MASSIVE IMMUNO MULTI-RESIDUE SCREENING OF WATER POLLUTANTS

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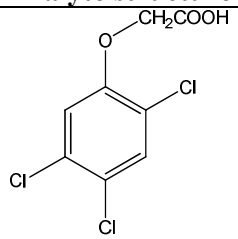
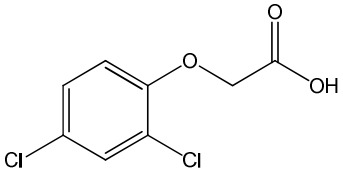
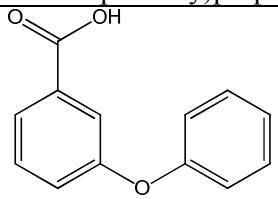
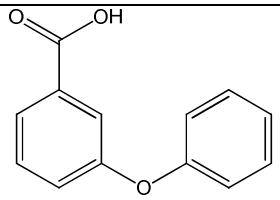
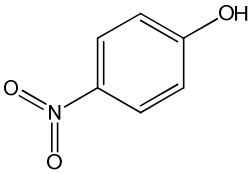
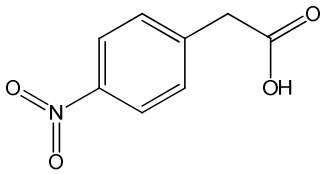
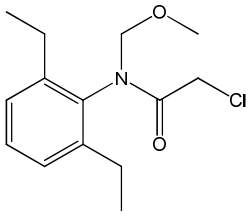
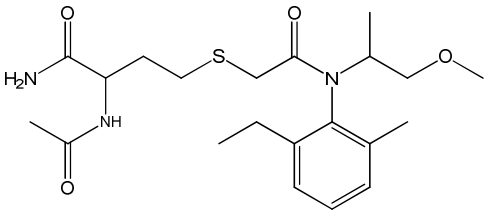
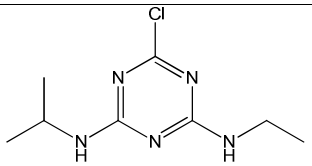
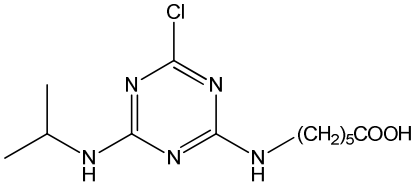
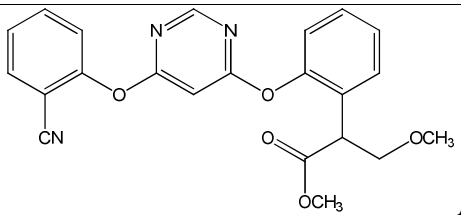
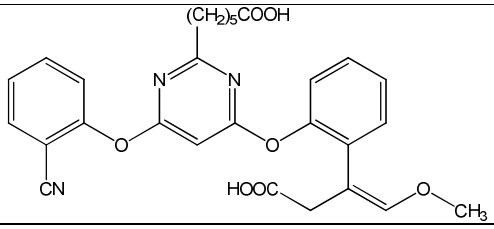
HPLC-MS/GC-MS determination.

Electrospray ionization (ESI) was applied in HPLC-MS in positive mode for analysis of ATZ, AZB, DIU, FCF, PBA, PYS and SSZ. Chromatographic separation was achieved using an HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a reversed phase analytical column of 150 mm-4.6 mm and 5 µm particle size (Zorbax Eclipse Plus C18). Column temperature was maintained at 55 °C. The sample injection volume was 100 µL. The mobile phase was composed of acetonitrile (solvent A) and methanol (solvent B) at a constant flow of 0.35 mL/min. The chromatographic method held the mobile phase composition A:B at 86:14.

For GC-MS determination of ALA, CLP and TCS residues, 100 mL water samples were extracted with PDMS stir bars by agitation at 500 rpm during 2h at room temperature. Later, the stir bars were removed from samples, dried, and introduced into glass desorption tubes. Desorption was carried out at 325 °C during 7 min under helium (flow-rate of 75 mL min-1) in the split less mode, maintaining a cryofocusing temperature of 20 °C in the PTV injector. The analyses were conducted using a HP-5 MS column (30m × 0.25 mm i.d., 0.25 µm film thickness, 5:95 phenyl- polydimethylsiloxane). The column was kept at 70 °C for 2 min. Later, a temperature ramp was applied, first until 150 °C at 25 °C min-1; when this temperature was reached a second ramp of 200 at 3 °C min-1 was applied; the third ramp was until 280 °C at 8 °C min-1 for 10 min. Detection was achieved using electron impact ionization (EI+) mode and full scan in the m/z range of 100–325. Characteristic ions were used to identify analytes, being quantified by the peak areas.

Table S1. Chemical structures of the analytes and haptens used in this work

Analyte structure	Hapten structure	Reference
 2-(2,4,5-trichlorophenoxy)propionic acid	 2,4D	1
 3-phenoxybenzoic acid	 3PBA	2
 4-nitrophenol	 4NP	3
 Alachlor	 Metolachlor	4
 Atrazine	 2d	5
 Azo		6

Analyte structure	Hapten structure	Reference
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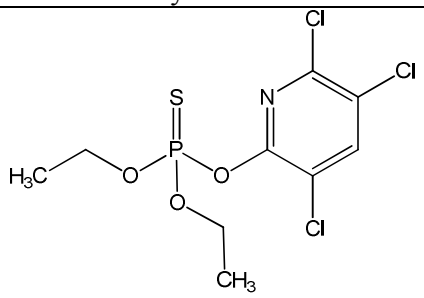
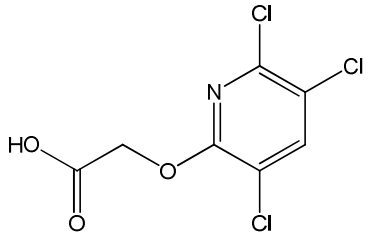
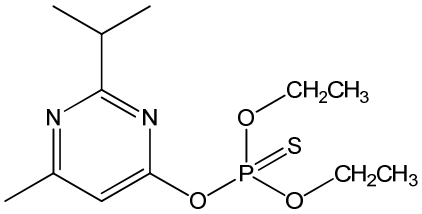
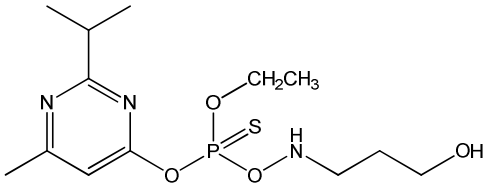
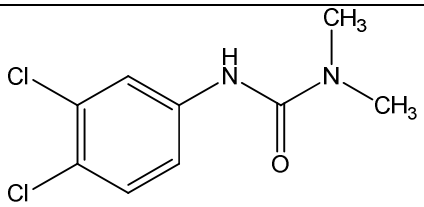
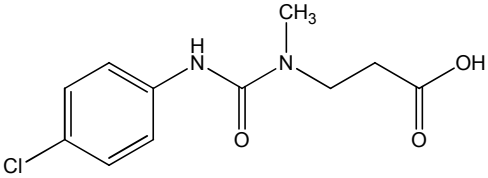
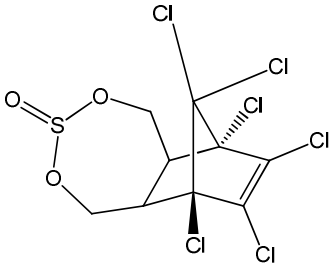
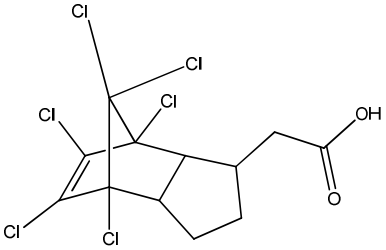
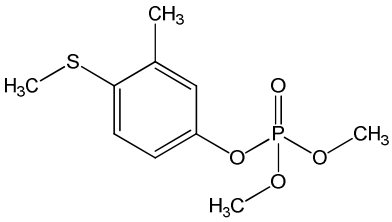
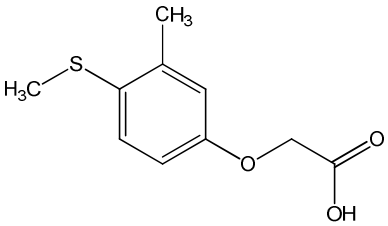
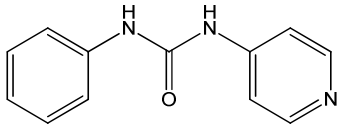
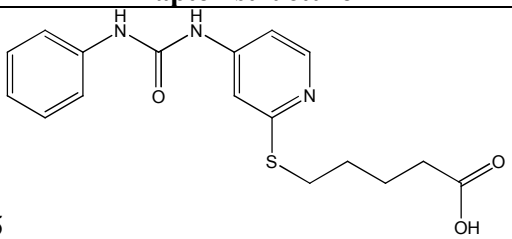
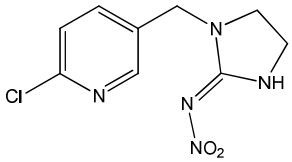
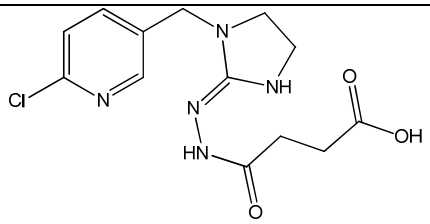
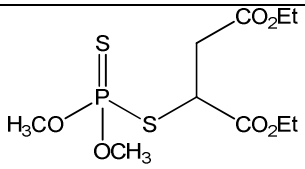
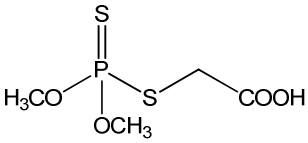
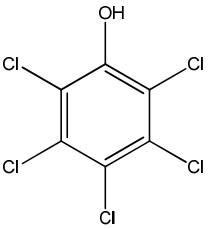
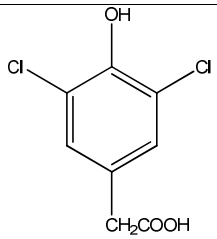
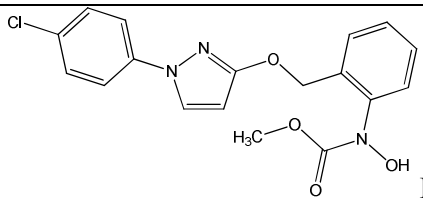
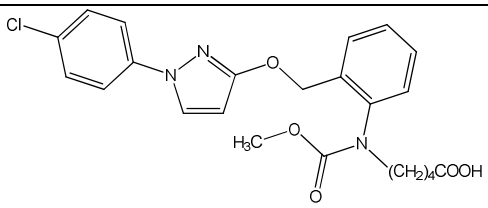
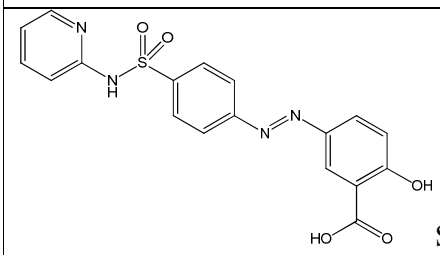
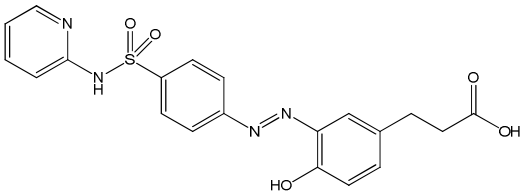
 Chlorpyrifos	 Triclopyr	7
 Diazinon	 D7	8
 Diuron	 H7	9
 Endosulfan	 CCD1	10
 Fention	 F1	11

Analyte structure	Hapten structure	Reference
 Forchlorfenuron	 s5	12
 Imidacloprid	 H1	13
 Malathion	 MA-2	14
 Pentachlorophenol	 CIP1	15
 Pyraclo strobin	 PYo5	16
 Sulfasal azine	 S8	17

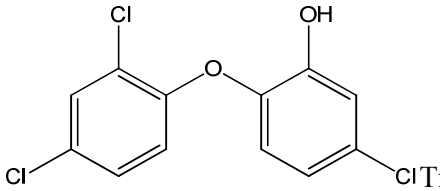
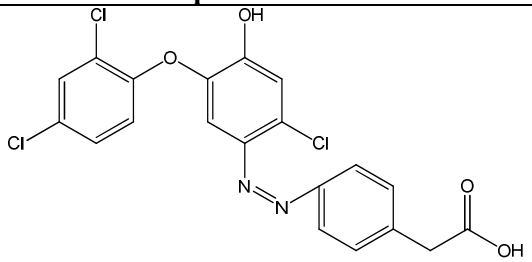
Analyte structure	Hapten structure	Reference
 Triclosan	 Tr7	18

Table S2. Optimal coating conjugate concentrations and gold labeled antibody dilutions

Analyte	Coating conjugate (mg/L)	Gold labeled antibody	
		[a]Dilution	[b]Optical density
2-(2,4,5 trichlorophenoxy)propionic acid	10	1/100	0.10
3-phenoxybenzoic acid	10	1/100	0.10
4-nitrophenol	10	1/50	0.20
Alachlor	40	1/50	0.20
Atrazine	2.5	1/500	0.02
Azoxystrobin	5.0	1/500	0.02
Chlorpyrifos	20	1/100	0.10
Diazinon	20	1/100	0.10
Diuron	5.0	1/100	0.10
Endosulfan	20	1/100	0.10
Fenthion	10	1/100	0.10
Forchlorfenuron	10	1/500	0.02
Imidaclorpid	10	1/250	0.04
Malathion	20	1/100	0.10
Pentachlorophenol	20	1/100	0.10
Pyraclostrobin	20	1/250	0.04
Sulfasalazine	10	1/500	0.02
Triclosan	50	1/50	0.20

[a]PBS-T as buffer (v/v). [b]Absorbance at 520 nm (a.u)

Table S3. Limit of detection (LOD), sensitivity (IC_{50}), and working range (WR) for the single assays

Analyte	LOD (µg/L)	IC_{50} (µg/L)	WR (µg/L)
2-(2,4,5-trichlorophenoxy) propionic acid (TPA)	0.08	1.35 ± 0.18	0.15 - 5.94
3-Phenoxybenozic acid (PBA)	0.26	1.95 ± 0.24	0.41 - 7.12
4-Nitrophenol (4NP)	10.9	72.7 ± 21.2	13.5 - 349
Alachlor (ALA)	0.28	2.61 ± 0.28	0.35 -7.21
Atrazine (ATZ)	0.16	1.93 ± 0.20	0.19 - 9.01
Azoxystrobin (AZB)	0.13	0.92 ± 0.13	0.18 - 4.92
Chlorpyrifos (CLP)	0.12	2.65 ± 0.31	0.26 - 18
Diazinon (DZN)	102	627 ± 331	125 - 1905
Diuron (DIU)	0.42	3.97 ± 0.85	0.54 – 11.7
Endosulfan (END)	1.8	20.2 ± 7.6	5.3 - 37.3
Fenthion (FTN)	1.7	8.4 ± 2.1	2.3 - 34.3
Forchlorfenuron (FCF)	0.05	0.15 ± 0.01	0.07 - 0.58
Imidacloprid (IMD)	0.08	0.32 ± 0.06	0.14 - 0.52
Malathion (MLT)	5.8	15.1 ± 2.4	7.52 - 41.4
Pentachlorophenol (PCP)	1.0	14.2 ± 3.1	2.1 - 25.3
Pyraclostrobin (PYS)	0.34	1.55 ± 0.11	0.41 - 5.15
Sulfasalazine (SSZ)	0.003	0.06 ± 0.01	0.005 - 0.15
Triclosan (TCS)	14	195 ± 25	44 - 388

The limit of detection (LOD) was calculated as the signal of the blank plus 3 times its standard deviation. The working range corresponds to the lineal part of the calibration curve (20-80%).

Table S4. Reactivity for gold labeled 4NP, TPA and CLP antibodies

	Coating conjugate			
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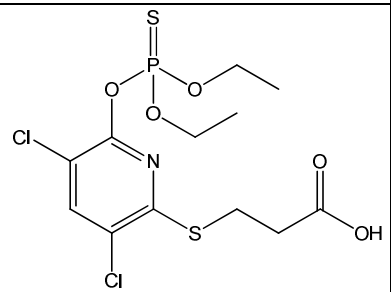
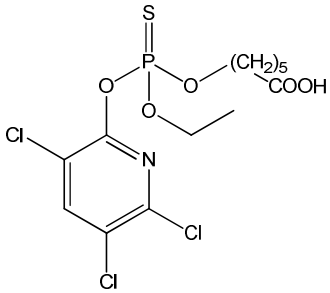
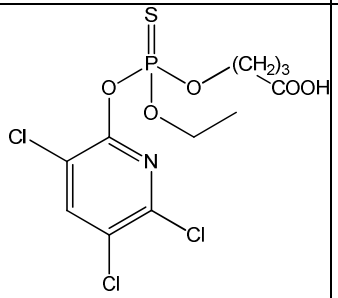
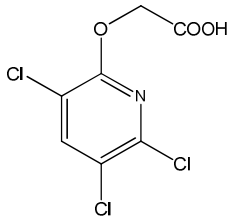
Antibody functionalized nanoparticle				
CLP (IC_{50}; µg/L)	-/+	++++ (1013)	++++ (474)	++++ (2.71)
4NP	++	-	++++	++
TPA	++	++++	++++	+++

Signal intensity (a.u.): <500; -/+: 1000-3000; +: 3000-6000; ++: 6000-8000; ++: 6000-8000; +++: 8000-10,000

Table S5. Chemical hapten structures for Forchlorfenuron

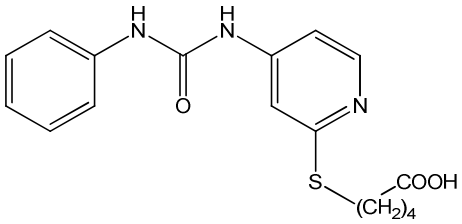
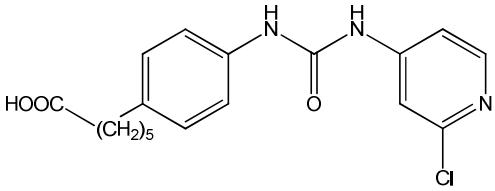
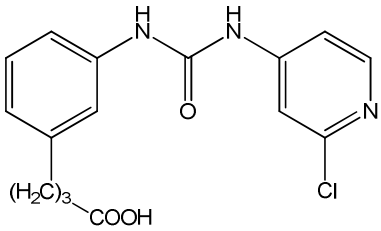
Hapten	Hapten structure
s5	
p6	
m6	

Table S6. Limit of detection (LOD), sensitivity (IC_{50}), working range (WR), slope, and linear regression coefficient (r^2) for the ten-plex assay

Analyte	LOD (µg/L)	IC_{50} (µg/L)	WR (µg/L)	Slope	r^2
3-Phenoxybenozic acid (PBA)	0.24	1.86 ± 0.27	0.39 - 6.04	-1.07	0.996
Alachlor (ALA)	0.21	2.28 ± 0.24	0.35 -7.21	-0.83	0.998
Atrazine (ATZ)	0.14	1.73 ± 0.31	0.18 - 8.81	-0.74	0.997
Azoxystrobin (AZB)	0.11	0.88 ± 0.11	0.15 - 4.12	-0.97	0.997
Chlorpyrifos (CLP)	0.11	2.71 ± 0.36	0.22 - 16	-0.61	0.995
Diuron (DIU)	0.36	3.52 ± 0.94	0.49 - 10.7	-0.68	0.994
Forchlorfenuron (FCF)	0.06	0.16 ± 0.01	0.06 - 0.52	-2.00	0.999
Pyraclostrobin (PYS)	0.39	1.78 ± 0.06	0.46 - 5.14	-1.4	0.999
Sulfasalazine (SSZ)	0.003	0.07 ± 0.008	0.004 - 0.12	-0.78	0.998
Triclosan (TCS)	12	182 ± 70	34 - 397	-0.65	0.995

The limit of detection (LOD) was calculated as the signal of the blank plus 3 times its standard deviation. The working range corresponds to the lineal part of the calibration curve (20-80%).

Table S7. Cross-reactivity values for structurally related compounds in the ten-plex immunoassay

Compound	IC_{50} (μg/L)	CR (%)	Compound	IC_{50} (μg/L)	CR (%)
Alachlor	2.48	100	**Forchlorfenuron**	0.16	100
Metolachlor	496	0.5	Thidiazuron	0.8	20
Acetochlor	99.2	2.5	Diphenylurea	>10000	< 0.01
Butachlor	107.8	2.3	Kinetin	>10000	< 0.01
Propachlor	496	0.5	*Trans*-Zeatin	>10000	< 0.01
Alachlor oxanilic acid	34.9	14.1	6-Benzylaminopurine	>10000	< 0.01
Atrazine	1.73	100	**3-Phenoxybenzoic acid**	1.86	100
Propazine	1.48	117	4-Fluoro-3-phenoxybenzoic acid	2.58	72
Terbutylazine	7.21	24	4-hydroxy-3-phenoxybenzoic acid	1.77	105
Simazine	8.81	16	3-phenoxybenzaldehyde	2.41	77
Ametryn	12.82	11	Cypermethrin	>10000	< 0.01
Prometryn	20.14	7	Fenvalerate	>10000	< 0.01
Azoxystrobin	0.88	100	**Pyraclostrobin**	1.78	100
Kresoxim-methyl	>10000	< 0.01	Dimoxystrobin	>10000	< 0.01
Dimoxystrobin,	>10000	< 0.01	Picoxystrobin	>10000	< 0.01
Picoxystrobin,	>10000	< 0.01	Azoxystrobin	>10000	< 0.01
Trifloxystrobin,	>10000	< 0.01	Trifloxystrobin	>10000	< 0.01
Pyraclostrobin,	>10000	< 0.01	Kresoxim-methyl	>10000	< 0.01
Chlorpyrifos	2.71	100	**Sulfasalazine**	0.07	100
Chlorpyrifos-methyl	5.42	50	Sulfathiazole	3.75	1.8
Chlorpyrifos-oxon	5.89	46	N^4-Phtalylsulfathiazole	4.28	1.6
Bromophos-methyl	10.84	25	Sulfapyridine	12	0.6
Bromophos-ethyl	677.5	0.4	Sulfamethoxazole	85.7	0.08
Diclophention	>10000	0.01	Sulfadiazine	120	0.06
Diuron	3.52	180	**Triclosan**	182	100
Monuron	1.95	100	Methyl-triclosan	>3033	<6
Linuron	6.1	58	2,4-Dichlorophenol	>10000	0.03
Monolinuron	8.4	42	2,5-Dichlorophenol	>10000	0.03
Fenuron	352	1	3,4-Dichlorophenol	>10000	0.03
Neburon	0.25	1400	2,4,6-Trichlorophenol	>10000	0.03

Table S8. Results obtained for the immuno multi-residue[a] and chromatographic[b] analysis (GC-MS or HPLC-MS) of spiked water samples

	Added (µg/L)	Found (µg/L)		Recovery (%)		Added (µg/L)	Found (µg/L)		Recovery (%)		Added (µg/L)	Found (µg/L)		Recovery (%)	
Sample	**1**					**2**					**3**				
ALA	2	[a]2.71 ± 0.48	[b]1.70 ± 0.25	136[a]	85[b]	1	1.29 ± 0.41	0.75 ± 0.15	129	75	0.5	0.61 ± 0.09	0.33 ± 0.10	122	66
ATZ	4	4.12 ± 1.04	3.08 ± 0.22	103	77	2	2.22 ± 0.54	1.44 ± 0.30	111	72	1	0.73 ± 0.2	0.82 ± 0.16	73	82
DIU	8	8.42 ± 0.86	6.82 ± 1.11	105	85	4	4.81 ± 0.72	3.40 ± 0.21	120	85	2	2.75 ± 0.58	0.67 ± 0.21	138	34
AZB	1	1.14 ± 0.16	0.78 ± 0.12	114	78	0.5	0.55 ± 0.06	0.37 ± 0.12	110	74	0.25	0.28 ± 0.04	0.18 ± 0.06	112	72
FCF	0.5	0.59 ± 0.05	0.42 ± 0.11	118	84	0.25	0.27 ± 0.04	0.20 ± 0.08	108	80	0.125	0.14 ± 0.09	0.09 ± 0.01	112	72
PYS	2	1.80 ± 0.28	1.69 ± 0.31	90	85	1	0.61 ± 0.11	0.70 ± 0.18	61	70	0.5	0.41 ± 0.05	0.44 ± 0.12	72	88
Sample	**4**					**5**					**6**				
ALA	2	2.73 ± 0.65	2.40 ± 0.31	137	120	1	1.44 ± 0.23	1.10 ± 0.28	144	110	0.5	0.51 ± 0.06	0.23 ± 0.15	102	46
ATZ	4	2.83 ± 0.12	3.29 ± 0.45	71	82	2	1.46 ± 0.25	1.68 ± 0.19	73	84	1	0.97 ± 0.13	0.83 ± 0.17	97	83
DIU	8	6.71 ± 0.52	7.96 ± 0.92	84	100	4	4.87 ± 0.36	4.12 ± 0.44	122	103	2	2.57 ± 0.63	2.03 ± 0.10	129	102
SSZ	2	1.95 ± 0.14	2.14 ± 0.33	98	107	1	0.92 ± 0.26	1.06 ± 0.11	92	106	0.5	0.46 ± 0.21	0.50 ± 0.04	92	100
PBA	4	4.41 ± 0.02	3.45 ± 0.56	110	86	2	2.64 ± 0.39	2.03 ± 0.28	132	102	1.00	1.17 ± 0.54	1.34 ± 0.17	117	134
CLP	2	2.36 ± 0.41	1.34 ± 0.41	118	67	1	0.99 ± 0.28	0.65 ± 0.16	99	65	0.5	0.66 ± 0.16	0.37 ± 0.07	132	74
Sample	**7**					**8**					**9**				
AZB	1	0.86 ± 0.19	0.87 ± 0.16	86	87	0.5	0.47 ± 0.16	0.47 ± 0.09	94	94	0.25	0.29 ± 0.07	0.25 ± 0.09	116	100
FCF	0.5	0.48 ± 0.20	0.57 ± 0.12	96	114	0.25	0.34 ± 0.05	0.28 ± 0.03	136	112	0.125	0.09 ± 0.03	0.13 ± 0.02	72	104
PYS	2	2.06 ± 0.53	1.84 ± 0.28	103	92	1	1.21 ± 0.45	0.93 ± 0.09	121	93	0.5	0.59 ± 0.29	0.43 ± 0.12	118	86
SSZ	2	2.15 ± 0.11	2.80 ± 0.20	108	140	1	1.11 ± 0.25	1.29 ± 0.11	111	129	0.5	0.46 ± 0.11	0.67 ± 0.07	92	134
TCS	2	< LOD	1.33 ± 0.16	-	67	1	< LOD	0.78 ± 0.14	-	78	0.5	< LOD	0.38 ± 0.09	-	76
Sample	**10**					**11**					**12**				
ALA	2	2.68 ± 0.39	1.33 ± 0.24	134	67	1	1.34 ± 0.16	0.75 ± 0.13	134	75	0.5	0.61 ± 0.13	0.31 ± 0.08	122	62
ATZ	4	3.80 ± 0.62	3.13 ± 0.49	95	78	2	2.34 ± 0.73	1.47 ± 0.68	117	74	1	0.92 ± 0.22	0.74 ± 0.13	92	74
DIU	8	8.93 ± 0.61	7.74 ± 0.96	112	97	4	3.76 ± 0.56	4.06 ± 0.62	94	102	2	2.75 ± 0.49	1.98 ± 0.38	138	99
AZB	1	1.20 ± 0.18	0.87 ± 0.11	120	87	0.5	0.43 ± 0.08	0.48 ± 0.06	86	96	0.25	0.23 ± 0.14	0.23 ± 0.14	92	92
FCF	0.5	0.63 ± 0.05	0.50 ± 0.06	126	100	0.25	0.33 ± 0.03	0.26 ± 0.01	132	104	0.125	0.11 ± 0.01	0.12 ± 0.01	88	96
PYS	2	1.66 ± 0.44	1.90 ± 0.18	83	95	1	0.65 ± 0.07	0.84 ± 0.05	65	84	0.5	0.52 ± 0.11	0.47 ± 0.12	104	94
PBA	4	4.00 ± 0.53	3.28 ± 0.53	100	82	2	1.58 ± 0.16	2.07 ± 0.11	79	104	1	1.19 ± 0.25	1.38 ± 0.24	119	138
CLP	2	1.46 ± 0.35	1.29 ± 0.28	73	64	1	1.16 ± 0.31	0.74 ± 0.22	116	74	0.5	0.55 ± 0.12	0.37 ± 0.12	110	74
SSZ	2	1.92 ± 0.61	2.83 ± 0.29	96	142	1	1.04 ± 0.41	1.17 ± 0.33	104	117	0.5	0.58 ± 0.24	0.69 ± 0.14	116	138
TCS	2	< LOD	1.33 ± 0.14	-	66	1	< LOD	0.67 ± 0.25	-	67	0.5	< LOD	0.39 ± 0.11	-	78
Sample	**13**					**14**					**15**				
AZB	1	0.99 ± 0.18	0.93 ± 0.08	99	93	0.5	0.41 ± 0.04	0.51 ± 0.03	82	102	0.25	0.14 ± 0.06	0.24 ± 0.03	56	96
FCF	0.5	0.44 ± 0.19	0.56 ± 0.06	88	112	0.25	0.29 ± 0.02	0.27 ± 0.01	116	108	0.125	0.11 ± 0.04	0.13 ± 0.01	88	104
PYS	2	2.50 ± 0.53	2.04 ± 0.18	125	102	1	0.82 ± 0.18	0.99 ± 0.09	82	99	0.5	0.42 ± 0.08	0.49 ± 0.07	84	98
PBA	4	4.82 ± 0.61	3.57 ± 0.47	121	89	2	2.38 ± 0.34	1.73 ± 0.21	119	87	1	1.32 ± 0.43	0.85 ± 0.11	132	85
CLP	2	2.21 ± 0.19	1.39 ± 0.19	111	70	1	1.26 ± 0.31	0.79 ± 0.28	126	79	0.5	0.62 ± 0.12	0.40 ± 0.08	124	80
SSZ	2	2.36 ± 0.16	3.46 ± 0.61	118	173	1	0.85 ± 0.27	1.91 ± 0.16	85	191	0.5	0.57 ± 0.09	1.40 ± 0.03	114	280
TCS	2	< LOD	1.32 ± 0.15	-	66	1	< LOD	0.72 ± 0.22	-	72	0.5	< LOD	0.38 ± 0.06	-	76

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