SUPPORTING INFORMATION

Metabolic profiling of chloroacetanilide herbicides in earthworm coelomic fluid using ^{1}H NMR and GC-MS

Corey M. Griffith[†], Melissa A. Morgan[‡], Meredith M. Dinges[‡], Caroline Mathon[‡,§], and Cynthia K. Larive[*,†,‡]

[†]Environmental Toxicology Graduate Program, University of California, Riverside, California 92521, United States

[‡]Department of Chemistry, University of California, Riverside, California 92521, United States

*Corresponding Author Information - E-mail: clarive@ucr.edu, Tel: 951-827-2990, Fax: 951-827-2435

[§]Present Address: Philip Morris International R&D, Phillip Morris Products S.A., 2000 Neuchâtel, Switzerland

TABLE OF CONTENTS

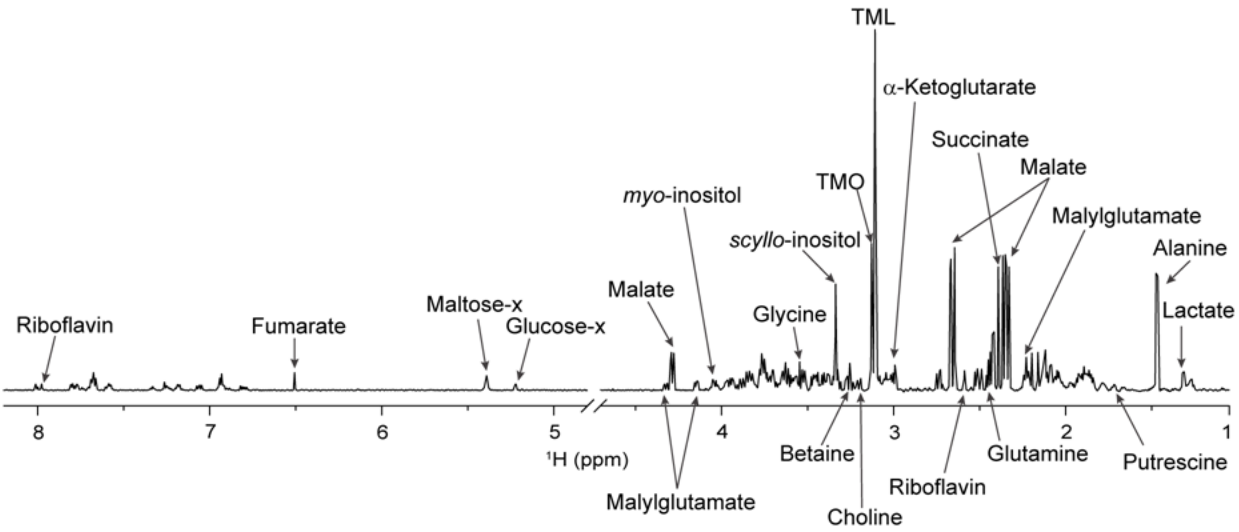

Figure S-1. Representative ^{1}H NMR spectrum of a control earthworm coelomic fluid sample. A subset of detected metabolites is annotated, with a full description of resonance assignments described in Griffith et al.[11] *TML = *N*ε,*N*ε,*N*ε-trimethyllysine; TMO= *N*δ,*N*δ,*N*δ-trimethylornithine.

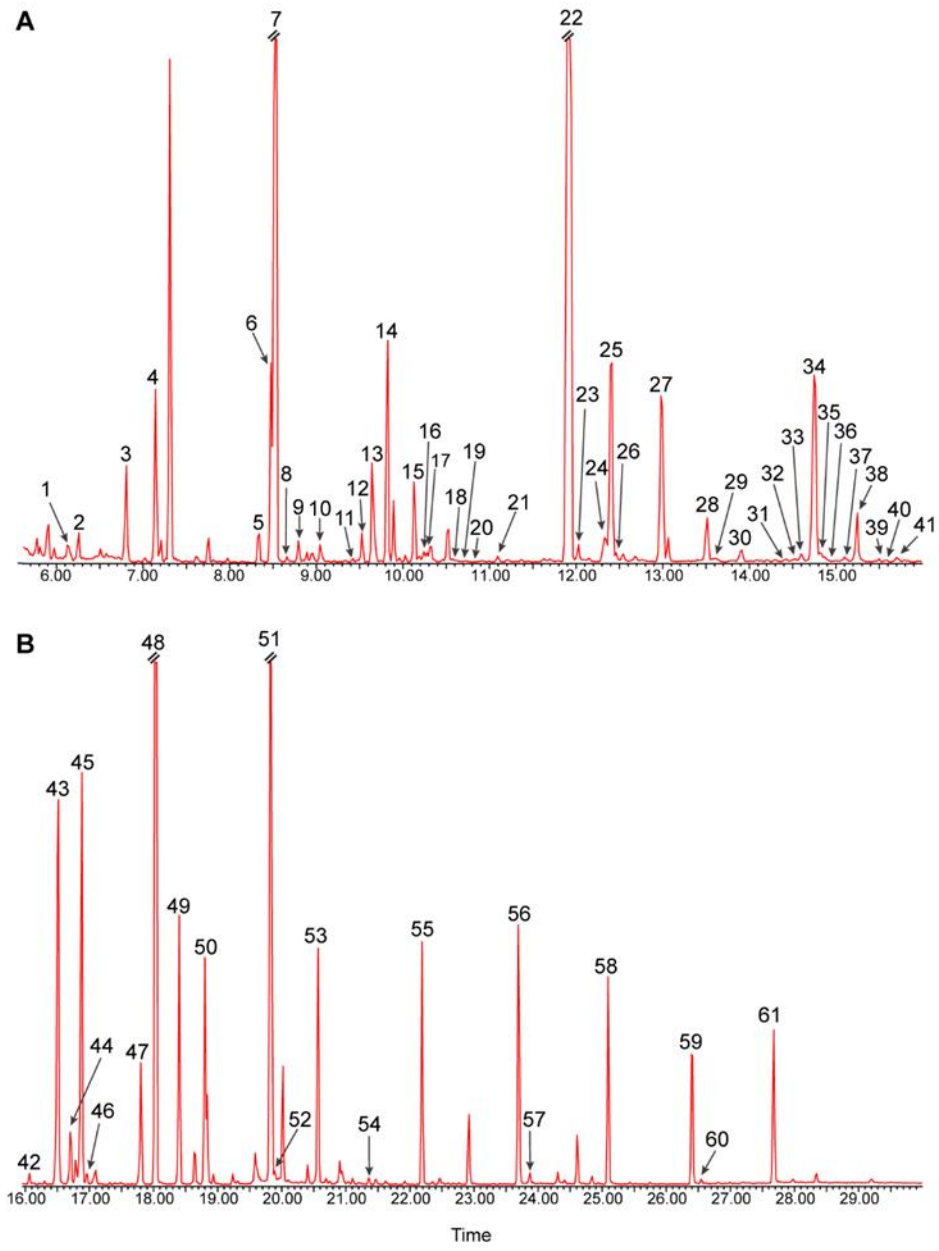

Figure S-2. Representative GC-MS total ion chromatogram (TIC) between A) 5.00 – 16.00 min and B) 16.00 – 30 min of a control earthworm coelomic fluid sample. Metabolites are annotated with numbers in Table S-1.

Table S-1. GC-MS metabolite assignment with retention time, calculated retention index, reported retention index, and *m/z* used for quantitation. Retention Index (RI) is reported from NIST 2017.

#	Metabolite	Retention Time	Retention Index (Calculated)	Retention Index (NIST)	*m/z* for quantitation
1	Pyruvate (1TMS)	6.126	1064	1037*	174.08
2	Lactate (2TMS)	6.248	1072	1066	117.09
3	Alanine (2TMS)	6.791	1109	1100	116.10
4	FAMES: C8	7.125	1132	1126	-----
5	Butylamine (2TMS)	8.312	1218	1123	130.12
6	FAMES: C9	8.451	1124	1225	-----
7	Ethanolamine (3TMS)	8.511	1130	1242	174.13
8	Urea (2TMS)	8.607	1238	1249	-----
9	Benzoate (1TMS)	8.778	1251	1249	179.06
10	Phosphate (3TMS)	9.044	1294	1292	-----
11	Proline (2TMS)	9.402	1296	1305	142.12
12	Glycine (3TMS)	9.518	1304	1314	174.14
13	Succinate (2TMS)	9.636	1313	1321	75.04
14	FAMES: C10	9.813	1325	1325	-----
15	Fumarate(2TMS)	10.127	1350	1353	245.10
16	Serine (3TMS)	10.236	1357	1368	204.15
17	Nonanoate (1TMS)	10.247	1361	1355	117.04
18	Threonine (3TMS)	10.578	1383	1367	218.17
19	Glutarate (2TMS)	10.876	1406	1409	75.03
20	2-Methylglutarate (2TMS)	10.992	1414	1423	239.20
21	Aspartate(2TMS)	11.092	1422	1420	75.03
22	Malate (3TMS)	11.916	1484	1497	133.03
23	Adipate (2TMS)	12.142	1502	1514	75.03
24	Aspartate (3TMS)	12.309	1513	1522	232.15
25	FAMES: C12	12.403	1521	1526	-----
26	Glutamate (2TMS)	12.448	1525	1527	84.06
27	α-Ketoglutarate	12.985	1571	1587	198.09
28	Glutamate (3TMS)	13.508	1616	1651	246.17
29	Phenylalanine (2TMS)	13.606	1624	1636	91.07
30	Dodecanoic acid (1TMS)	13.905	1651	1655	117.05
31	Suberate (2TMS)	14.424	1696	1707	75.04
32	Lysine (3TMS)	14.513	1703	1718	84.09
33	2-Aminoadipate	14.621	1711	1719	260.18
34	FAMES: C14	14.751	1724	1725	-----
35	Glutamine (4TMS)	14.763	1725	1721*	227.17
36	Putrescine (4TMS)	14.835	1731	1733	174.14
37	Glycerol-3-phospate	15.097	1757	1778	-----
38	Glutamine (3TMS)	15.241	1771	1768	156.11
39	Azelate (2TMS)	15.505	1795	1806	317.20
40	Ornithine	15.707	1815	1812	174.14

41	Citrate (3TMS)	15.711	1815	1845	273.14
42	Tetradecanoate (1TMS)	16.061	1849	1850	285.24
43	Glucose	16.509	1891	1865	205.13
44	Lysine (4TMS)	16.780	1918	1933	174.14
45	FAMES: C16	16.872	1927	1926	-----
46	Tyrosine (3TMS)	16.956	1935	1959	218.13
47	*scyllo*-Inositol (6TMS)	17.798	2019	2027*	318.20
48	Hexadecanoate (1TMS)	18.034	2070	2049	-----
49	*myo*-Inositol (6TMS)	18.397	2078	2129	217.14
50	FAMES: C18	18.796	2117	2128	-----
51	Octadecanoate (1TMS)	19.833	2277	2234	-----
52	Spermidine (5TMS)	19.879	2249	2245	174.14
53	FAMES: C20	20.559	2333	2329	-----
54	Eicosanoate (1TMS)	21.462	2443	2447	117.04
55	FAMES: C22	22.183	2531	2528	-----
56	FAMES: C24	23.688	2737	2728	-----
57	Maltose	23.704	2739	2733	361.22
58	FAMES: C26	25.087	2940	2935	-----
59	FAMES: C28	26.396	3112	3126	-----
60	Cholesterol (1TMS)	26.543	3131	3150	-----
61	FAMES: C30	27.666	3279	3323	-----
62	Triacontanoate (1TMS)	28.338	3366	3426	117.04
63	Maltotriose (11TMS)	29.201	3481	3508*	204.13

*RIs from the Golm Metabolome Database
**---- = not quantified

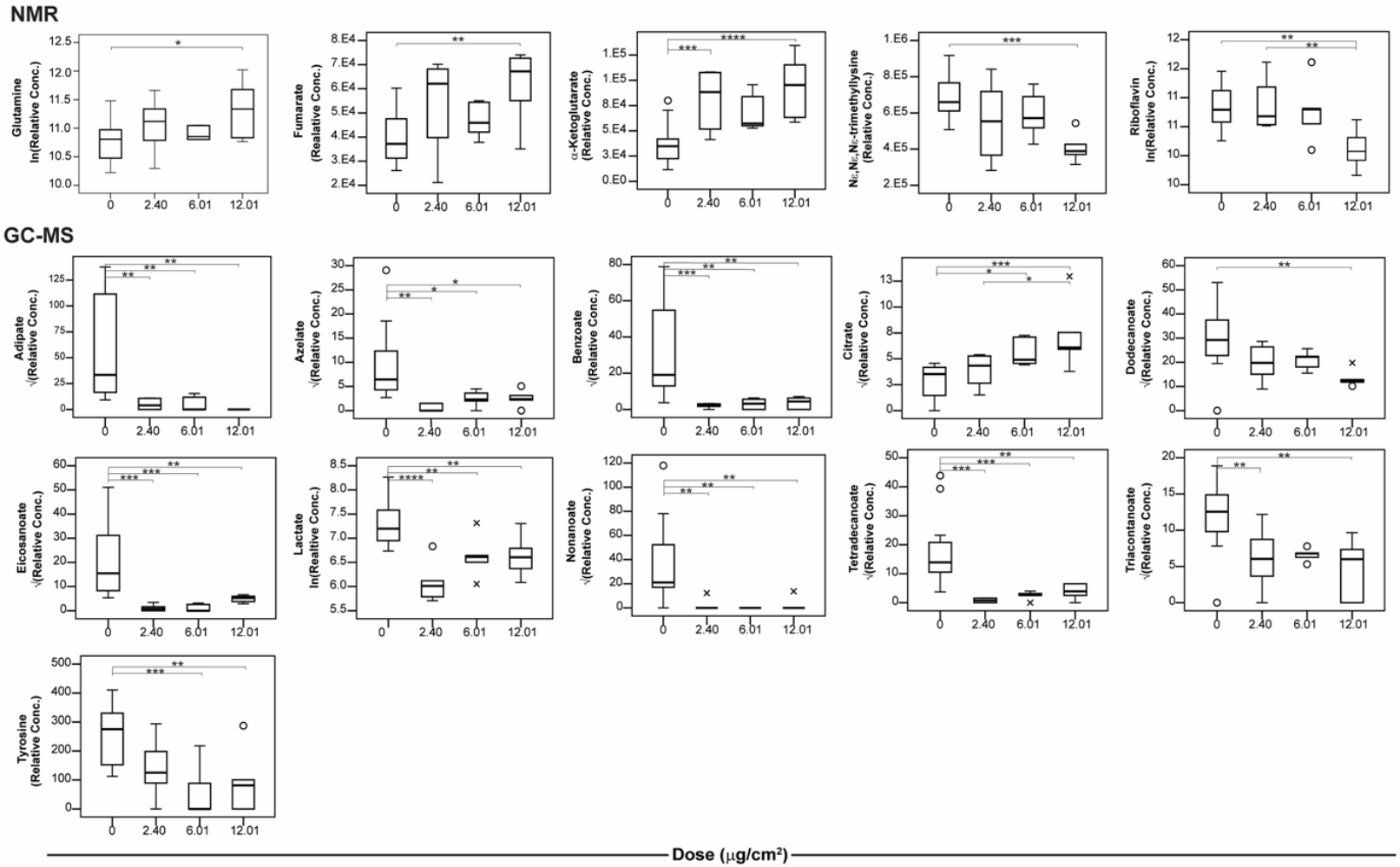

Figure S-3. Subset of metabolic changes observed in coelomic fluid of earthworms exposed to acetochlor. Tukey's HSD pairwise comparisons are annotated to show the significance level (* = $p \leq 0.10$; ** = $p \leq 0.05$; *** = $p \leq 0.01$; and **** = $p \leq 0.001$).

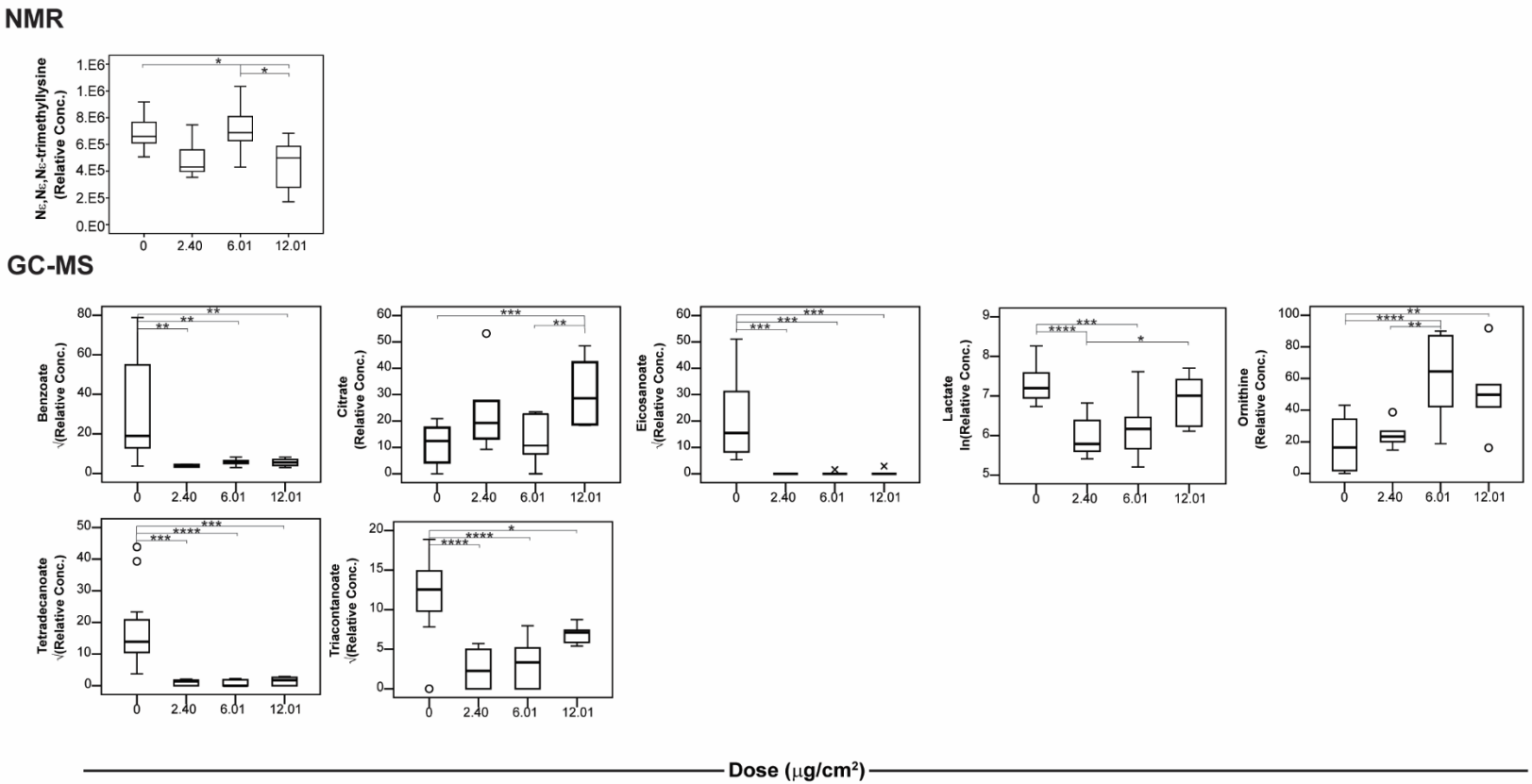

Figure S-4. Subset of metabolic changes observed in coelomic fluid of earthworms exposed to alachlor. Tukey's HSD pairwise comparisons are annotated to show the significance level (* = $p \leq 0.10$; ** = $p \leq 0.05$; *** = $p \leq 0.01$; and **** = $p \leq 0.001$).

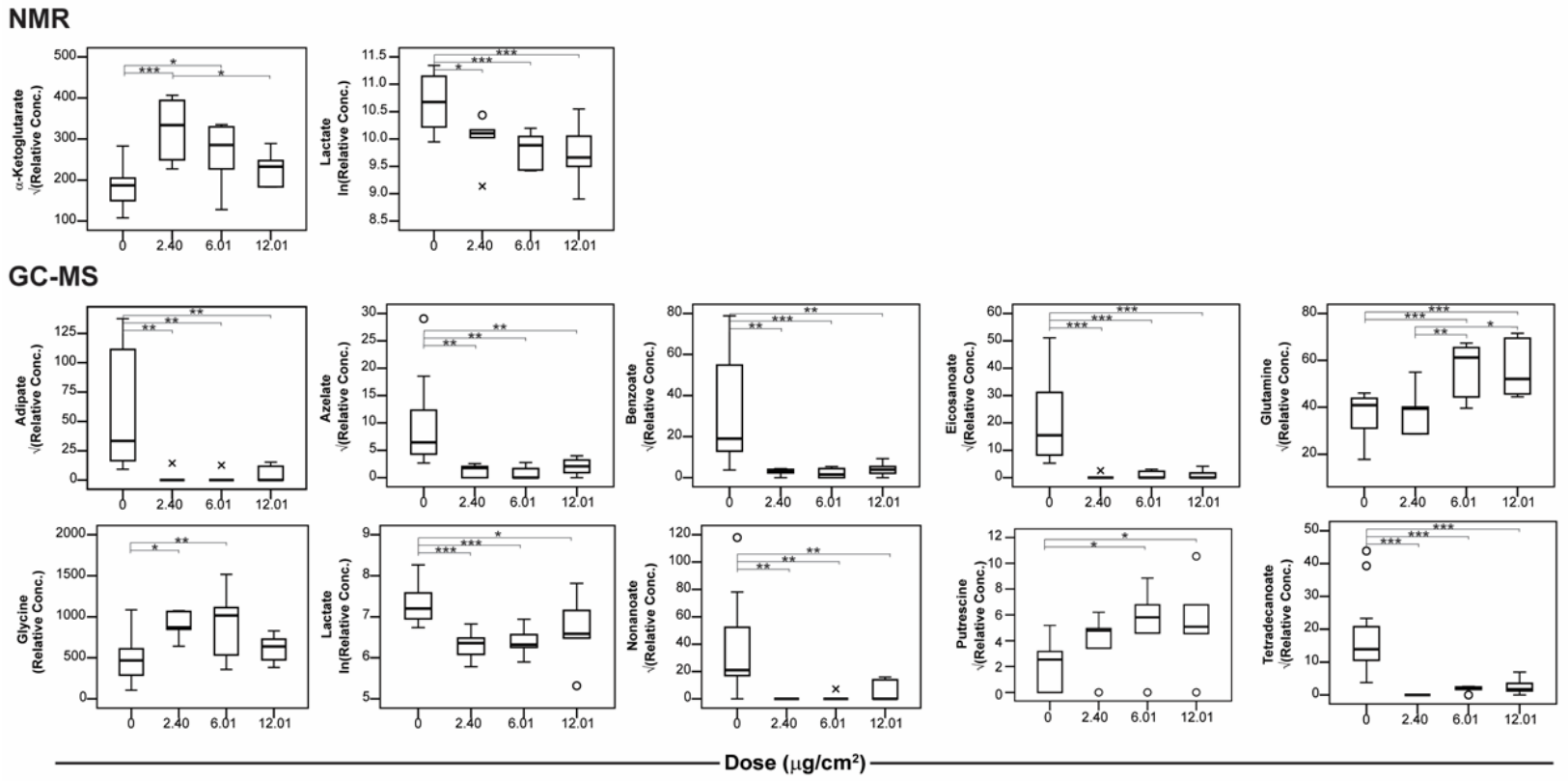

Figure S-5. Subset of metabolic changes observed in coelomic fluid of earthworms exposed to butachlor. Tukey's HSD pairwise comparisons are annotated to show the significance level (* = $p \leq 0.10$; ** = $p \leq 0.05$; *** = $p \leq 0.01$; and **** = $p \leq 0.001$).

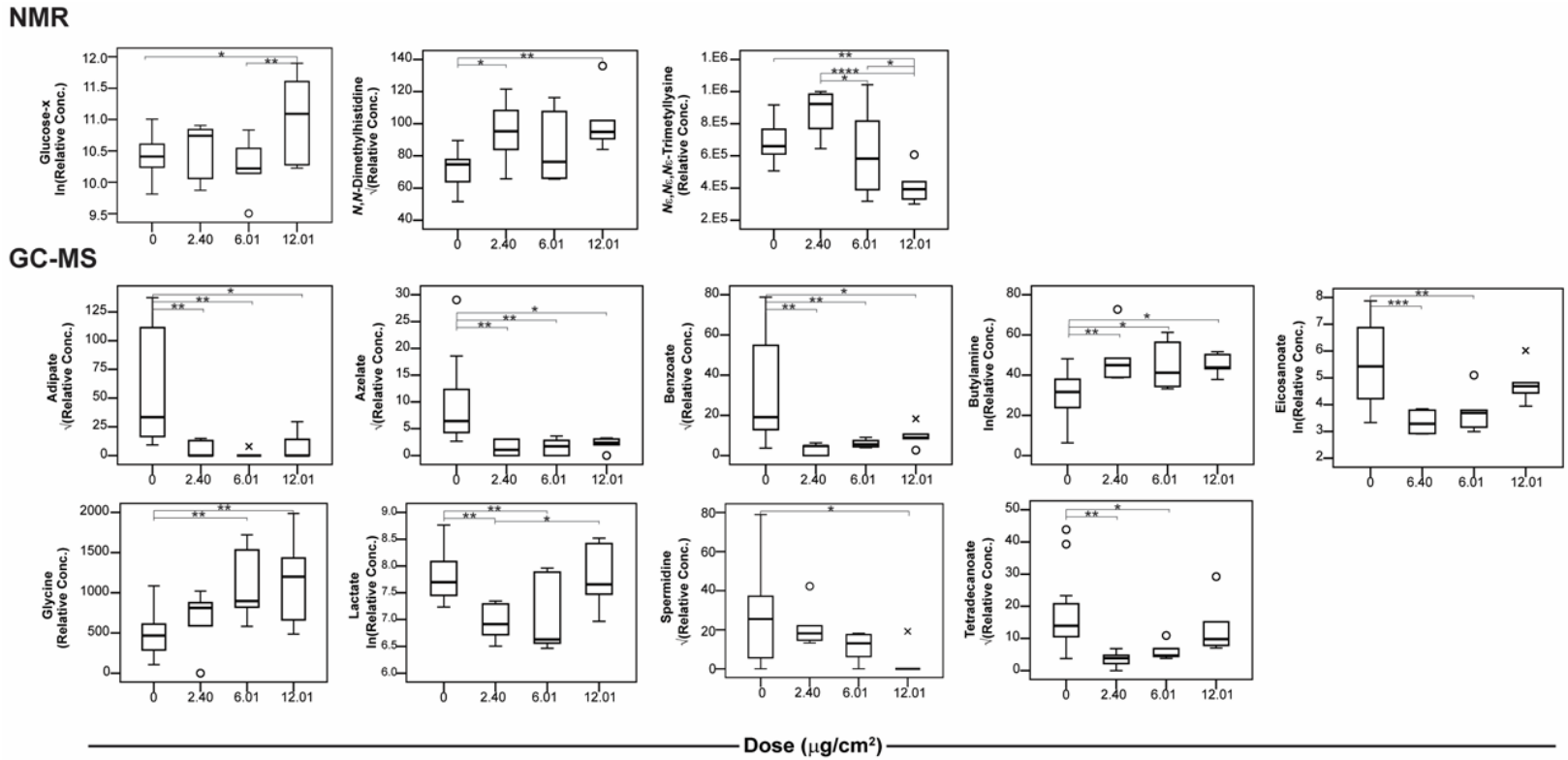

Figure S-6. Subset of metabolic changes observed in coelomic fluid of earthworms exposed to metolachlor. Tukey's HSD pairwise comparisons are annotated to show the significance level (* = $p \leq 0.10$; ** = $p \leq 0.05$; *** = $p \leq 0.01$; and **** = $p \leq 0.001$).

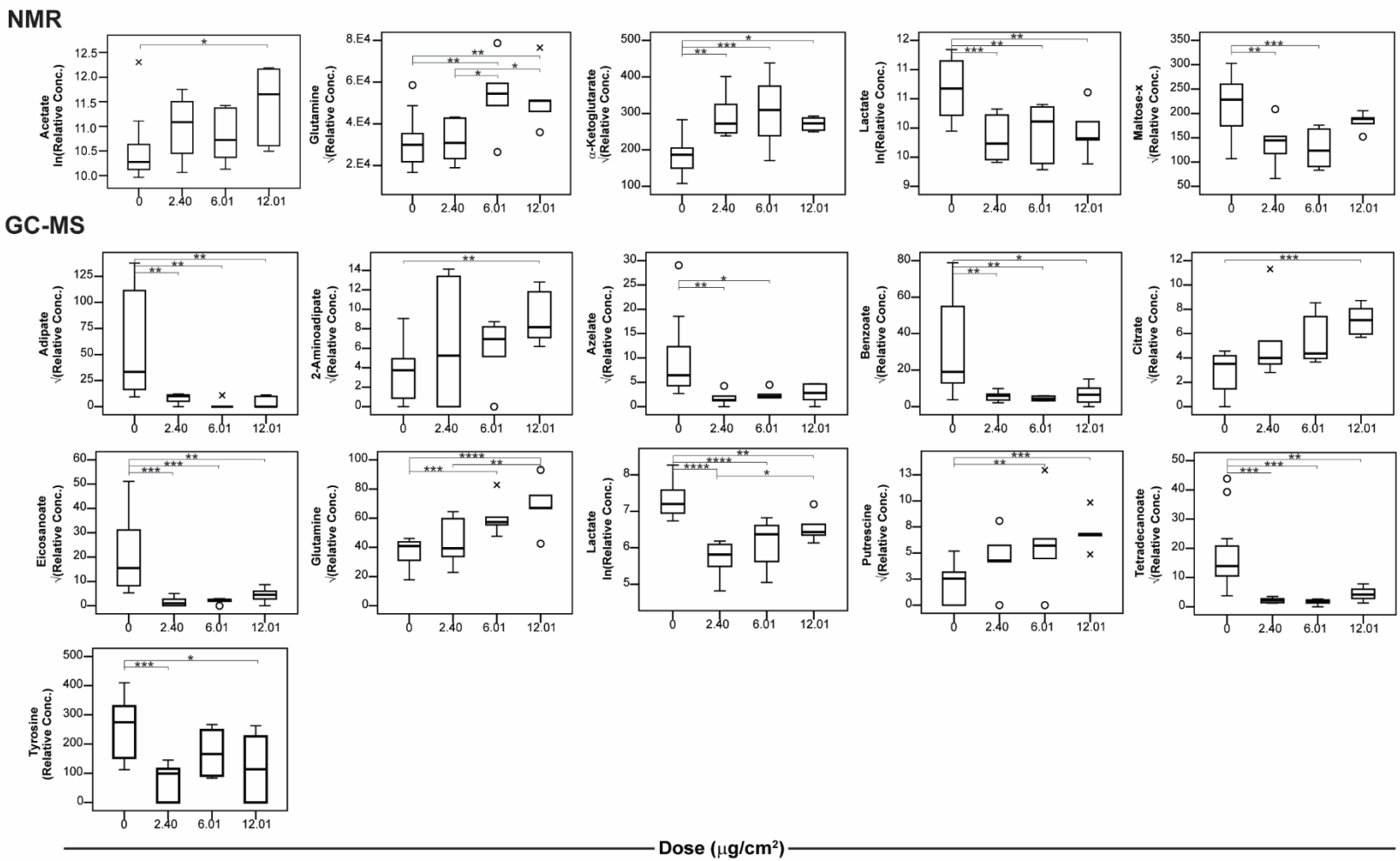

Figure S-7. Subset of metabolic changes observed in coelomic fluid of earthworms exposed to S-metolachlor. Tukey's HSD pairwise comparisons are annotated to show the significance level (* = $p \leq 0.10$; ** = $p \leq 0.05$; *** = $p \leq 0.01$; and **** = $p \leq 0.001$)..

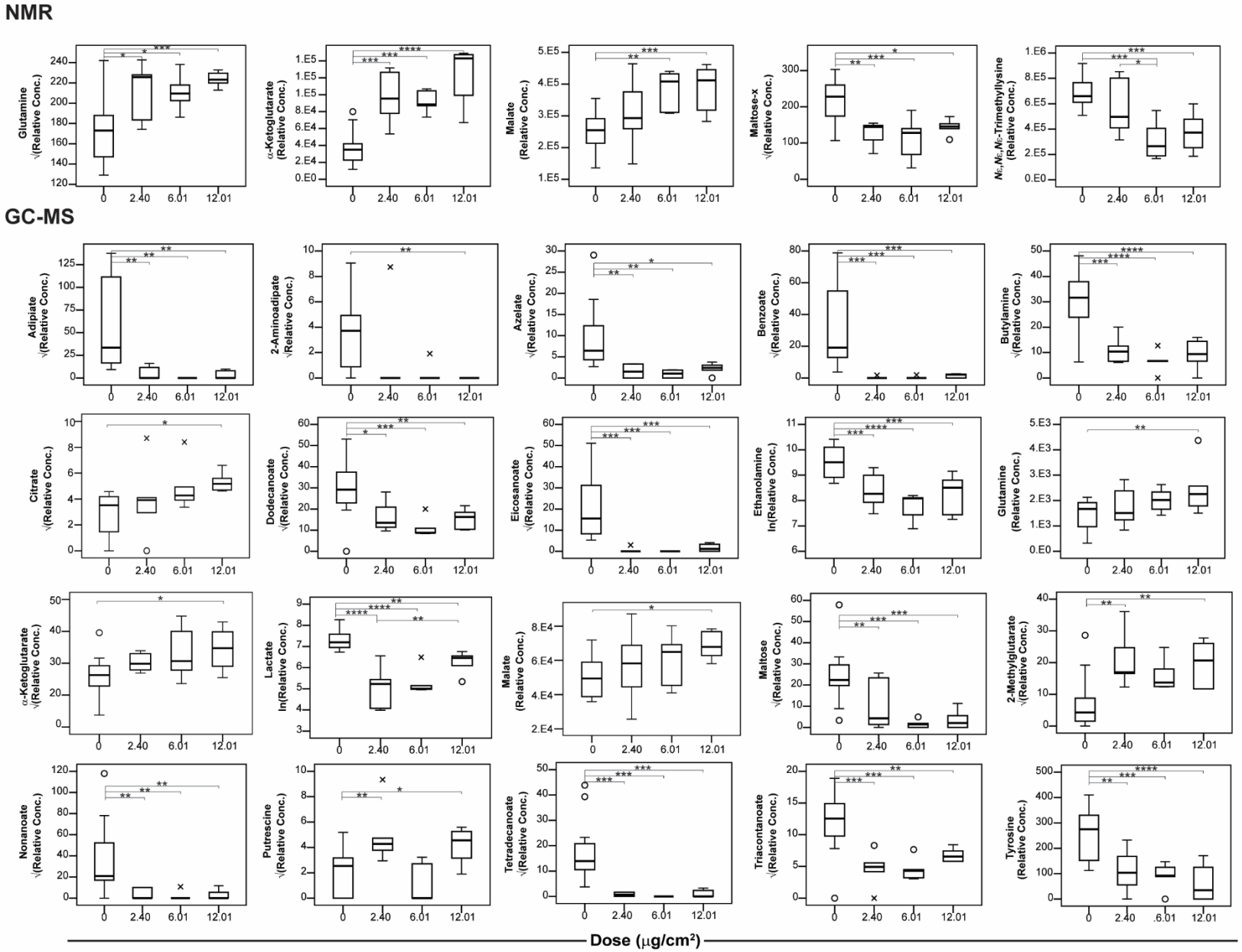

Figure S-8. Subset of metabolic changes observed in coelomic fluid of earthworms exposed to propachlor. Tukey's HSD pairwise comparisons are annotated to show the significance level (* = $p \leq 0.10$; ** = $p \leq 0.05$; *** = $p \leq 0.01$; and **** = $p \leq 0.001$).

Table S-2. ANOVA and Tukey's HSD results ($p \leq 0.10$) and Glass' Δ of metabolic perturbations detected by ^{1}H NMR and GC-MS for earthworms exposed to acetochlor.

	NMR						GC-MS					
Dose (μg/cm^2)	2.40		6.01		12.01		2.40		6.01		12.01	
	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*
Acetate			2.51	0.00					nd			
Citrate			nd						1.56	0.07	2.35	0.00
Fumarate					1.86	0.03						
α-Ketogluatarate	2.01	0.01			2.59	0.00						
Lactate							-2.48	0.00	-1.39	0.04	-1.39	0.03
Malate												
Glucose			nq									
Glucose-x									n/a			
Maltose			nq						-1.54	0.04		
Maltose-x									n/a			
myo-Inositol												
Alanine												
Aspartate												
Glutamine					1.36	0.06						
Glycine												
Leucine									nd			
Phenylalanine												
Tyrosine									-1.96	0.01	-1.65	0.02
N,*N*-Dimethylhistidine									nd			
Nε,*N*ε,*N*ε-Trimethyllysine					-2.24	0.00			nd			
Nδ,*N*δ,*N*δ-Trimethylornithine									nd			
Ornithine			nd									
Putrescine			nq				3.58	0.03				
Spermidine			nq									
2-Aminoadipate			nd									
Butylamine			nd									
Ethanolamine			nd									
Riboflavin					-1.82	0.01			nd			
Nonanoate (C9:0)			nd				-1.03	0.02	-1.09	0.03	-1.02	0.03
Dodecanoate (C12:0)			nd								-1.23	0.01
Tetradecanoate (C14:0)			nd				-1.37	0.00	-1.23	0.01	-1.11	0.01
Eicosanoate (C20:0)			nd				-1.25	0.01	-1.25	0.01	-1.01	0.03
Triacontanoate (C30:0)			nd				-1.19	0.05			-1.44	0.01
Adipate			nd				-1.04	0.02	-1.03	0.04	-1.14	0.01
Azelate			nd				-1.14	0.01	-0.89	0.09	-0.88	0.07
Benzoate			nd				-1.16	0.01	-1.12	0.02	-1.10	0.02
Glutarate			nd									
2-Methylglutarate			nd									

*nd = not detected; nq = not quantifiable; n/a = not applicable

Table S-3. ANOVA and Tukey's HSD results ($p \leq 0.10$) and Glass' Δ of metabolic perturbations detected by ^{1}H NMR and GC-MS for earthworms exposed to alachlor.

	NMR						GC-MS					
Dose (μg/cm^2)	2.40		6.01		12.01		2.40		6.01		12.01	
	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*
Acetate									nd			
Citrate			nd								2.58	0.01
Fumarate	2.01	0.03										
α-Ketogluatarate												
Lactate							-2.71	0.00	-2.22	0.01		
Malate	1.97	0.02										
Glucose			nq						2.11	0.03		
Glucose-x			1.63	0.02					n/a			
Maltose			nq									
Maltose-x	-1.78	0.03							n/a			
myo-Inositol												
Alanine												
Aspartate												
Glutamine												
Glycine												
Leucine									nd			
Phenylalanine												
Tyrosine												
N,*N*-Dimethylhistidine									nd			
Nε,*N*ε,*N*ε-Trimethyllysine					-1.94	0.05			nd			
Nδ,*N*δ,*N*δ-Trimethylornithine									nd			
Ornithine			nd						2.50	0.00	1.88	0.02
Putrescine			nq				3.50	0.01				
Spermidine			nq									
2-Aminoadipate			nd									
Butylamine			nd									
Ethanolamine			nd									
Riboflavin	-2.09	0.02							nd			
Nonanoate (C9:0)			nd									
Dodecanoate (C12:0)			nd				-0.99	0.05				
Tetradecanoate (C14:0)			nd				-1.34	0.00	-1.37	0.00	-1.31	0.00
Eicosanoate (C20:0)			nd				-1.32	0.00	-1.31	0.00	-1.29	0.00
Triacontanoate (C30:0)			nd				-1.91	0.00	-1.76	0.00	-1.02	0.06
Adipate			nd									
Azelate			nd									
Benzoate			nd				-1.09	0.01	-1.02	0.02	-1.03	0.02
Glutarate			nd									
2-Methylglutarate			nd									

*nd = not detected; nq = not quantifiable; n/a = not applicable

Table S-4. ANOVA and Tukey's HSD results ($p \leq 0.10$) and Glass' Δ of metabolic perturbations detected by ^{1}H NMR and GC-MS for earthworms exposed to butachlor.

	NMR						GC-MS					
Dose (μg/cm^2)	2.40		6.01		12.01		2.40		6.01		12.01	
	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*
Acetate									nd			
Citrate			nd									
Fumarate												
α-Ketogluatarate	2.44	0.00	1.43	0.09								
Lactate	-1.36	0.06	-1.68	0.01	-1.85	0.00	-2.03	0.01	-1.87	0.01	-1.33	0.09
Malate												
Glucose			nq									
Glucose-x									n/a			
Maltose			nq									
Maltose-x	-2.53	0.01							n/a			
myo-Inositol												
Alanine									1.74	0.07		
Aspartate									2.58	0.00		
Glutamine									2.12	0.01	2.05	0.01
Glycine							1.37	0.07	1.46	0.03		
Leucine									nd			
Phenylalanine									-1.28	0.04		
Tyrosine												
N,*N*-Dimethylhistidine			1.86	0.10					nd			
Nε,*N*ε,*N*ε-Trimethyllysine									nd			
Nδ,*N*δ,*N*δ-Trimethylornithine									nd			
Ornithine			nd									
Putrescine			nq						1.80	0.07	1.82	0.06
Spermidine			nq									
2-Aminoadipate			nd									
Butylamine			nd						1.32	0.09		
Ethanolamine			nd									
Riboflavin									nd			
Nonanoate (C9:0)			nd				-1.09	0.03	-1.06	0.02	-0.94	0.04
Dodecanoate (C12:0)			nd									
Tetradecanoate (C14:0)			nd				-1.35	0.00	-1.12	0.00	-0.94	0.01
Eicosanoate (C20:0)			nd				-1.29	0.01	-1.27	0.00	-1.26	0.01
Triacontanoate (C30:0)			nd						-1.23	0.05		
Adipate			nd				-1.08	0.03	-1.10	0.02	-1.05	0.02
Azelate			nd				-1.04	0.04	-1.11	0.01	-0.95	0.05
Benzoate			nd				-1.13	0.02	-1.15	0.01	-1.08	0.02
Glutarate			nd									
2-Methylglutarate			nd									

*nd = not detected; nq = not quantifiable; n/a = not applicable

Table S-5. ANOVA and Tukey's HSD results ($p \leq 0.10$) and Glass' Δ of metabolic perturbations detected by ^{1}H NMR and GC-MS for earthworms exposed to metolachlor.

	NMR						GC-MS					
Dose (μg/cm^2)	2.40		6.01		12.01		2.40		6.01		12.01	
	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*
Acetate			1.56	0.06					nd			
Citrate			nd						1.51	0.04		
Fumarate												
α-Ketogluatarate			1.47	0.09								
Lactate							-1.74	0.02	-1.60	0.03		
Malate												
Glucose			nq									
Glucose-x					1.80	0.09			n/a			
Maltose			nq									
Maltose-x									n/a			
myo-Inositol												
Alanine												
Aspartate												
Glutamine												
Glycine									1.98	0.04	2.25	0.03
Leucine			-1.13	0.06					nd			
Phenylalanine												
Tyrosine												
N,*N*-Dimethylhistidine	1.96	0.06			2.40	0.01			nd			
Nε,*N*ε,*N*ε-Trimethyllysine					-2.20	0.02			nd			
Nδ,*N*δ,*N*δ-Trimethylornithine	1.73	0.02							nd			
Ornithine			nd				1.48	0.08				
Putrescine			nq						2.65	0.00		
Spermidine			nq								-0.92	0.10
2-Aminoadipate			nd									
Butylamine			nd				1.49	0.02	1.20	0.08	1.26	0.08
Ethanolamine			nd									
Riboflavin									nd			
Nonanoate (C9:0)			nd									
Dodecanoate (C12:0)			nd									
Tetradecanoate (C14:0)			nd				-1.14	0.02	-0.96	0.07		
Eicosanoate (C20:0)			nd				-1.41	0.00	-1.15	0.02		
Triacontanoate (C30:0)			nd									
Adipate			nd				-1.05	0.02	-1.11	0.01	-0.97	0.06
Azelate			nd				-1.03	0.03	-1.00	0.03	-0.93	0.07
Benzoate			nd				-1.11	0.01	-1.01	0.03	-0.87	0.10
Glutarate			nd				-0.81	0.09	-0.81	0.09		
2-Methylglutarate			nd									

*nd = not detected; nq = not quantifiable; n/a = not applicable

Table S-6. ANOVA and Tukey's HSD results ($p \leq 0.10$) and Glass' Δ of metabolic perturbations detected by ^{1}H NMR and GC-MS for earthworms exposed to S-metolachlor.

	NMR						GC-MS					
Dose (μg/cm^2)	2.40		6.01		12.01		2.40		6.01		12.01	
	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*
Acetate					1.31	0.09			nd			
Citrate			nd								2.37	0.01
Fumarate												
α-Ketogluatarate	1.91	0.02	2.17	0.01	1.53	0.09						
Lactate	-1.67	0.01	-1.42	0.03	-1.41	0.04	-3.23	0.00	-2.36	0.00	-1.54	0.05
Malate												
Glucose			nq									
Glucose-x									n/a			
Maltose			nq									
Maltose-x	-1.23	0.03	-1.41	0.01					n/a			
myo-Inositol									-1.09	0.10		
Alanine												
Aspartate												
Glutamine			1.55	0.02	1.49	0.04			2.52	0.01	3.46	0.00
Glycine									2.05	0.01		
Leucine									nd			
Phenylalanine												
Tyrosine							-1.80	0.00			-1.36	0.06
N,*N*-Dimethylhistidine									nd			
*N*ε,*N*ε,*N*ε-Trimethyllysine									nd			
*N*δ,*N*δ,*N*δ-Trimethylornithine									nd			
Ornithine			nd						1.53	0.03		
Putrescine			nq						2.10	0.03	2.71	0.01
Spermidine			nq									
2-Aminoadipate			nd								2.02	0.05
Butylamine			nd									
Ethanolamine			nd									
Riboflavin									nd			
Nonanoate (C9:0)			nd						-0.98	0.03		
Dodecanoate (C12:0)			nd									
Tetradecanoate (C14:0)			nd				-1.25	0.01	-1.30	0.00	-1.07	0.03
Eicosanoate (C20:0)			nd				-1.22	0.01	-1.19	0.01	-1.04	0.03
Triacontanoate (C30:0)			nd									
Adipate			nd				-0.98	0.03	-1.10	0.01	-1.06	0.03
Azelate			nd				-0.99	0.04	-0.89	0.07		
Benzoate			nd				-1.02	0.03	-1.07	0.02	-0.98	0.05
Glutarate			nd						-0.81	0.10		
2-Methylglutarate			nd									

*nd = not detected; nq = not quantifiable; n/a = not applicable

Table S-7. ANOVA and Tukey's HSD results ($p \leq 0.10$) and Glass' Δ of metabolic perturbations detected by ^{1}H NMR and GC-MS for earthworms exposed to propachlor.

	NMR						GC-MS					
Dose (μg/cm^2)	2.40		6.01		12.01		2.40		6.01		12.01	
	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*	Δ	*p*
Acetate									nd			
Citrate			nd								1.37	0.08
Fumarate					1.29	0.09						
α-Ketogluatarate	2.74	0.00	2.52	0.00	4.02	0.00					1.43	0.08
Lactate							-4.47	0.00	-4.01	0.00	-2.09	0.02
Malate			2.05	0.03	2.19	0.01					1.49	0.07
Glucose			nq									
Glucose-x									n/a			
Maltose			nq				-1.09	0.05	-1.70	0.00	-1.56	0.00
Maltose-x	-1.44	0.02	-1.66	0.01	-1.14	0.05			n/a			
myo-Inositol												
Alanine												
Aspartate									-1.28	0.05		
Glutamine	1.04	0.09	1.05	0.09	1.40	0.01					1.67	0.04
Glycine												
Leucine									nd			
Phenylalanine												
Tyrosine							-1.46	0.01	-1.66	0.01	-1.96	0.00
N,*N*-Dimethylhistidine									nd			
Nε,*N*ε,*N*e-Trimethyllysine			-2.95	0.00	-2.47	0.01			nd			
Nδ,*N*δ,*N*δ-Trimethylornithine									nd			
Ornithine			nd									
Putrescine			nq				1.57	0.02			1.19	0.10
Spermidine			nq									
2-Aminoadipate			nd								-1.25	0.04
Butylamine			nd				-1.55	0.00	-1.91	0.00	-1.68	0.00
Ethanolamine			nd				-1.88	0.01	-2.90	0.00	-2.03	0.00
Riboflavin									nd			
Nonanoate (C9:0)			nd				-0.99	0.03	-1.03	0.04	-1.01	0.03
Dodecanoate (C12:0)			nd				-1.00	0.05	-1.37	0.01	-1.05	0.04
Tetradecanoate (C14:0)			nd				-1.37	0.00	-1.43	0.00	-1.35	0.00
Eicosanoate (C20:0)			nd				-1.29	0.00	-1.32	0.01	-1.22	0.01
Triacontanoate (C30:0)			nd				-1.48	0.00	-1.50	0.00	-1.05	0.04
Adipate			nd				-1.05	0.02	-1.14	0.02	-1.08	0.02
Azelate			nd				-1.00	0.03	-1.08	0.03	-0.92	0.05
Benzoate			nd				-1.22	0.01	-1.22	0.01	-1.18	0.01
Glutarate			nd				-0.81	0.09			-0.81	0.09
2-Methylglutarate			nd				1.55	0.01			1.45	0.02

*nd = not detected; nq = not quantifiable; n/a = not applicable