
555 **Supplemental Information**

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557 **Online Solid Phase Extraction High-Performance Liquid Chromatography – Isotope Dilution –**
558 **Tandem Mass Spectrometry Quantification of Organophosphate Pesticides, Synthetic**
559 **Pyrethroids, and Selected Herbicide metabolites in human Urine.**

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564 Table S1. Native Standards
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Analyte Abbreviation	Source	Catalog number	CAS#	Purity (%)
2,4-D	Cambridge Isotopes	ULM-7418-1.2	94-75-7	98
3-PBA	Cambridge Isotopes	ULM-6781-SA-1.2	3739-38-6	98
4FP	Cambridge Isotopes	ULM-7391-1.2	77279-89-1	98
cis-DBCA	Cambridge Isotopes	ULM-10693-S	63597-73-9	95
cis-DCCA	Cambridge Isotopes	ULM-9176-1.2	55701-05-8	98
IMPy	Cambridge Isotopes	ULM-7432-1.2	2814-20-2	98
MDA	LGC Standards	DRE-C14713000	1190-28-9	99
PNP	Cambridge Isotopes	ULM-8892-1.2	100-02-7	98
TCPy	Cambridge Isotopes	ULM-9204-1.2	6515-38-4	98
trans-DCCA	Cambridge Isotopes	ULM-9176-1.2	55701-05-8	98

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568 Table S2. Labeled Internal Standards

Analyte Abbreviation	Label	Source	Catalog number	Purity (%)
2,4-D_L	¹³ C6	Cambridge Isotopes	CLM-1858-1.2	98
3-PBA_L	¹³ C6	Cambridge Isotopes	CLM-4542-SA-1.2	98
4FP_L	¹³ C6	Cambridge Isotopes	CLM-7389-1.2	98
<i>cis</i> -DBCA_L	¹³ C2, 1D	Cambridge Isotopes	CDLM-10692	98
<i>cis</i> -DCCA_L	¹³ C2, 1D	Cambridge Isotopes	CDLM-9205-1.2	98
IMPy_L	¹³ C4	Cambridge Isotopes	CLM-4538-1.2	97
MDA_L	¹³ C4	Cambridge Isotopes	CLM-9050-MT-1.2	97
PNP_L	¹³ C6	Cambridge Isotopes	CLM-789-1.2	98
TCPy_L	¹³ C3	Cambridge Isotopes	CLM-9049-1.2	97
<i>trans</i> -DCCA_L	¹³ C2, 1D	Cambridge Isotopes	CDLM-9206-1.2	95

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573 Table S3. Online solid-phase extraction (SPE) and analytical separation conditions. Solvent A: 0.1% acetic
 574 acid in water, Solvent B: methanol

<u>SPE Pump</u>			<u>Analytical Pump</u>		
Time (min)	Flow rate (mL/min)	%B	Time (min)	Flow rate (mL/min)	%B
0.0	2.00	1.0	0.0	0.75	20.0
3.1	2.00	1.0	6.1	0.75	20.0
3.1	0.20	1.0	6.1	0.75	20.0
5.0	0.20	1.0	8.0	0.75	70.0
7.0	0.20	1.0	10.0	0.40	70.0
8.0	0.75	100	20.0	0.40	70.0
13.0	0.75	100	20.0	0.40	100
14.0	0.50	1.0	23.0	0.40	100
25.0	0.50	1.0	23.5	0.75	20.0
27.0	2.00	1.0	27.0	0.75	20.0

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578 Table S4. Total, within run and between run precision expressed as % RSD, n = 20 runs using two spiked
 579 urine materials (QC1 and QC2 concentrations shown in Table 3) analyzed in duplicate in each run.

ANALYTE	QC 1			QC 2		
	Within Run	Between Run	Total	Within Run	Between Run	Total
2,4-D	3.2	7.9	8.6	3.9	6.7	8.0
3-PBA	3.7	7.9	8.7	3.2	6.6	7.3
4FP	3.5	7.5	8.3	4.1	6.1	7.3
<i>cis</i>-DBCA	11.3	2.3	11.5	6.6	7.2	9.8
<i>cis</i>-DCCA	5.1	7.9	9.4	5.3	7.6	9.3
IMPy	3.1	5.9	6.7	4.3	4.1	5.9
MDA	5.5	10.1	11.5	3.8	6.4	7.4
PNP	3.5	7.0	7.8	3.2	5.5	6.4
TCPy	4.7	10.3	11.3	3.2	8.7	9.3
<i>trans</i>-DCCA	3.0	7.0	7.6	3.8	7.0	8.0.

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Table S5. Stability results expressed as percent relative standard deviations of the concentrations of the materials used for the test; n=3 for all measurements

ANALYTE	Material 1			Material 2		
	Three freeze-thaw cycles ^a	Bench-top stability ^b	Processed sample stability ^c	Three freeze-thaw cycles ^a	Bench-top stability ^b	Processed sample stability ^c
2,4-D	12.8	12.8	0.0	7.0	7.6	-1.9
3-PBA	13.5	12.5	-5.2	6.9	5.7	-4.4
4FP	10.5	13.7	-2.1	5.8	9.7	0.6
cis-DBCA	5.4	9.7	-1.1	5.7	11.5	-10.2
cis-DCCA	11.7	10.6	-5.3	9.4	7.5	-10.6
IMPy	10.8	12.9	3.2	3.8	4.5	-8.9
MDA	11.7	13.8	-5.3	7.4	5.6	-8.0
PNP	7.4	11.7	-5.3	10.1	10.1	-5.7
TCPy	9.4	10.4	-5.2	5.4	5.4	-9.2
trans-DCCA	10.5	10.5	0.0	8.0	9.9	-3.1

^a Freeze-thaw stability was determined by comparing analyte concentrations of two quality control materials before storing samples at -70°C and after three subsequent freeze-thaw cycles.

^b Bench-top stability was assessed by comparing analyte concentrations of two quality control materials before and after materials were left on the bench at room temperature for one day.

^c Processed sample stability was assessed for short-term stability of processed samples and included 24 hr. resident time in autosampler at room temperature.

593 Table S6. The mean slope of calibration curve in water and in urine for each analyte, and % difference
594 between the slopes.

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Analyte	Water Slope	Matrix Slope	% Difference	Weighting
2,4-D	0.15	0.16	-3.2%	1/X
3-PBA	0.17	0.18	-3.5%	1/X
4FP	0.08	0.08	-2.0%	1/X
<i>cis</i> -DBCA	0.09	0.09	-3.9%	1/X
<i>cis</i> -DCCA	0.19	0.19	-2.6%	1/X
IMPy	0.09	0.10	-3.7%	1/X
MDA	0.18	0.17	1.7%	1/X
PNP	0.15	0.16	-3.3%	1/X
TCPy	0.04	0.04	-5.0%	1/X
<i>trans</i> -DCCA	0.31	0.33	-4.5%	1/X

596 N=3

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598 Table S7. German External Quality Assessment Scheme (GEQUAS) and External Quality Assessment
599 Scheme for Organic substances in Urine (OSEQAS) results (2021-2023).

Analyte	Quality Assurance Program	Round	Year	Sample ID	Reported Value (ng/mL)	Reference Value (ng/mL)	Tolerance Range (ng/mL)
2,4-D	OSEQAS	2021-01	2021	OS-U-E2101	0.44	0.513	0.288 - 0.738
		2021-01	2021	OS-U-E2102	0.45	0.508	0.291 - 0.725
		2021-01	2021	OS-U-E2103	0.42	0.515	0.279 - 0.751
		2021-02	2021	OS-U-E2104	0.39	0.407	0.243 - 0.571
		2021-02	2021	OS-U-E2105	0.48	0.49	0.293 - 0.687
		2021-02	2021	OS-U-E2106	0.2	0.246	0.116 - 0.376
		2022-01	2022	OS-U-E2201	0.37	0.408	0.239 - 0.577
		2022-01	2022	OS-U-E2202	0.88	0.947	0.557 - 1.34
		2022-01	2022	OS-U-E2203	1.1	1.23	0.72 - 1.74
		2022-02	2022	OS-U-E2204	1.9	1.84	1.1 - 2.58
		2022-02	2022	OS-U-E2205	0.78	0.816	0.488 - 1.14
		2022-02	2022	OS-U-E2206	6.7	6.19	3.66 - 8.72
		2023-01	2023	OS-U-E2301	0.5	0.526	0.314 - 0.738
		2023-01	2023	OS-U-E2302	1.4	1.59	0.917 - 2.26
		2023-01	2023	OS-U-E2303	2	2.26	1.32 - 3.2
3-PBA	GEQUAS	RV67	2021	A:	2.433	2.552	2.000 - 3.104
		RV67	2021	B:	9.85	9.57	7.818 - 11.322
		RV68	2021	A:	0.93	1.087	0.811 - 1.363
		RV68	2021	B:	5.7	6.255	5.073 - 7.437
		RV69	2022	A:	2.633	2.6	1.967 - 3.233
		RV69	2022	B:	14.833	14.082	11.337 - 16.827
		RV70	2022	A:	0.72	0.75	0.579 - 0.921
		RV70	2022	B:	8.1	7.714	6.139 - 9.289
4FP	GEQUAS	RV67	2021	A:	0.57	0.53	0.38 - 0.68
		RV67	2021	B:	4.27	4.36	3.43 - 5.29
		RV68	2021	A:	5.7	6.03	4.65 - 7.41
		RV68	2021	B:	1.8	2	1.49 - 2.51
		RV69	2022	A:	0.61	0.59	0.44 - 0.74
		RV69	2022	B:	2	1.91	1.46 - 2.36
		RV70	2022	A:	0.42	0.41	0.32 - 0.50
		RV70	2022	B:	2.8	2.79	2.16 - 3.42
<i>cis</i> -DBCA	GEQUAS	RV67	2021	A:	0.25	0.466	0.298 - 0.634
		RV67	2021	B:	4.4	4.56	3.246 - 5.874
		RV68	2021	A:	0.25	0.261	0.177 - 0.345
		RV68	2021	B:	1.1	1.099	0.781 - 1.417

		RV69	2022	A:	0.458	0.427	0.292 - 0.562
		RV69	2022	B:	3.233	3.05	2.153 - 3.947
		RV70	2022	A:	0.58	0.684	0.483 - 0.885
		RV70	2022	B:	2	1.204	0.874 - 1.534
<i>cis</i> -DCCA	GEQUAS	RV67	2021	A:	0.25	0.438	0.342 - 0.534
		RV67	2021	B:	3.3	3.223	2.506 - 3.940
		RV68	2021	A:	0.25	0.262	0.187 - 0.337
		RV68	2021	B:	2.5	2.466	1.920 - 3.012
		RV69	2022	A:	1.083	1.091	0.737 - 1.445
		RV69	2022	B:	3.433	3.48	2.769 - 4.191
		RV70	2022	A:	0.41	0.274	0.199 - 0.349
		RV70	2022	B:	1.5	1.281	0.951 - 1.611
PNP	GEQUAS	RV67	2021	A:	49.8	60.8	45.8 - 75.8
		RV67	2021	B:	233.3	298.8	226.2 - 371.4
		RV68	2021	A:	11	13.5	9.6 - 17.4
		RV68	2021	B:	87	100.9	77.5 - 124.3
		RV69	2022	A:	29.7	32.5	26.2 - 38.8
		RV69	2022	B:	263.3	288.5	252.2 - 324.8
		RV70	2022	A:	17	15.9	12.6 - 19.2
		RV70	2022	B:	78	73.6	61.0 - 86.2
TCPy	GEQUAS	RV67	2021	A:	1.22	1.28	0.95 - 1.61
		RV67	2021	B:	8.07	8.51	6.20 - 10.82
		RV68	2021	A:	4.5	4.86	3.66 - 6.06
		RV68	2021	B:	15	16.71	13.14 - 20.28
		RV69	2022	A:	4.08	3.95	3.14 - 4.76
		RV69	2022	B:	12	11.8	9.79 - 13.81
		RV70	2022	A:	1.9	1.79	1.31 - 2.27
		RV70	2022	B:	9.9	9.74	7.49 - 11.99
<i>trans</i> -DCCA	GEQUAS	RV67	2021	A:	1.15	1.036	0.739 - 1.333
		RV67	2021	B:	4.183	3.97	3.172 - 4.768
		RV68	2021	A:	0.3	0.37	0.274 - 0.466
		RV68	2021	B:	4.9	4.604	3.665 - 5.543
		RV69	2022	A:	1.6	1.408	1.048 - 1.768
		RV69	2022	B:	7.55	6.793	5.467 - 8.119
		RV70	2022	A:	0.41	0.348	0.237 - 0.459
		RV70	2022	B:	2.2	1.945	1.462 - 2.428

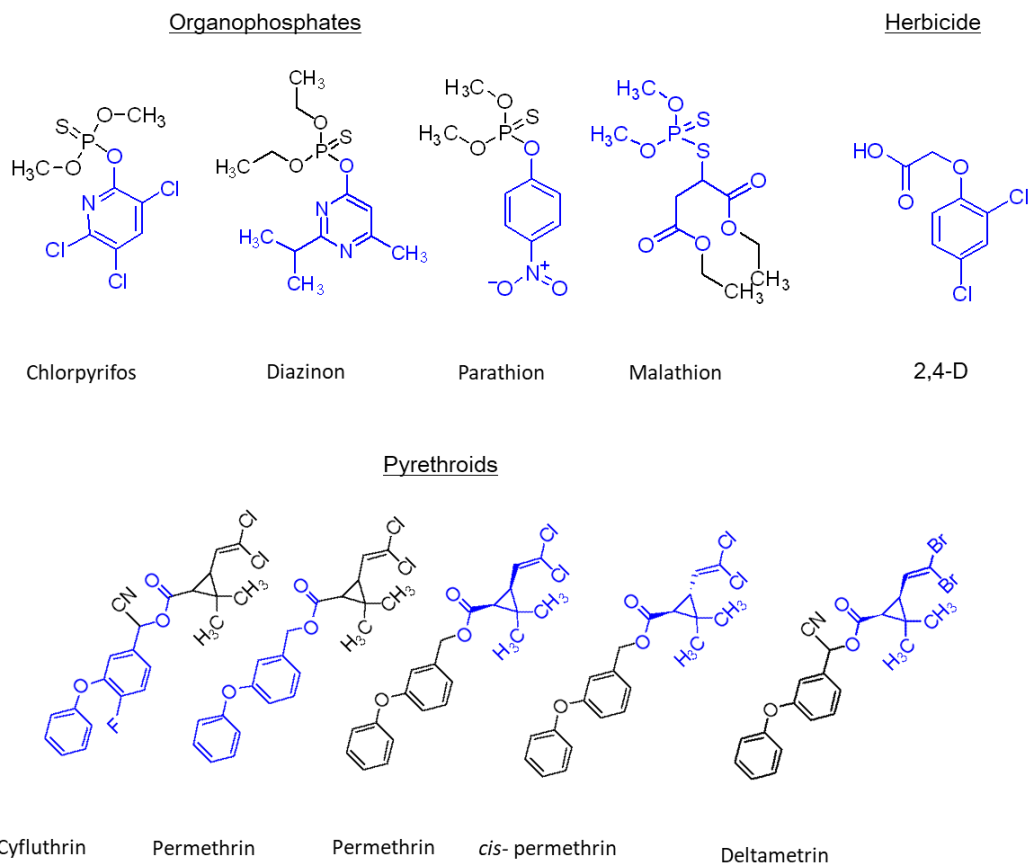
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Figure S1.

Parent pesticides examples and their metabolites shown in blue.



Parent pesticide	Metabolite
Chlorpyrifos, chlorpyrifos methyl	TCPy
Diazinon	IMPy
Parathion, methyl parathion	PNP:
Malathion	MDA
2,4-dichlorophenoxyacetic acid (and it's esters)	2,4-D
Cyfluthrin, Flumethrin	4FP
Cyhalothrin, Cypermethrin, Deltamethrin, Fenpropathrin, Permethrin, Tralomethrin, Esfenvalerate, Phenothrin	3-PBA
<i>cis</i> - and <i>trans</i> - Permethrin, Cypermethrin, Cyfluthrin	<i>cis</i> - and <i>trans</i> -DCCA
Deltamethrin	<i>cis</i> -DBCA