Supplementary Material

Development and application of a mini-QuEChERS method for the determination of pesticide residues in anuran adipose tissues

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Methods for determining pesticide residues in frog tissues

Table S1. Parameters of some methods for determining pesticide residues in frog samples reported in the literature.

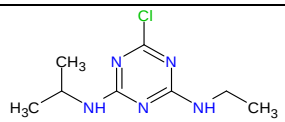
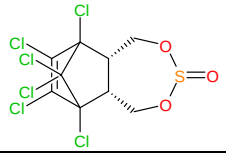
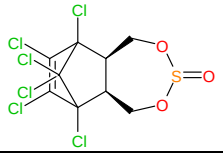
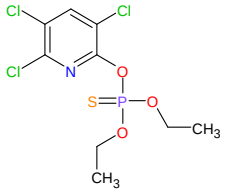
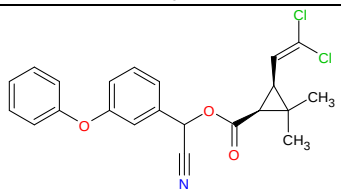
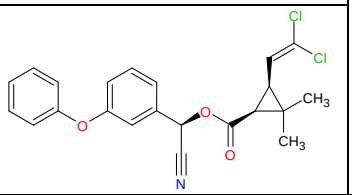
Anuran samples	Extraction Method	Analytical Technique	Pesticides	Recovery (%)	LoQ (µg kg^{-1})	Solvent (volume)	Sample size (mg)	REF
Adipose tissue	QuEChERS	HPLC-DAD	Atrazine	102.3	10	ACN (1.5 mL)	500	this work
			Endosulfan	86.4	75			
			Chlorpyriphos	101.8	55			
			Cypermethrin	96.3	55			
Skin (whole body)	QuEChERS SPE	LC-MS/MS	Acetamiprid	-	0.03	ACN (20 mL) Hexane (3 mL) Methanol (0.2 mL)	200	[1]
			Clothianidin	49.2	0.03			
			Dinotefuran	-	0.12			
			Imidacloprid	-	0.03			
			Nitempy	-	0.02			
			Thiacloprid	-	0.02			
			Thiamethoxam	-	0.06			
Tissues	SLE	LC-MS	Imidacloprid	-	-	Methanol (10 mL) Distilled water (10 mL) MTBE (3 mL)	1,000	[2]
			Atrazine	-	-			
			Triadimefon	-	-			
			Fipronil	-	-			
			Pendimethalin	-	-			
Tissues	Ultrasonic extraction (EPA	GC-MS	Tricyclazole	-	-	Hexane (25 mL) Acetone	10,000	[3]
			Fipronil	-	-			
			Diazinon	-	-			

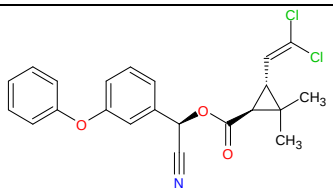
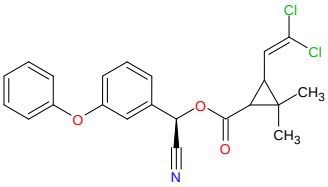
	Method 3550)		Butachlor	-	-	(25 mL)		
			Bentazone	-	-			
			Fenitrothion	-	-			
Tissues	SLE	GC-ECD	Various	-	0.004-0.018	Hexane (20 mL) Acetone (20 mL)	10,000	[4]

SPE: solid-phase extraction, SLE: Solid liquid extraction, LC: liquid chromatography, MS: mass spectrometry, GC: gas chromatography, ECD: electron capture detector, ACN: acetonitrile, MTBE: methyl-tert-butyl ether

Pesticides studied and their physicochemical properties

Table S2. Pesticides under investigation and some chemical and physicochemical properties.[5]

Pesticides	**Molecular structure**	**Chemical class**	**Water solubility at 25 °C** (mg L^{-1})	**LogP**	**Half-life at 25 °C in wet soil** (days)	**Henry's Law at 20-25 °C** (atm-cu m/mol)
Atrazine		Triazine	33.00	2.61	28-35	2.6×10^{-9}
α-endosulfan		Organochlorine	0.32 (α) 0.33 (β)	3.83 (α) 3.62 (β)	32	6.5×10^{-5}
β-endosulfan						
Chlorpyriphos		Organophosphate	1.40	4.96	33-56	3.6×10^{-5}
α-cypermethrin		Pyrethroid	0.01	6.94	18-56	2.4×10^{-7}
β-cypermethrin						

θ-cypermethrin						
ζ-cypermethrin						

Optimization of mini-QuEChERS method for extraction of pesticide residues from anuran adipose tissue samples

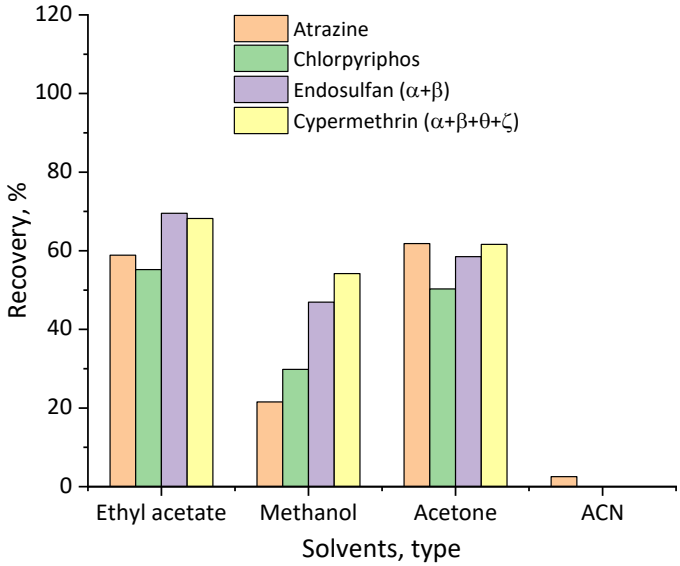
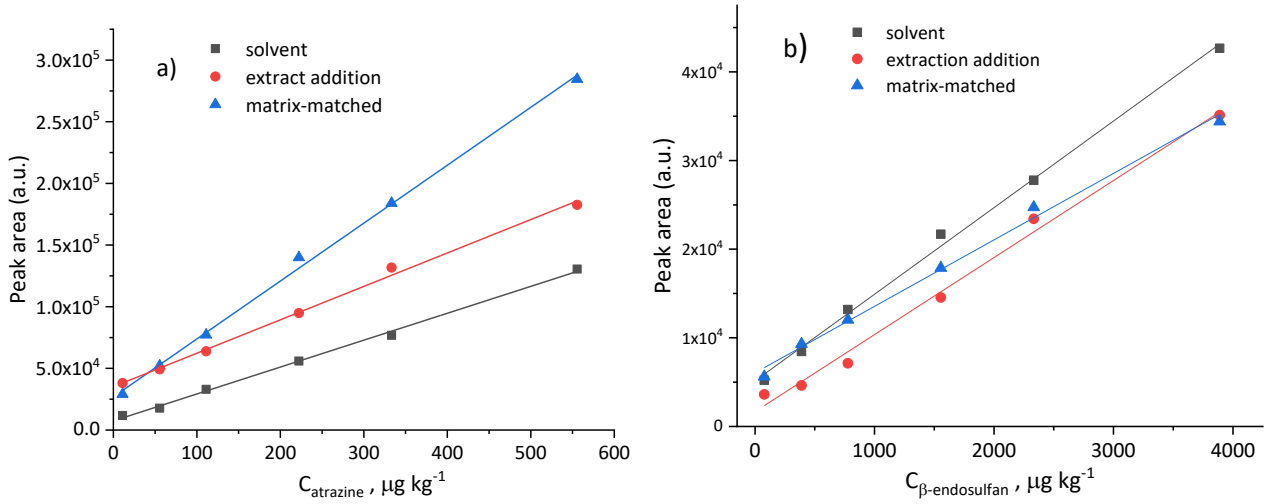
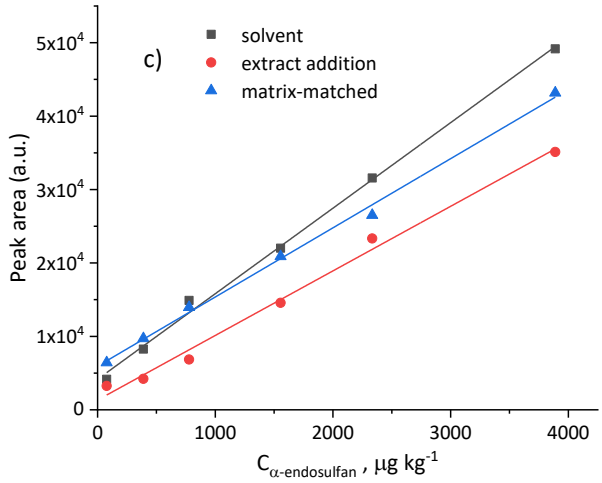
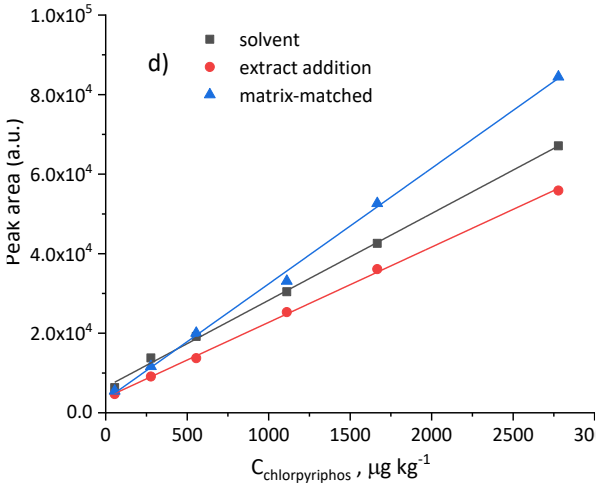


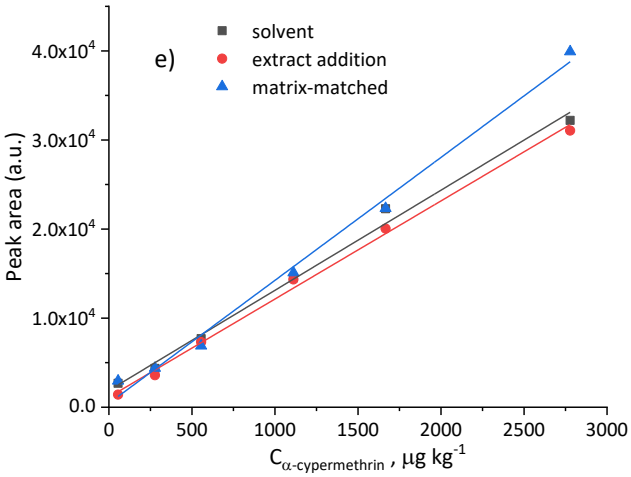
Figure S1. Recovery of pesticides from adipose tissue samples by mini-QuEChERS method. Selection of extraction solvents.

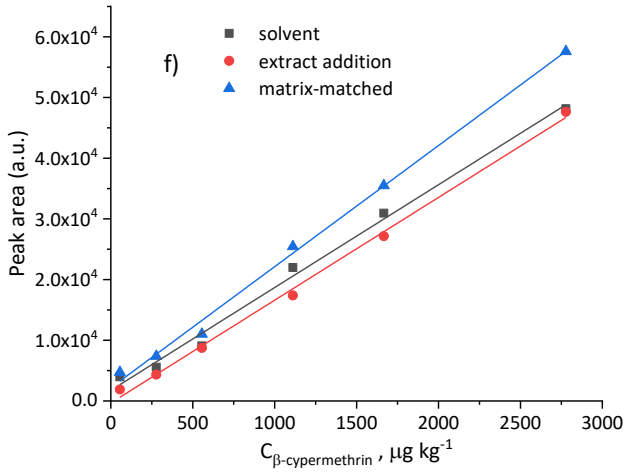
Analytical curves for the studied pesticides

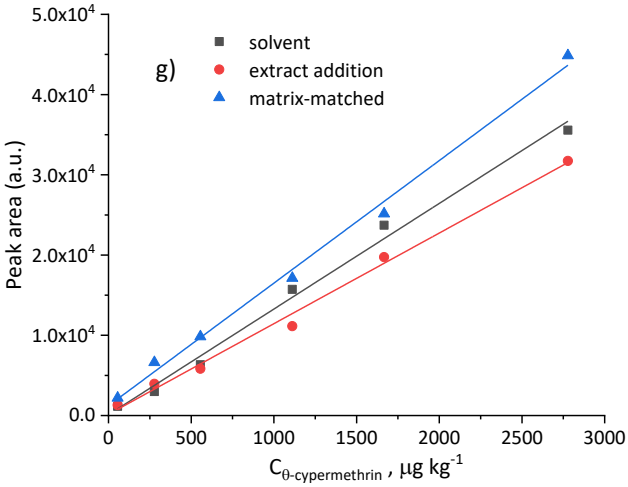


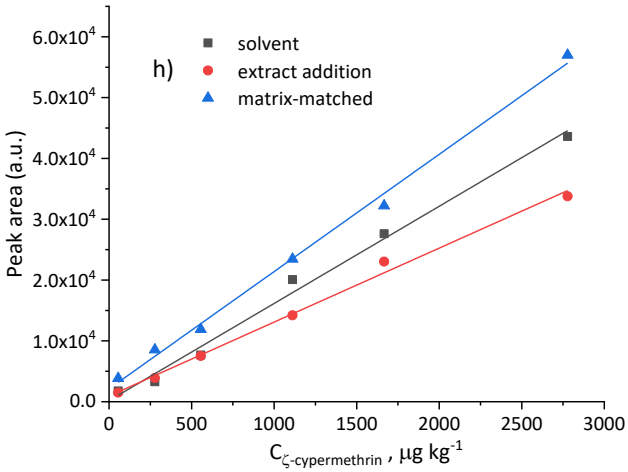

c)
solvent
extract addition
matrix-matched
Peak area (a.u.)
$C_{\alpha\text{-endosulfan}}$, µg kg^{-1}


d)
solvent
extract addition
matrix-matched
Peak area (a.u.)
$C_{chlorpyriphos}$, µg kg^{-1}


e)
solvent
extract addition
matrix-matched
Peak area (a.u.)
$C_{\alpha\text{-cypermethrin}}$, µg kg^{-1}


f)
solvent
extract addition
matrix-matched
Peak area (a.u.)
$C_{\beta\text{-cypermethrin}}$, µg kg^{-1}


g)
solvent
extract addition
matrix-matched
Peak area (a.u.)
$C_{\theta\text{-cypermethrin}}$, µg kg^{-1}


h)
solvent
extract addition
matrix-matched
Peak area (a.u.)
$C_{\zeta\text{-cypermethrin}}$, µg kg^{-1}

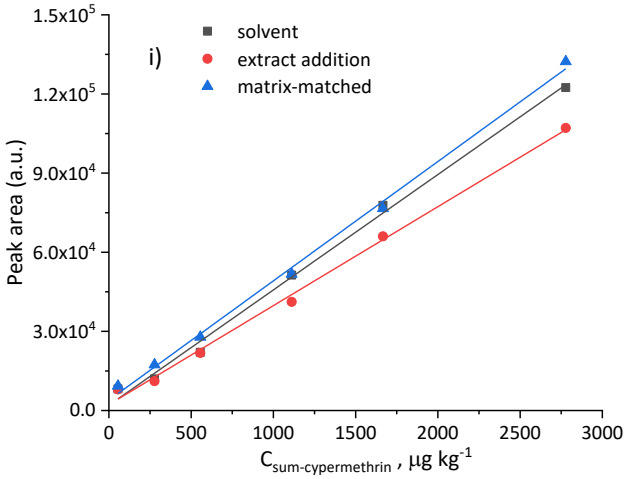


Figure S2. Analytical curves for a) atrazine, b) α-endosulfan, c) β-endosulfan, d) chlorpyriphos, e) α-cypermethrin, f) β-cypermethrin, g) θ-cypermethrin, h) ζ-cypermethrin, and i) sum of cypermethrin isomers, obtained by the external standard, in-extract addition and matrix-matched calibration methods.

Table S3. Analytical parameters of calibration curves for the pesticides in anuran adipose tissue samples.

Pesticides	**Analytical curves**		**Range (µg kg^{-1})**	**R^2**	**r**
Atrazine	Solvent	$A = 242385*C_{Atrazine} + 7437$	11.1 to 555.6	0.998	0.999
	Extract addition	$A = 301152*C_{Atrazine} + 35174$		0.997	0.998
	Matrix-matched	$A = 521763*C_{Atrazine} + 26899$		0.998	0.999
α-endosulfan	Solvent	$A = 10854*C_{\alpha\text{-endosulfan}} + 5144$	77.8 to 3888.9	0.997	0.998
	Extract addition	$A = 8689*C_{\alpha\text{-endosulfan}} + 1677$		0.992	0.996
	Matrix-matched	$A = 8830*C_{\alpha\text{-endosulfan}} + 6053$		0.994	0.997
β-endosulfan	Solvent	$A = 12496*C_{\beta\text{-endosulfan}} + 4159$	77.8 to 3888.9	0.997	0.999
	Extract addition	$A = 10469*C_{\beta\text{-endosulfan}} + 5943$		0.997	0.998
	Matrix-matched	$A = 9774*C_{\beta\text{-endosulfan}} + 1330$		0.992	0.996
Chlorpyriphos	Solvent	$A = 24243*C_{chlorpyriphos} + 6470$	55.6 to 2777.8	0.998	0.999
	Extract addition	$A = 21042*C_{chlorpyriphos} + 3796$		0.999	0.999
	Matrix-matched	$A = 32307*C_{chlorpyriphos} + 3337$		0.998	0.999
α-cypermethrin	Solvent	$A = 12488*C_{\alpha\text{-cypermethrin}} + 1886$	55.6 to 2777.8	0.994	0.997
	Extract addition	$A = 15334*C_{\alpha\text{-cypermethrin}} + 444$		0.992	0.996
	Matrix-matched	$A = 12238*C_{\alpha\text{-cypermethrin}} + 1131$		0.997	0.998
β-cypermethrin	Solvent	$A = 18825*C_{\beta\text{-cypermethrin}} + 1745$	55.6 to 2777.8	0.993	0.997
	Extract addition	$A = 18814*C_{\beta\text{-cypermethrin}} - 328$		0.997	0.999
	Matrix-matched	$A = 22172*C_{\beta\text{-cypermethrin}} + 2175$		0.996	0.998
θ-cypermethrin	Solvent	$A = 14629*C_{\theta\text{-cypermethrin}} + 104$	55.6 to 2777.8	0.992	0.996
	Extract addition	$A = 12549*C_{\theta\text{-cypermethrin}} + 153$		0.994	0.997
	Matrix-matched	$A = 16975*C_{\theta\text{-cypermethrin}} + 1223$		0.995	0.997
ζ-cypermethrin	Solvent	$A = 17755*C_{\zeta\text{-cypermethrin}} + 174$	55.6 to 2777.8	0.992	0.996
	Extract addition	$A = 13513*C_{\zeta\text{-cypermethrin}} + 929$		0.994	0.997
	Matrix-matched	$A = 21421*C_{\zeta\text{-cypermethrin}} + 2116$		0.996	0.998
Cypermethrin (Sum of α, β, θ, and ζ)	Solvent	$A = 48597*C_{sum\text{-cypermethrin}} + 1977$	55.6 to 2777.8	0.995	0.998
	Extract addition	$A = 41668*C_{sum\text{-cypermethrin}} + 2259$		0.996	0.998
	Matrix-matched	$A = 50249*C_{sum\text{-cypermethrin}} + 3934$		0.997	0.999

Table S4. Pesticide residues in adipose tissue samples from two anuran species determined by the mini-QuEChERS-HPLC-DAD method and confirmed by LC-MS/MS analysis.

Sample	Species	Concentration of pesticide residues (μg kg^{-1})							
		ATRA	α-ENDO	β-ENDO	CHLOR	α-CYPER	β- CYPER	θ- CYPER	ζ- CYPER
1	*L. Macrosternum*	-	-	-	-	-	-	-	-
2	*L. Macrosternum*	-	-	-	-	-	-	-	-
3	*L. Macrosternum*	-	-	-	-	-	-	-	-
4	*L. Macrosternum*	-	-	-	-	-	-	-	-
5	*L. Macrosternum*	-	-	-	-	-	-	-	-
6	*L. Macrosternum*	-	-	-	-	-	-	-	-
7	*L. Macrosternum*	-	-	-	-	-	-	-	-
8	*L. Macrosternum*	-	-	-	-	-	-	-	-
9	*S. x-signatus**	-	-	-	-	-	-	-	-
10	*S. x-signatus**	-	-	-	-	-	-	-	-
11	*S. x-signatus**	-	-	-	-	-	-	-	-
12	*L. Macrosternum*	-	-	-	-	-	-	-	-
13	*L. Macrosternum*	-	-	-	-	-	-	-	-
14	*L. Macrosternum*	-	-	-	-	-	-	-	-
15	*L. Macrosternum*	-	-	-	-	-	-	-	-
16	*L. Macrosternum*	-	-	-	-	-	-	-	-
17	*L. Macrosternum*	-	-	-	-	-	-	-	-
18	*L. Macrosternum*	-	-	-	-	-	-	-	-
19	*L. Macrosternum*	-	-	-	-	-	-	-	-
20	*L. Macrosternum*	-	-	-	-	-	-	-	-
21	*L. Macrosternum*	-	1.2	8.4	-	-	-	-	-
22	*L. Macrosternum*	-	-	-	-	-	-	-	-
23	*L. Macrosternum*	-	-	-	-	-	-	-	-
24	*L. Macrosternum*	-	-	1.6	-	-	-	-	-
25	*S. x-signatus**	-	-	-	-	-	-	-	-
26	*S. x-signatus**	-	5.0	-	-	-	-	-	-
27	*S. x-signatus**	-	-	-	-	-	-	-	-
28	*L. Macrosternum*	-	-	1.3	-	-	-	-	-
29	*L. Macrosternum*	-	-	-	-	-	-	-	-
30	*L. Macrosternum*	-	-	-	-	-	-	-	-
31	*L. Macrosternum*	-	1.6	-	-	-	-	-	-
32	*L. Macrosternum*	-	-	-	-	-	-	-	-
33	*L. Macrosternum*	-	4.7	2.2	1.9	-	-	-	-
34	*L. Macrosternum*	-	2.8	2.7	-	-	-	-	-
35	*L. Macrosternum*	-	-	-	2.5	-	-	-	-
36	*L. Macrosternum*	-	5.7	1.2	-	-	-	-	-
37	*L. Macrosternum*	-	-	4.9	-	-	-	-	-
38	*L. Macrosternum*	-	-	-	-	-	-	-	-
39	*L. Macrosternum*	-	-	-	-	-	-	-	-
40	*L. Macrosternum*	-	2.1	6.3	-	-	-	-	-
41	*L. Macrosternum*	-	-	-	-	-	-	-	-

42	*L. Macrosternum*	-	-	-	-	-	-	-	-
43	*L. Macrosternum*	-	-	-	-	-	-	-	-
44	*L. Macrosternum*	-	3.6	1.6	-	-	-	-	-
45	*L. Macrosternum*	-	-	-	-	-	-	-	-
46	*L. Macrosternum*	-	-	-	-	-	-	-	-
47	*L. Macrosternum*	-	-	-	-	-	-	-	-
48	*L. Macrosternum*	-	-	-	-	-	-	-	-
49	*L. Macrosternum*	-	5.5	2.7	-	-	-	-	-
50	*L. Macrosternum*	-	-	-	-	-	-	-	-
51	*L. Macrosternum*	-	2.5	-	-	-	-	-	-
52	*L. Macrosternum*	-	-	-	-	-	-	-	-
53	*L. Macrosternum*	-	-	-	-	-	-	-	-
54	*L. Macrosternum*	-	4.2	2.5	-	-	-	-	-
55	*L. Macrosternum*	-	-	-	-	-	-	-	-
56	*L. Macrosternum*	-	-	-	-	-	-	-	-
57	*L. Macrosternum*	-	-	-	-	-	-	-	-
58	*L. Macrosternum*	-	4.4	3.9	-	-	-	-	-
59	*L. Macrosternum*	-	2.0	1.4	-	-	-	-	-
60	*L. Macrosternum*	-	1.7	1.4	-	-	-	-	-
61	*L. Macrosternum*	-	2.9	5.8	-	-	-	-	-
62	*L. Macrosternum*	-	-	-	-	-	-	-	-
63	*L. Macrosternum*	-	-	-	-	-	-	-	-
64	*L. Macrosternum*	-	-	-	-	-	-	-	-
65	*L. Macrosternum*	-	2.5	-	1.6	-	-	-	-
66	*L. Macrosternum**	-	-	-	-	-	-	-	-
67	*L. Macrosternum**	-	-	-	-	-	-	-	-

ATRA: atrazine, ENDO: endosulfan, CHLOR: chlorpyriphos, CYPER: cypermethrin. *: pooled samples

References

1. S. Shiya, F. Nishibe, Y. B. Yohannes, M. Ishizuka, S. M. Nakayama, Y. Ikenaka, *Aquatic Toxicology*, 2023, **257**, 106437.
2. R. J. van Meter, D. A. Glinski, S. T. Purucker, W. M. Henderson, *Environmental Toxicology and Chemistry*, 2022, **41**, 122-133.
3. N. Shojaei, S. Naderi, E. Yasari, N. Moradi, *Environmental Science and Pollution Research*, 2021, **28**, 33557-33569.
4. O. Edo-Taiwo, M. S. O. Aisien, *Journal of Applied Sciences and Environmental Management*, 2023, **27**, 509-517.
5. PubChem, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health. Available at: https://pubchem.ncbi.nlm.nih.gov/. Accessed in: Sep, 2023.