

Relating Metatranscriptomic Profiles to the Micropollutant Biotransformation Potential of Complex Microbial Communities

Supporting Information

Stefan Achermann,^{1,2} Cresten B. Mansfeldt,¹ Marcel Müller,^{1,3} David R. Johnson,¹ Kathrin Fenner*,^{1,2,4}

¹Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland. ²Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, 8092 Zürich, Switzerland. ³Institute of Atmospheric and Climate Science, ETH Zürich, 8092 Zürich, Switzerland. ⁴Department of Chemistry, University of Zürich, 8057 Zürich, Switzerland.

*Corresponding author (email: kathrin.fenner@eawag.ch)

S.A and C.B.M contributed equally to this work.

This supporting information (SI) is organized in 4 sections (S1-S4) with a total of 10 pages and comprises 7 figures (Figure S1-S7) and 4 tables (Table S1-S4).

S1 Data normalization

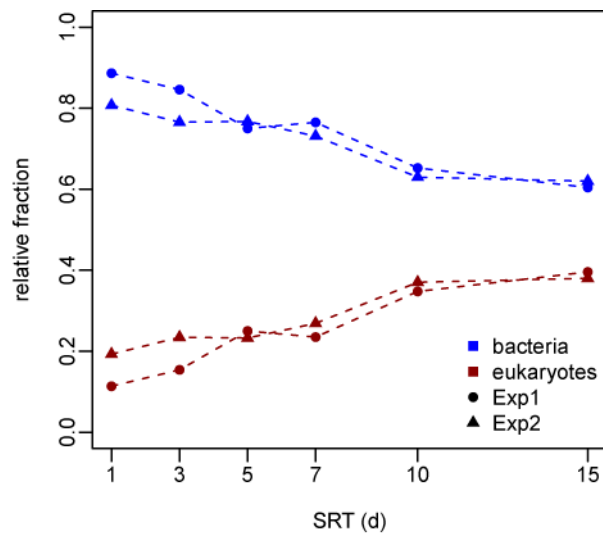


Figure S1. Relative fractions of gene transcripts originating from eukaryotes and bacteria.

Table S1. Relative standard deviation (RSD) for commonly used reference genes across all samples (n=12).

EC number	mean fraction bacteria (%)	RSD (%)	RSD bacteria (%)	RSD eukaryotes (%)
2.7.7.6 (RNAP)	80	16	6	nd ^a
5.99.1.2 (DNA topoisomerase)	90	11	9	nd ^a
5.99.1.3 (DNA gyrase)	92	16	10	nd ^a
1.2.1.12 (GAPDH)	37	39	6	32

^and indicates not determined.

S2 Nitrile hydration

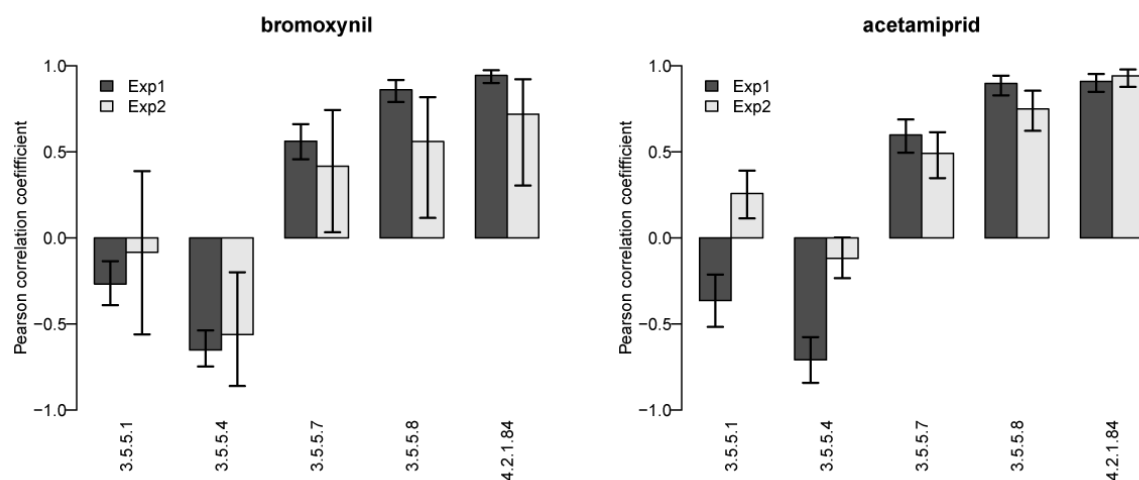


Figure S2: Pearson correlation coefficients r for rate constants of bromoxynil and acetamiprid with gene transcripts of ECs describing nucleophilic reactions of water with nitriles. Rate constants (k_{bio}) obtained from Monte Carlo error propagation described elsewhere¹ allowed to calculate a distribution of correlation coefficients (repeated sampling ($n=1000$) of k_{bio} values and calculation of r). Bars represent median values and estimated 95% confidence intervals, showing that the inferior correlation for bromoxynil in Exp2 may originate from uncertainties in k_{bio} .

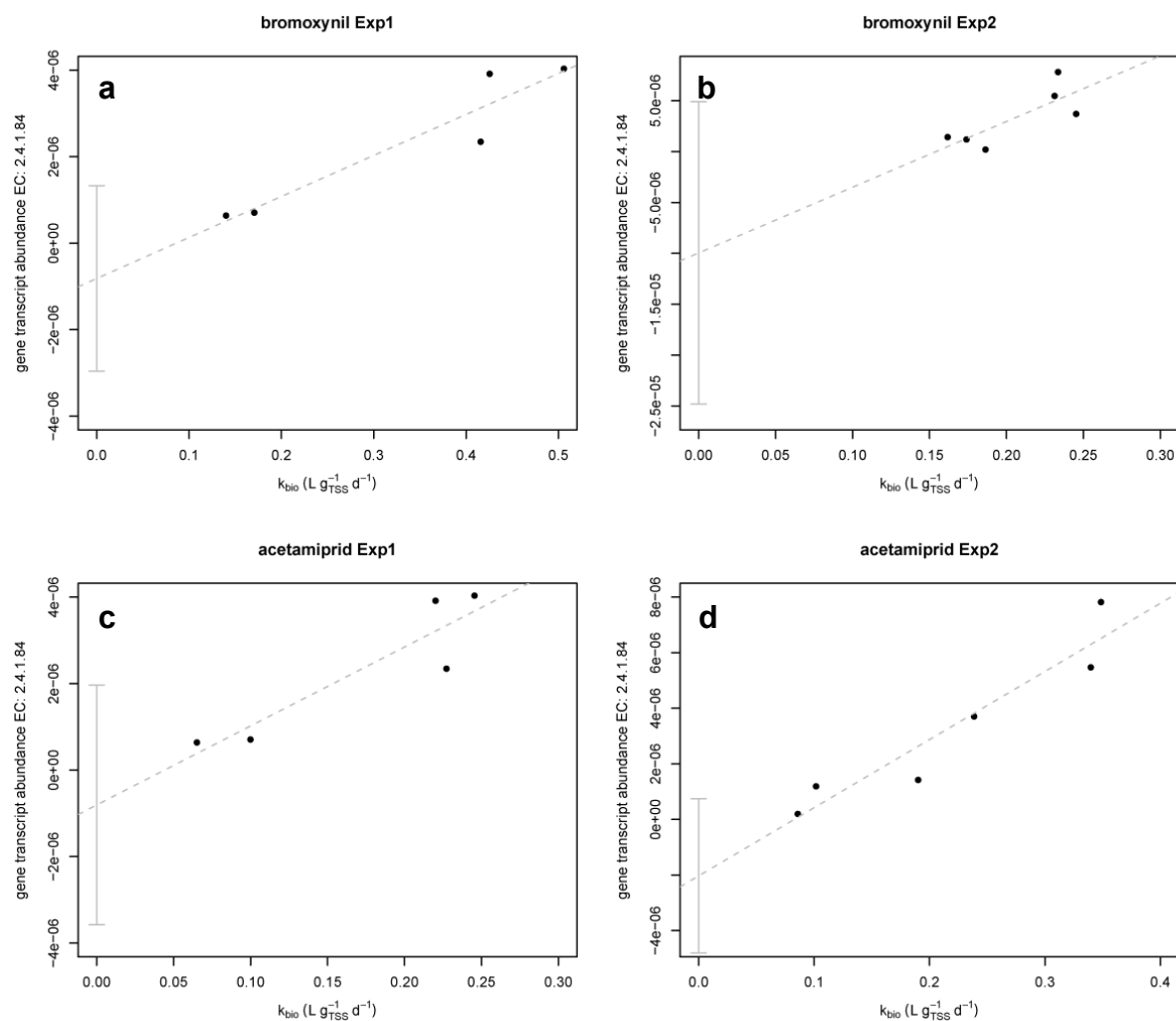
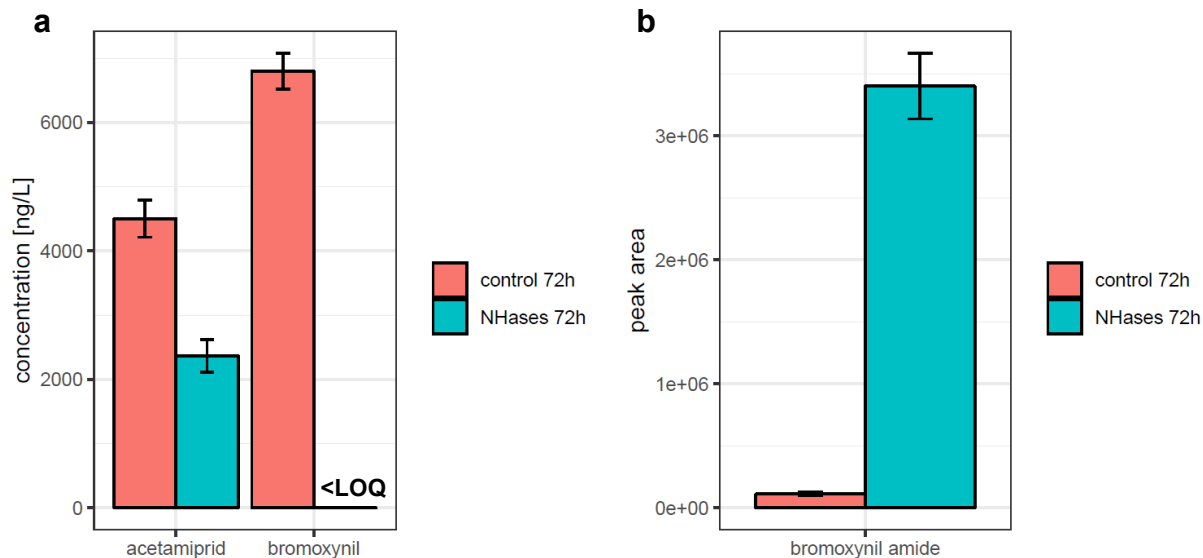


Figure S3: The relative abundance of nitrile hydratase (EC: 2.4.1.84) gene transcripts in dependence of k_{biol} -values for bromoxynil (a and b) and acetamiprid (c and d) in Exp1 (a and c) and Exp2 (b and d). The dashed lines were obtained from linear regression analysis providing estimates for the confidence interval (95% confidence level) of the estimated intercept (indicated by gray bar).

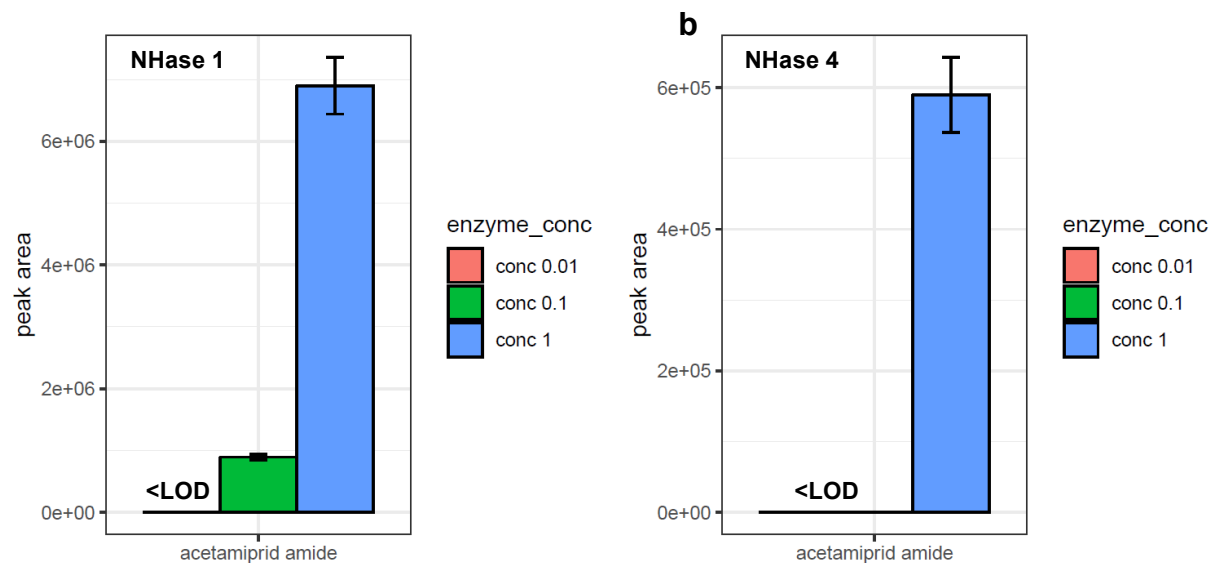
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60 **Figure S4:** (a) Measured concentrations (triplicate measurements) of acetamidiprid and bromoxynil with
61 and without a blend of nitrile hydratases (NHases) after 72 h of incubation. In presence of NHases,
62 bromoxynil concentrations were below the limit of quantification (which was below 300 ng/L but not
63 exactly determined). (b) In parallel, higher peak areas at the expected m/z values of bromoxynil amide
64 were detected after 72 h in the NHase treated reactor as compared to the control.

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67 **Figure S5:** Evidence for the formation of acetamidiprid amide was detected in a separate experiment
68 with two individual nitrile hydratases at different dilutions of the enzymes (i.e., 10- and 100-fold
69 dilution, with the highest enzyme concentration "conc 1" corresponding to 3 mg/mL). The bars
70 represent peak areas at the expected m/z values of acetamidiprid amide, showing higher values at
71 higher enzyme concentrations at which also a higher degree of acetamidiprid removal was observed.

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S3 Oxidation reactions

Table S2: Sub-subclasses containing monooxygenases and dioxygenases.

sub-subclass	number of detected 4 th level ECs
1.12.99	1
1.13.11	28
1.13.12	5
1.13.99	1
1.14.11	14
1.14.12	13
1.14.13	45
1.14.14	9
1.14.15	7
1.14.16	4
1.14.17	3
1.14.18	3
1.14.99	12

Table S3: Median values of distributions of correlation coefficients between oxidative reactions (19 micropollutants) and different selections of EC categories.

EC selection ^a	sub-subclasses ^b	EC ^c	ECs filtered ^d	Pearson's r ^e oxidation
all ECs		7341	2760	0.12
sub-subclasses monoox. ^f	9	476	101	0.45
sub-subclasses diox. ^f	8	508	118	0.43
peroxygenase	1.11.2	0	0	
sub-subclasses mono- or diox. ^{f,g}	13	598	144	0.47

^aMonooxygenases, dioxygenases and peroxygenases were selected based on the EC descriptions obtained from BRENDA database. ^bIndicates the code or the number of sub-subclasses when the selection was based on sub-subclasses. ^cNumber of ECs selected directly or contained within the selected sub-subclasses indicated in the respective column on the left. ^dNumber of ECs detected (after applying relative abundance threshold filter (10⁻⁶)). ^eMedian values of distributions of correlation coefficients (r values) obtained when correlating filtered ECs with 19 micropollutants or with SRT. ^fAll detected 4th level ECs of sub-subclasses containing at least one 4th level EC of the respective category (mono- or dioxygenase) were considered. ^gSelection of sub-subclasses containing either mono- or dioxygenase ECs. Due to sub-subclasses containing both mono-, and dioxygenases, the total of selected sub-subclasses and ECs in this category is less than the sum of the selection of only mono- or dioxygenase containing sub-subclasses.

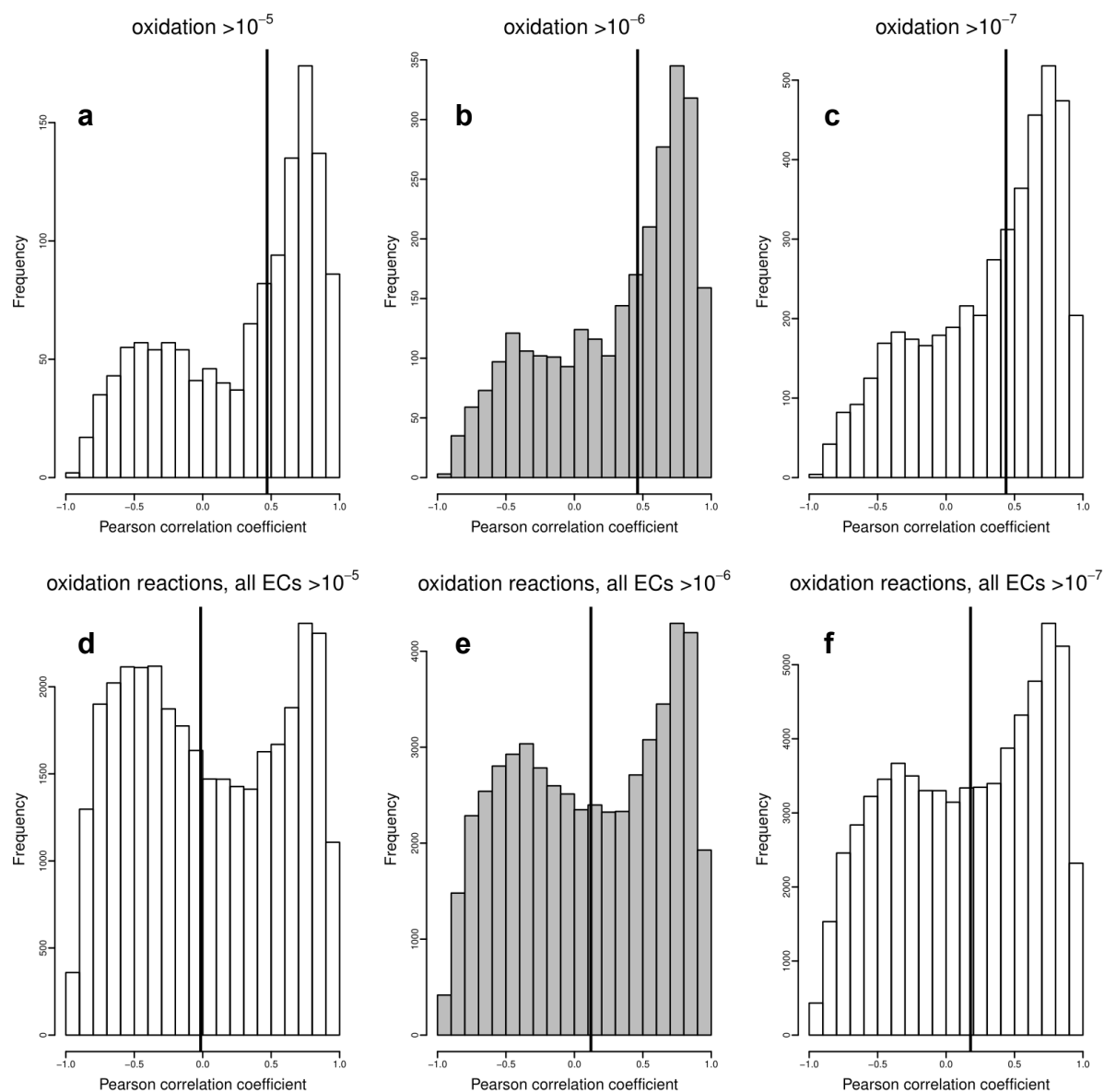


Figure S6: (a-c) Distribution of correlation coefficients (Pearson's r) for oxidative biotransformation reactions (19 micropollutants and 144 ECs associated with oxygenases) and (d-f) distribution of correlation coefficients between all detected ECs and the 19 micropollutants undergoing oxidative reactions. In all distributions (a-f), ECs were selected using the indicated relative abundance threshold values (10^{-5} , 10^{-6} or 10^{-7}) applied as detailed in the methods section.

Table S4: ECs in sub-subclasses associated with mono- or dioxygenases

EC	EC description	EC	EC description
1.12.99.6	hydrogenase (acceptor)	1.14.12.10	benzoate 1,2-dioxygenase
1.13.11.1	catechol 1,2-dioxygenase	1.14.12.11	toluene dioxygenase
1.13.11.11	tryptophan 2,3-dioxygenase	1.14.12.12	naphthalene 1,2-dioxygenase
1.13.11.15	3,4-dihydroxyphenylacetate 2,3-dioxygenase	1.14.12.13	2-halobenzoate 1,2-dioxygenase
1.13.11.16	3-carboxyethylcatechol 2,3-dioxygenase	1.14.12.14	2-aminobenzenesulfonate 2,3-dioxygenase
1.13.11.18	persulfide dioxygenase	1.14.12.15	terephthalate 1,2-dioxygenase
1.13.11.2	catechol 2,3-dioxygenase	1.14.12.17	nitric oxide dioxygenase
1.13.11.20	cysteine dioxygenase	1.14.12.18	biphenyl 2,3-dioxygenase
1.13.11.24	quercetin 2,3-dioxygenase	1.14.12.19	3-phenylpropanoate dioxygenase
1.13.11.27	4-hydroxyphenylpyruvate dioxygenase	1.14.12.21	benzoyl-CoA 2,3-dioxygenase
1.13.11.29	stizolobate synthase	1.14.12.3	benzene 1,2-dioxygenase
1.13.11.3	protocatechuate 3,4-dioxygenase	1.14.12.7	phthalate 4,5-dioxygenase
1.13.11.32	2-nitropropane dioxygenase	1.14.13.1	salicylate 1-monooxygenase
1.13.11.33	arachidonate 15-lipoxygenase	1.14.13.100	25/26-hydroxycholesterol 7 α -hydroxylase
1.13.11.34	arachidonate 5-lipoxygenase	1.14.13.106	epi-isozizaene 5-monooxygenase
1.13.11.37	hydroxyquinol 1,2-dioxygenase	1.14.13.107	limonene 1,2-monooxygenase
1.13.11.39	biphenyl-2,3-diol 1,2-dioxygenase	1.14.13.111	methanesulfonate monooxygenase
1.13.11.4	gentisate 1,2-dioxygenase	1.14.13.113	FAD-dependent urate hydroxylase
1.13.11.40	arachidonate 8-lipoxygenase	1.14.13.114	6-hydroxynicotinate 3-monooxygenase
1.13.11.49	chlorite O ₂ -lyase	1.14.13.122	chlorophyllide-a oxygenase
1.13.11.5	homogentisate 1,2-dioxygenase	1.14.13.127	3-(3-hydroxyphenyl)propanoate hydroxylase
1.13.11.52	indoleamine 2,3-dioxygenase	1.14.13.129	beta-carotene 3-hydroxylase
1.13.11.53	acireductone dioxygenase (Ni ²⁺ -requiring)	1.14.13.131	dimethyl-sulfide monooxygenase
1.13.11.54	acireductone dioxygenase [iron(II)-requiring]	1.14.13.132	squalene monooxygenase
1.13.11.6	3-hydroxyanthranilate 3,4-dioxygenase	1.14.13.133	pentalene oxygenase
1.13.11.63	beta-carotene 15,15'-dioxygenase	1.14.13.142	3-ketosteroid 9 α -monooxygenase
1.13.11.64	5-nitrosalicylate dioxygenase	1.14.13.149	phenylacetyl-CoA 1,2-epoxidase
1.13.11.79	5,6-dimethylbenzimidazole synthase	1.14.13.151	linalool 8-monooxygenase
1.13.11.8	protocatechuate 4,5-dioxygenase	1.14.13.163	6-hydroxy-3-succinoylpyridine 3-monooxygenase
1.13.12.16	nitronate monooxygenase	1.14.13.172	salicylate 5-hydroxylase
1.13.12.19	2-oxoglutarate dioxygenase (ethylene-forming)	1.14.13.178	methylxanthine N1-demethylase
1.13.12.21	tetracenomycin-F1 monooxygenase	1.14.13.199	docosahexaenoic acid omega-hydroxylase
1.13.12.4	lactate 2-monooxygenase	1.14.13.2	4-hydroxybenzoate 3-monooxygenase
1.13.99.1	inositol oxygenase	1.14.13.20	2,4-dichlorophenol 6-monooxygenase
1.14.11.1	gamma-butyrobetaine dioxygenase	1.14.13.205	long-chain fatty acid omega-monooxygenase
1.14.11.16	peptide-aspartate beta-dioxygenase	1.14.13.208	benzoyl-CoA 2,3-epoxidase
1.14.11.17	taurine dioxygenase	1.14.13.22	cyclohexanone monooxygenase
1.14.11.18	phytanoyl-CoA dioxygenase	1.14.13.225	F-actin monooxygenase
1.14.11.2	procollagen-proline dioxygenase	1.14.13.24	3-hydroxybenzoate 6-monooxygenase
1.14.11.21	clavamate synthase	1.14.13.25	methane monooxygenase (soluble)
1.14.11.23	flavonol synthase	1.14.13.30	leukotriene-B ₄ 20-monooxygenase
1.14.11.27	[histone-H3]-lysine-36 demethylase	1.14.13.38	anhydrotetracycline monooxygenase
1.14.11.35	1-deoxypentalenic acid 11 β -hydroxylase	1.14.13.40	anthraniloyl-CoA monooxygenase
1.14.11.4	procollagen-lysine 5-dioxygenase	1.14.13.48	(S)-limonene 6-monooxygenase
1.14.11.42	tRNA ^{Phe} (7-(3-amino-3-carboxypropyl)wyosine37-C2)-hydroxylase	1.14.13.49	(S)-limonene 7-monooxygenase
1.14.11.45	L-isoleucine 4-hydroxylase	1.14.13.50	pentachlorophenol monooxygenase
1.14.11.7	procollagen-proline 3-dioxygenase	1.14.13.67	quinine 3-monooxygenase
1.14.11.8	trimethyllysine dioxygenase	1.14.13.7	phenol 2-monooxygenase (NADPH)
1.14.12.1	anthranilate 1,2-dioxygenase (deaminating, decarboxylating)	1.14.13.70	sterol 14 α -demethylase

Table S4: (continued)

EC	EC description	EC	EC description
1.14.13.72	methylsterol monooxygenase	1.14.14.17	squalene monooxygenase
1.14.13.8	flavin-containing monooxygenase	1.14.14.18	heme oxygenase (biliverdin-producing)
1.14.13.81	magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase	1.14.14.3	bacterial luciferase
1.14.13.82	vanillate monooxygenase	1.14.14.46	pimeloyl-[acyl-carrier protein] synthase
1.14.13.84	4-hydroxyacetophenone monooxygenase	1.14.14.5	alkanesulfonate monooxygenase
1.14.13.9	kynurenine 3-monooxygenase	1.14.14.9	4-hydroxyphenylacetate 3-monooxygenase
1.14.13.90	zeaxanthin epoxidase	1.14.15.1	camphor 5-monooxygenase
1.14.13.92	phenylacetone monooxygenase	1.14.15.11	pentalenic acid synthase
1.14.99.1	prostaglandin-endoperoxide synthase	1.14.15.12	pimeloyl-[acyl-carrier protein] synthase
1.14.99.28	linalool 8-monooxygenase	1.14.15.13	pulcherriminic acid synthase
1.14.99.29	deoxyhypusine monooxygenase	1.14.15.21	zeaxanthin epoxidase
1.14.99.3	heme oxygenase (biliverdin-producing)	1.14.15.3	alkane 1-monooxygenase
1.14.99.33	DELTA12-fatty acid dehydrogenase	1.14.15.7	choline monooxygenase
1.14.99.36	beta-carotene 15,15'-monooxygenase	1.14.16.1	phenylalanine 4-monooxygenase
1.14.99.39	ammonia monooxygenase	1.14.16.2	tyrosine 3-monooxygenase
1.14.99.44	diaplycopene oxygenase	1.14.16.4	tryptophan 5-monooxygenase
1.14.99.46	pyrimidine oxygenase	1.14.16.5	alkylglycerol monooxygenase
1.14.99.5	stearoyl-CoA desaturase	1.14.17.1	dopamine beta-monooxygenase
1.14.99.50	gamma-glutamyl hercynylcysteine S-oxide synthase	1.14.17.3	peptidylglycine monooxygenase
1.14.99.7	squalene monooxygenase	1.14.17.4	aminocyclopropanecarboxylate oxidase
1.14.14.1	unspecific monooxygenase	1.14.18.1	tyrosinase
1.14.14.11	styrene monooxygenase	1.14.18.3	methane monooxygenase (particulate)
1.14.14.12	3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione monooxygenase	1.14.18.5	sphingolipid C4-monooxygenase

S4 Substitution-type reactions

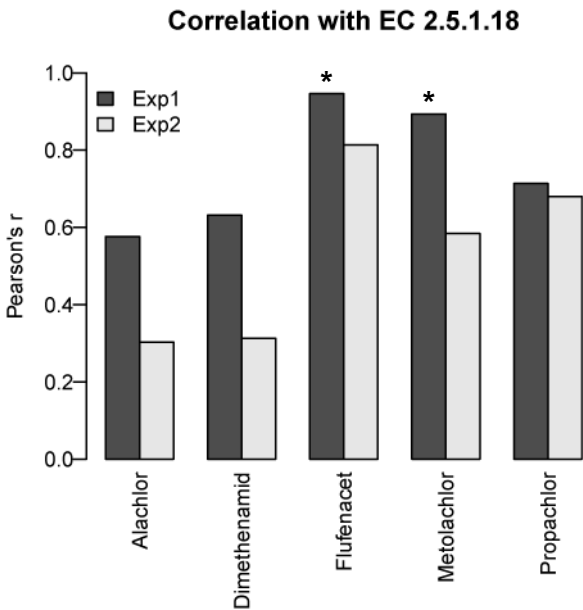


Figure S7: Correlation between the 5 investigated acetanilides and glutathione-S-transferase (EC 2.5.1.18). Significant correlations ($P < 0.05$) are indicated with an asterisk.

108 **References**

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