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Supplementary Materials

Bioaccumulation and biotransformation of plasticisers diisononyl phthalate and di(2-ethylhexyl) terephthalate in black soldier fly larvae reared on (micro)plastic-contaminated food waste

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All chemicals were of analytical reagent grade with the purity greater than 98 % and are together with all used materials in this study listed below in Table S1.

Supplementary Table S1. Chemicals, reagents, and materials used in the current study.

Chemical, reagent or material	Company
Di(2-ethylhexyl) terephthalate (DEHT) Diisononyl phthalate (DINP) Labelled dibenzyl phthalate (DBzP-d4)	Accustandard Inc.
Mono-hydroxy-isononyl phthalate (OH-MINP) Mono-carboxy-isononyl phthalate (cx-MINP) Mono(2-ethyl-5-hydroxyhexyl) terephthalate (5-OH-MEHTP)	Cambridge Isotope Laboratories

Mono(2-ethyl-5-carboxyhexyl) terephthalate (cx-MEHTP) Labelled mono (2-ethylhexyl) phthalate (MEHP-d4) Mono(2-ethyl-5-hydroxyhexyl) phthalate (5-OH-MEHP-d4) Mono(2-ethyl-5-carboxyhexyl) terephthalate (5-cx-MEHTP-d4)	
Mono (2-ethylhexyl) terephthalate (MEHTP) Mono isononyl phthalate (MINP)	Da Vinci Europe
Labelled di(2-ethylhexyl) phthalate (DEHP-d4) Di-n-butyl phthalate (DnBP-d4) Sodium chloride Ammonium hydroxide Formic acid Acetic acid	Sigma-Aldrich
Labelled triphenyl phosphate (TPHP-d15) Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP-d15) Tris(2-butoxyethyl) phosphate (TBOEP-d6) Tris(2-chloroethyl) phosphate (TCEP-d12)	Dr. Vladimir Belov, Max Planck Institute for biophysical Chemistry
Labelled mono isononyl cyclohexane-1,2-dicarboxylate (MINCH-d2) Mono-hydroxy-isononyl phthalate (7-OH-MINP-d4)	kindly provided by Dr. Koch
Chlorobiphenyl (CB-207)	Dr. Ehrenstorfer Laboratories
C18 sorbent powder Primary-secondary amine (PSA) Florisil ENVI (500 mg, 3 mL) cartridges	Supelco
OASIS MAX (60 mg, 3 mL) cartridges	Waters
Centrifugal filters of 0.22 and 0.45 μm	VWR
n-Hexane	Acros Organics

Ethyl acetate Dichloromethane Isooctane Toluene	Merck
Methanol Acetonitrile	Biosolve

S2. Identification of additive compounds in plastic food contact materials

S2.1. Extraction procedure

For the liquid chromatographic (LC) analysis, 200 µL extract was transferred to a glass tube and evaporated using a gentle nitrogen flow (Reacti-Therm III, Thermo Fisher Scientific). The extract was then reconstituted with 50 µL internal standard (IS) solution for plasticisers (DBzP-d4, DEHP-d4, DnBP-d4, 10 ng/µL in MeOH) and 50 µL IS solution for PFRs (TBOEP-d6, TCEP-d12, TDCIPP-d15, TPHP-d15, 2 ng/µL in MeOH). Finally, the obtained extract was centrifuged (Sigma Aldrich) for 5 min at 10 000 rpm using centrifugal filters (0.22 µm, VWR), and transferred to LC injection vials. For the gas chromatographic (GC) analysis, a subaliquot of 75 µL was transferred to a GC injection vial and mixed with 25 µL IS solution (BDzP-d4, 10 ng/µL; DEHP-d4, 10 ng/µL; DnBP-d4, 10 ng/µL in isooctane)[33].

S2.2. Instrumental analysis

The mobile phases (flow rate: 0.4 mL/min) used during the suspect screening analysis in ESI+ mode were ultrapure water with 0.1 % formic acid (A) and methanol/water (80:20, v/v) with 0.1 % formic acid (B), while formic acid was substituted with acetic acid in ESI- mode. The injection volume was 5 µL, while the column was set at 30 °C[34]. After data acquisition, features were extracted from the raw data files using the Agilent MassHunter Qualitative software (version B.07.00). These describe a combination of *m*/*z* values representing an isotopic pattern occurring (as a peak) at a defined retention time and, if available, including a fragmentation spectrum. After filtering based on abundance and comparison with solvent blanks (ensuring an at least five-fold difference in intensity between blanks and samples), the remaining features

were matched against a predefined in-house suspect list (HECHIER)[34]. This suspect list contains the name, molecular formula, monoisotopic mass and at least one additional identifier (SMILES, CAS number, InChi, etc.) of more than 2100 additive chemicals suspected to be present in the samples, including phthalates, PFRs, and alternative plasticisers (e.g. citrates, adipates, trimellitates, azelates, etc.). Matched features were then further processed using the Agilent MassHunter Qualitative Analysis B.07.00, aiming at confirming the matched suspect and assigning a level of confidence (1 to 5) according to the scheme introduced by Schymanski et al. (2014)[41]. Level 1 is reached when the structure is confirmed with a reference standard, while level 2 results in a tentative structure based on a library spectrum match or by diagnostic evidence. Level 3 represent a potential candidate of the chemical structure based on the MS data, while level 4 and 5 represent an unequivocal molecular formula based on the isotope pattern and the exact mass of the compound, respectively[41].

To confirm the results from the SSA, the extracts were quantitatively analysed by a gas chromatography (GC) coupled to an Agilent 5973 mass spectrometer (MS) operated in electron ionisation mode (EI) according to Malarvannan et al. (2019)[33] and by a liquid chromatographic (LC) system coupled to an Agilent 6410 Triple Quadrupole mass spectrometer, according to Christia et al. (2019)[37].

Briefly, the GC was equipped with an HT-8 capillary column (25.0 m × 220 µm, 0.25 µm), while the oven was initially set at 60 °C. After 3 min, the oven temperature was increased to 300 °C at a rate of 10 °C/min and held for 15 min. Helium was used as carrier gas at a flow rate of 1 mL/min.

The LC was equipped with a Kinetex Biphenyl column (100 mm × 2.1 mm, 2.6 µm) and operated at 40 °C. The mobile phases (flow rate: 0.25 mL/min) were ultrapure water with 5 mmol/L ammonium formate (A) and methanol with 5 mmol/L ammonium formate (B)[33,37].

S3. Quantification of the plasticisers diisononyl phthalate and di(2-ethylhexyl) terephthalate and their biotransformation products in black soldier fly larvae and respective substrate/residue mixtures

S3.1. Extraction procedure

Briefly, approximately 100 to 150 mg of sample was mixed with 100 mg NaCl and 3 mL of an acetonitrile/toluene mixture (9:1, v/v) in pre-cleaned glass tubes. The obtained suspension was vortexed (DVX-2500, VWR) for 1 min, placed in an ultrasonic bath (5800, Branson) for 5 min, and centrifuged (3000 rpm, 5810, Eppendorf) for 3 min. The supernatant was then transferred to clean glass tubes and the extraction using fresh acetonitrile/toluene was repeated. The combined extracts were well homogenised and divided into two aliquots of the same volume for subsequent clean-up of the (i) parent compounds and (ii) biotransformation products.

The former aliquot (i) was spiked with 50 µL of internal standard solution (DBzP-d4, DEHP-d4 and DnBP-d4, all at 10 ng/µL) and concentrated to 2 mL under a gentle nitrogen flow. Then, the extract underwent a dispersive solid phase extraction (d-SPE) with the addition of 50 mg PSA and 100 mg C18. The clean extract was then evaporated until dryness and reconstituted in 1 mL of *n*-hexane. Finally, the extract was further purified by passage onto Florisil ENVI cartridges (pre-cleaned with 6 mL ethyl acetate and 6 mL hexane). A first fraction was eluted with 12 mL hexane/dichloromethane (4:1, v/v), which was discarded, and the compounds of interest (DINP and DEHT) were eluted with 10 mL ethyl acetate. This second fraction was evaporated and resolubilised in 50 µL recovery standard (CB-207, 50 pg/µL) and 50 µL isooctane, and stored at - 20 °C until GC-EI/MS analysis was performed.

The latter aliquot (ii) was spiked with 25 µL internal standard solution (MINCH-d2, 7-OH-MINP-d4, 5-OH-MEHP-d4, and 5-cx-MEHTP-d4, all at 500 pg/µL in acetonitrile), evaporated to dryness and reconstituted in 1 mL water/acetonitrile (95:5, v/v) containing 5 % ammonium hydroxide. The obtained solution was loaded onto OASIS MAX cartridges (pre-cleaned with 3 mL of dichloromethane, methanol, and ultrapure water, respectively). After loading, the cartridge was washed with 3 mL ultrapure water containing 5 % ammonium hydroxide and 1 mL ultrapure water. The analytes were eluted with 8 mL methanol containing 2 % formic acid. The fraction was evaporated to near dryness, reconstituted in 100 µL acetonitrile/ultrapure water (1:1, v/v), filtered using 0.45 µm nylon centrifugal filters, and finally transferred to LC injection vials, and stored at - 20 °C until LC-MS/MS analysis.

S3.2. Gas chromatographic analysis of parent compounds

Briefly, for the GC analysis an Agilent GC coupled to an Agilent 5973 mass spectrometer (MS) operated in electron ionisation mode (EI) was used, equipped with a GC HT-8 capillary column (25.0 m × 220 µm, 0.25 µm), electronic pressure control, and a programmable-temperature vaporiser inlet (splitless mode). The injection temperature was 90 °C, which increased to 180 °C, with a ramping rate of 10 °C/min, and was finally held for 25 min. The injection (injection volume: 1 µL) was executed under 14.4 psi for 1.25 min, and a purge flow to split vent of 50.0 mL/min. The column initially started at 90 °C and after 1.50 min ramped to 180 °C (10 °C/min) and further ramped to 310 °C (30 °C/min), whereafter a holding time of 10 min was applied. The carrier gas was helium, which was kept at a flow rate of 1 mL/min. The MS operated in selected ion monitoring mode with 2 characteristic ions acquired for each analyte and for the IS (DEHP-d4). Lastly, the calibration ranges were between 21.5 – 21500 ng and 2 – 1500 ng for DINP and DEHT, respectively (Table S2).

S3.3. Liquid chromatographic analysis of biotransformation products

Briefly, the LC instrument was equipped with a 2.5 µm Synergi Polar reverse phase column (100 mm × 2 mm, 100 Å, 00D-4371-B0, Phenomenex) held at 40 °C. The injection volume was 5 µL. The mobile phase (flow rate: 0.3 mL/min) was ultrapure water containing 0.1 % acetic acid (A) and acetonitrile containing 0.1 % acetic acid (B). The separation gradient started at 85 % (A) and went to 70 % (A) in 4 min, to 55 % (A) in 6 min, to 2 % (A) in 3 min, which was held for 3 min. Afterwards, the mobile phase gradient went back to 85 % (A) in 0.1 min, which was held for 4 min. The source parameters of the MS were set as follows; gas temperature: 340 °C, gas flow: 10 L/min, nebulizer pressure: 40 psi, and capillary voltage: 5 kV in negative electrospray ionisation mode. The calibration ranges were between 0.1 – 50 ng for MINP, OH-MINP, MEHTP, cx-MEHTP and 5-OH-MEHTP, and 0.025 – 12.5 ng for cx-MINP. The Agilent Mass Hunter Quantitative analysis software B.06.00 was used for the data analysis.

S3.4. In-house method validation

First, two calibration curves were prepared, one for the parent compounds in isooctane and one for the biotransformation products in acetonitrile and ultrapure water (1:1, v/v). The calibration ranges were selected according to the expected contents in the insect and substrate/residue samples. The accuracy, recovery and precision within and between experiments were determined by the fortification of solvent blanks, BSF larvae and substrate/residue mixtures (Table S3 and S4). For all matrices, a sample was spiked in triplicate with a low- (LL) and high-level (HL) mass of the specific compounds, while three non-spiked samples were used as blank control, which were subtracted from the spiked samples. The accuracy was estimated by calculating the ratio between the obtained and spiked concentration, while the precision was determined. Further, the recovery was assessed by calculating the fraction of the mean of the spiked samples and the mean of the samples which were spiked after the extraction. Based on these results, a correction factor was applied, if necessary.

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Supplementary Table S2. Chromatographic information for the targeted compounds and internal standards (ISTD). MF and MW are the molecular formula and weight, respectively, while RT is the retention time. The Q- and q-ion are the quantitative and qualitative ions, while FV and CE are the fragmentor voltage and collision energy, respectively. The primary and secondary biotransformation products are presented with (*) and (**).

Analyte	Acronym	Type	MF	MW (g/mol)	RT (min)	Q-ion (m/z)	q-ion (m/z)	ISTD
Bis (2-ethylhexyl) phthalate-d4	DEHP-D4	ISTD	$C_{24}D_4H_{34}O_4$	394.6	14.813	153	283	/
Bis (2-ethylhexyl) terephthalate	DEHT	Target	$C_{24}H_{38}O_4$	390.5	14.820	279	261	DEHP-D4
Diisononyl phthalate	DINP	Target	$C_{26}H_{42}O_4$	418.6	15-16	293	149	DEHP-D4

Analyte	Acronym	Type	MF	MW (g/mol)	RT (min)	Q-ion (m/z)	q-ion (m/z)	FV (V)	CE (eV)	ISTD
13C4-Mono(2-ethylhexyl) phthalate	13C4-MEHP	ISTD	$C_{12}{}^{13}C_4H_{22}O_4$	281.3	12.55	281.1	137	110	12	/
Mono isononyl phthalate*	MINP	Target	$C_{17}H_{24}O_4$	292.4	12.68	291.1	141	110	12	13C4-MEHP
Mono (2-ethylhexyl) terephthalate*	MEHTP	Target	$C_{16}H_{22}O_4$	278.3	12.81	277.1	233	110	8	13C4-MEHP

13C4- Mono hydroxy isononyl phthalate	13C4-OH-MINP	ISTD	$C_{13}{}^{13}C_4H_{24}O_5$	312.4	9.18	311	124	110	15	/
Mono hydroxy isononyl phthalate**	OH-MINP	Target	$C_{17}H_{24}O_5$	308.4	9.18	307	159	110	10	13C4-OH-MINP
cyclohexane-1,2-dicarboxylic mono carboxyisooctyl ester-d2	D2-cx-MINCH	ISTD	$C_{17}D_2H_{26}O_6$	330.4	10.01	329.2	175.2	110	12	/
Mono carboxy isononyl phthalate**	Cx-MINP	Target	$C_{18}H_{24}O_6$	336.4	9.21	321	173	90	10	D2-cx-MINCH
Mono(2-ethyl-5-hydroxyhexyl) phthalate	D4-5-HO-MEHP	ISTD	$C_{18}D_4H_{18}O_5$	298.3	8.15	297	123.9	110	15	/
Mono hydroxy (2-ethylhexyl) terephthalate**	OH-MEHTP	Target	$C_{16}H_{22}O_5$	294.3	8.79	293.1	121	110	15	D4-5-HO-MEHP

Analyte	Acronym	Type	MF	MW (g/mol)	RT (min)	Q-ion (m/z)	q-ion (m/z)	FV (V)	CE (eV)	ISTD
Mono-2-ethyl-5-carboxypentyl Terephthalate-d4	D4-cx-MEPTP	ISTD	$C_{16}D_4H_{16}O_6$	312.4	8.97	311	169	90	10	/
Mono (2-ethyl-5-carboxypentyl) terephthalate**	Cx-MEPTP	Target	$C_{16}H_{20}O_6$	308.4	9	307	165	90	10	D4-cx-MEPTP

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158 **Supplementary Table S3. The in-house validation parameters for the parent compounds of a blank, substrate and BSF larvae matrix. A,**
159 **R, and P are the accuracy, recovery, and precision, while WI and B represent the accuracy/recovery within and between a run,**
160 **respectively. CF represent the correction factor applied when required. All experiments were executed in triplicate (*n* = 3).**

	Blank			**Substrate**							**BSF Larvae**						
Analyte	A [%]	R [%]	P [%]	A [%]		R [%]		P [%]		CF	A [%]		R [%]		P [%]		CF
DINP	WI 53 ± 11	WI 95 ± 12	WI 90 ± 15	WI 48 ± 9	B 51 ± 14	WI 95 ± 4	B 87 ± 11	WI 90 ± 15	B 85 ± 17	2	WI 44 ± 11	B 42 ± 18	WI 93 ± 5	B 81 ± 14	WI 82 ± 21	B 77 ± 22	2

DEHT	93 ± 6	89 ± 21	95 ± 4	83 ± 7	81 ± 13	98 ± 8	88 ± 14	95 ± 4	90 ± 11	-	89 ± 4	77 ± 19	95 ± 9	82 ± 15	92 ± 9	84 ± 20	-

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Supplementary Table S4. The in-house validation parameters for the biotransformation products of a blank, substrate and BSF larvae matrix. A, R, and P are the accuracy, recovery, and precision, while WI and B represent the accuracy/recovery within and between a run, respectively. CF represent the correction factor applied when required. All experiments were executed in triplicate (*n* = 3).

	Blank			**Substrate**							**BSF Larvae**						
Analyte	A [%]	R [%]	P [%]	A [%]		R [%]		P [%]		CF	A [%]		R [%]		P [%]		CF
MINP	WI 105 ± 19	WI 86 ± 17	WI 96 ± 4	WI 88 ± 6	B 97 ± 12	WI 96 ± 7	B 93 ± 5	WI 99 ± 1	B 96 ± 1	1	WI 140 ± 34	B 105 ± 65	WI 67 ± 12	B 69 ± 11	WI 96 ± 5	B 89 ± 7	1.4
Cx-MINP	78 ± 9	81 ± 16	84 ± 11	103 ± 24	98 ± 19	102 ± 3	101 ± 7	97 ± 2	95 ± 1	1	614 ± 379	496 ± 641	3 ± 2	4 ± 1	67 ± 41	77 ± 26	3.3
OH-MINP	108 ± 4	88 ± 8	98 ± 3	107 ± 13	104 ± 9	108 ± 2	107 ± 4	97 ± 1	99 ± 1	1	127 ± 10	144 ± 16	4 ± 3	7 ± 1	85 ± 3	86 ± 3	1
MEHTP	85 ± 26	76 ± 14	92 ± 5	83 ± 1	126 ± 4	96 ± 8	105 ± 14	86 ± 11	78 ± 15	1	210 ± 57	203 ± 171	90 ± 9	109 ± 25	95 ± 4	68 ± 16	1.6
Cx-	58 ±	64 ±	98 ± 1	45 ±	49 ±	61 ±	70 ±	97 ±	93 ±	1	92 ±	94 ±	/	12 ±	84 ± 3	85 ± 3	1

MEHTP	72	1		75	65	43	27	1	9		31	14		14			
OH-MEHTP	96 ± 7	84 ± 9	98 ± 2	84 ± 1	90 ± 5	95 ± 3	99 ± 6	93 ± 3	93 ± 3	1	1058 ± 874	1210 ± 353	13 ± 11	15 ± 5	87 ± 10	58 ± 24	0.13

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166 **Supplementary Table S5. The targeted compounds, limit of quantification (based**
167 **on wet weight), accuracy and repeatability. The primary and secondary**
168 **biotransformation products are presented with (*) and (**), respectively.**

Abbreviation	Full name	LOQ [ng/g]	Accuracy [%]	RSD [%]
Parent compounds (gas chromatography)				
DEHT	di (2-ethylhexyl) terephthalate	20	91	6
DINP	Diisononyl phthalate	47	55	20
Biotransformation products (liquid chromatography)				
MINP*	Mono isononyl phthalate	0.44	103	4
MEHTP*	Mono (2-ethylhexyl) terephthalate	2.51	82	12
OH-MINP**	Mono hydroxy isononyl phthalate	0.25	84	2
Cx-MINP**	Mono carboxy isononyl phthalate	0.22	42	6
OH-MEHTP**	Mono hydroxy (2-ethylhexyl) terephthalate	0.26	78	4
Cx-MEPTP**	Mono (2-ethyl-5-carboxypentyl) terephthalate	1.67	99	7

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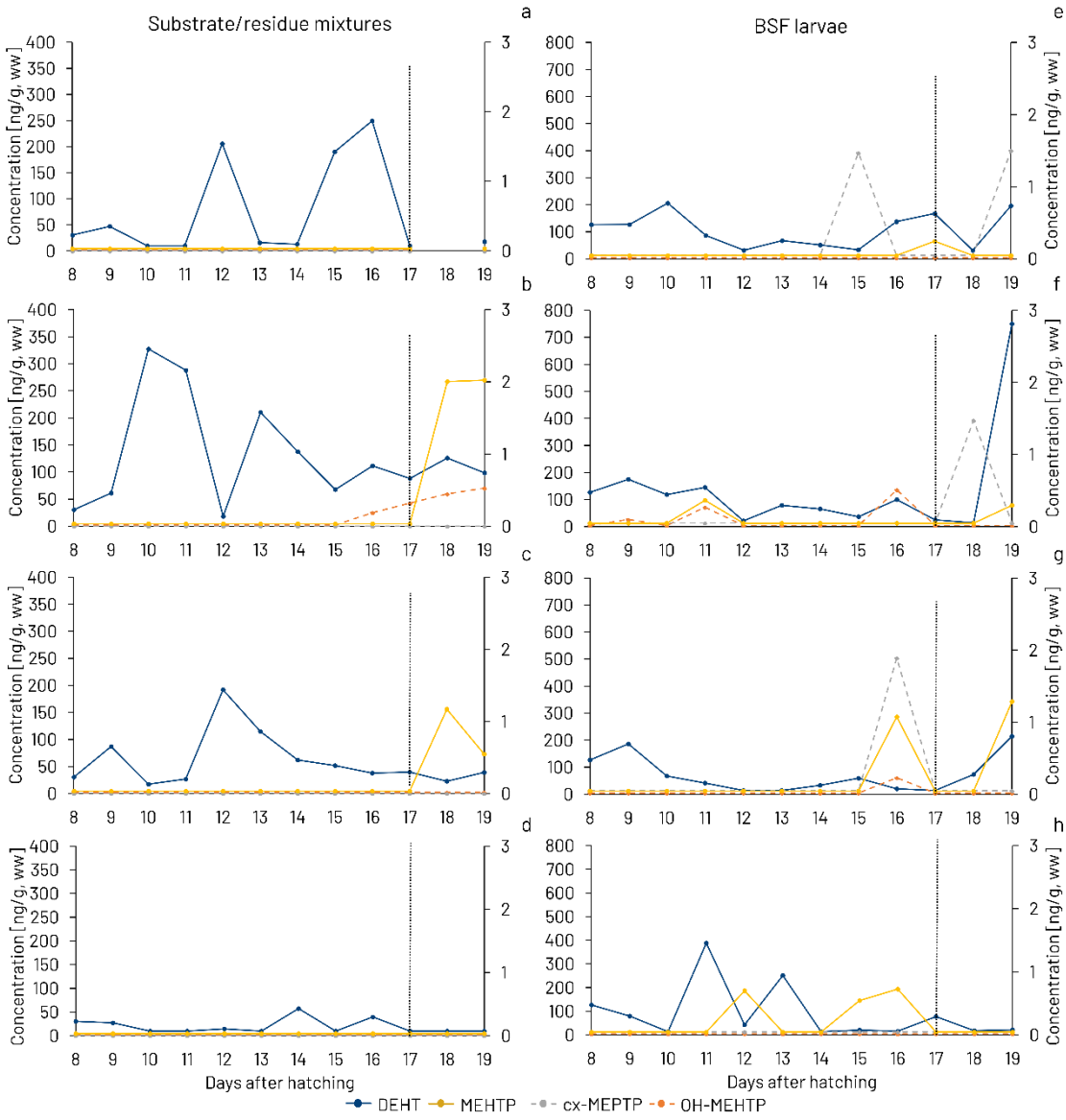
Supplementary Table S6. Confidence levels (CL) according to Schymanski et al. (2014)[41] obtained for the suspect screening of 16 different plastic materials.

Compound group	Compound and/or formula	CL
Phthalates	Diisononyl phthalate (DINP); $C_{26}H_{42}O_4$	1
	Di(2-propylheptyl) phthalate (DPHP); $C_{28}H_{46}O_4$	3
	Dimethyl phthalate (DMP); $C_{10}H_{10}O_4$	3
	Diethyl phthalate (DEP); $C_{12}H_{14}O_4$	3
	Diisobutyl phthalate (DIBP); $C_{16}H_{22}O_4$	3
	$C_{20}H_{14}O_4$	4
Alternative plasticisers	1,2- Cyclohexane dicarboxylic acid diisononyl ester (DINCH); $C_{26}H_{48}O_4$	3
	Tri-n-hexyl trimellitate (THTM); $C_{24}H_{42}O_6$	3
	Diethylhexyl adipate (DEHA); $C_{22}H_{42}O_4$	3
	Diisobutyl adipate (DIBA); $C_{14}H_{26}O_4$	3
	Dibutyl sebacate (DBS); $C_{18}H_{34}O_4$	3
	tributyl 2-acetyloxypropane-1,2,3-tricarboxylate (ATBC); $C_{20}H_{34}O_8$	3
	Tris(2-ethylhexyl) trimetallite (TOTM); $C_{33}H_{54}O_6$	3
	Di(2-ethylhexyl) terephthalate DEHT; $C_{24}H_{38}O_4$	3
	Butyryl trihexyl citrate (BTHC); $C_{28}H_{50}O_8$	3
	$C_{14}H_{22}O_8$	4
PFRs	$C_{21}H_{21}O_4P$	4
	$C_{14}H_{23}O_4P$	4
	$C_{12}H_{27}O_4P$	4
	Isodecyl diphenyl phosphate (iDPP); $C_{22}H_{31}O_4P$	3
	Triethyl phosphate (TEP); $C_6H_{15}O_4P$	3
	Bis(2-butoxyethyl) phosphate (BBOEP); $C_{12}H_{26}O_6P$	3
	$C_{39}H_{34}O_8P_2$	4

$C_{12}H_{10}O_5P$	4
Di(2-ethylhexyl) phenyl phosphate (BEHPP); $C_{22}H_{39}O_4P$	3
$C_8H_{19}O_4P$	4
$C_{18}H_{15}O_4P$	4
$C_{24}H_{51}O_4P$	4
$C_{30}H_{39}O_4P$	4
$C_9H_{15}Cl_6O_4P$	4
$C_{18}H_{39}O_7P$	4



Supplementary Figure S1. Variations in the concentrations (ng/g ww) of DEHT, primary biotransformation product (MEHTP), and secondary biotransformation products (OH-MEHTP and cx-MEPTP, dashed lines) over time in the substrate/residue mixtures and BSF larvae. Figures a, b, c, and d, are the control, macroplastic, mesoplastic, and microplastic substrates, while figures e, f, g, and h, are BSF larvae reared on the control, macroplastic, mesoplastic, and microplastic substrates, respectively. Vertical dotted lines represent the start of the starvation. The concentration of DEHT can be found on the left y-axis, while the concentration of the biotransformation products can be found on the right y-axis. The data is based on wet weight, and each data point is the mean of three biological replicates.