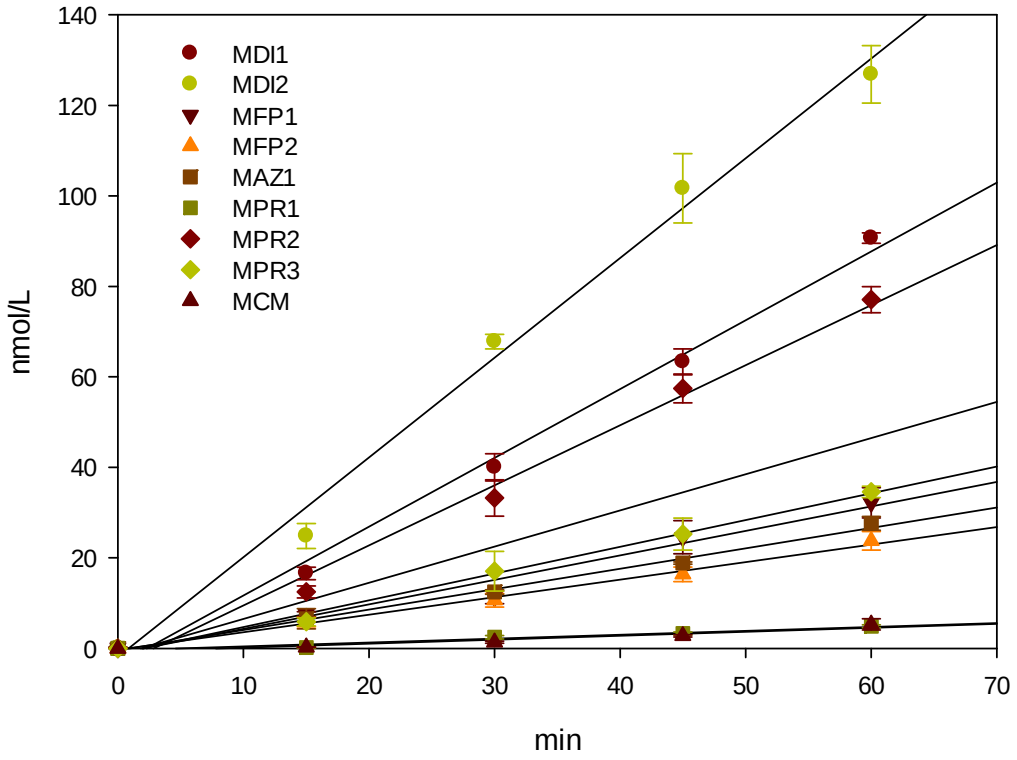


Figure S8. Ratio of metabolite amount to the parent, diazinon with increasing carbamazepine concentration. (Error bars indicate standard deviation between replicates, n=3)

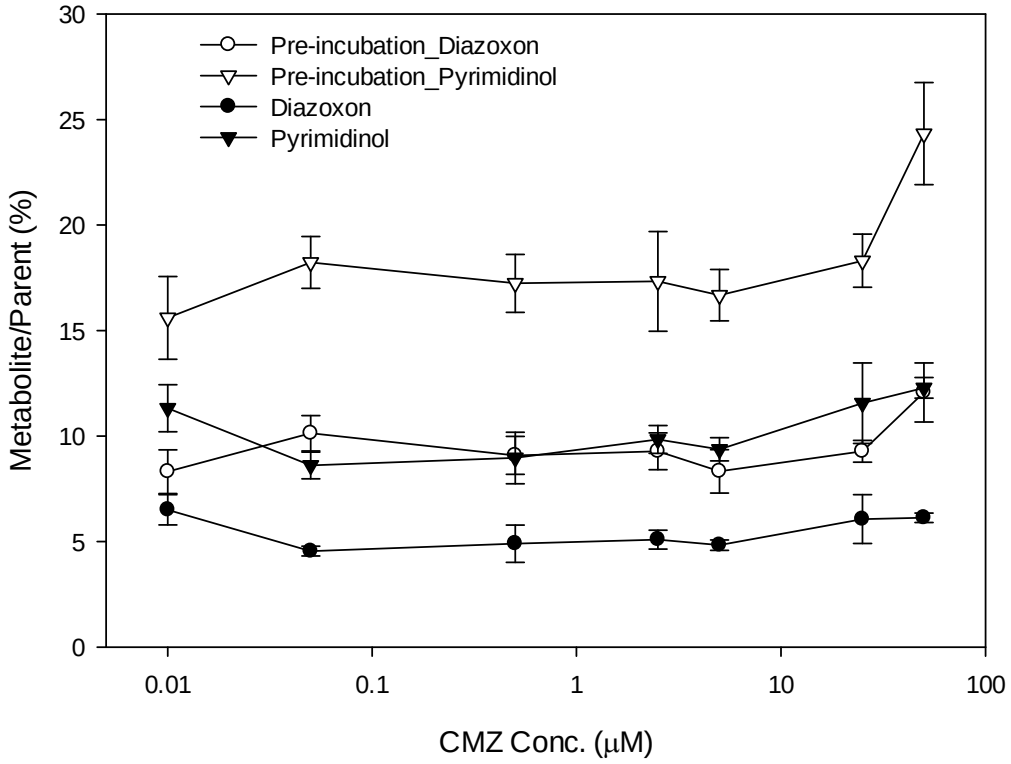
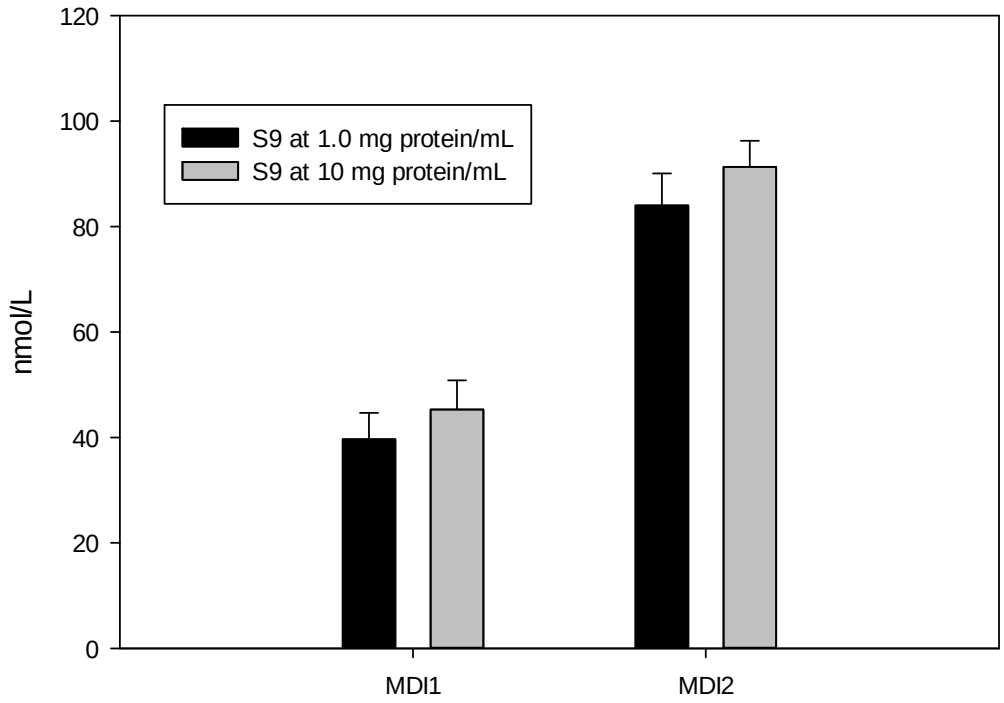


Figure S9. Formation of MDI1 and MDI2 at different S9 protein concentrations after 30 min incubation. (Error bars indicate standard deviation between replicates, n=3)



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Jeon, J., Kurth, D., Ashauer, R., Hollender, J., 2013. Comparative toxicokinetics of organic micropollutants in freshwater crustaceans. Environ. Sci. Technol. 47. doi:10.1021/es400833g