Anaerobic defluorination of chlorine-substituted per- and polyfluorinated carboxylic acids triggered by microbial dechlorination

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Supplemental Methods

Determination of the biotransformation rate constant and half-life

For most of the investigated Cl-PFCAs, no significant abiotic transformation and passive adsorption was observed. For those Cl-PFCAs, the parent compound decay data were fitted into a pseudo first-order reaction model in MATLAB:[1]

$$C = C_0 \cdot e^{-kt}$$

where C represents the concentration of the parent compound (μM) at time t; C_0 is the initial concentration (μM); k is the pseudo-first order removal rate constant (d^{-1}); t is the incubation time (d). The removal half-life of the parent compound was:

$$t_{1/2} = \frac{ln\,(2)}{k}$$

Microbial acute toxicity test using luminescent bacteria (*Vibrio fischeri*)

The microbial acute toxicity test was carried out using a BioTox Watertox Standard Kit (EBPI, Mississauga, ON, Canada). The procedure was modified based on the ISO method 11348-3[2] and conducted in 96-well plates according to manufacturer's instructions. Briefly, the freeze-dried luminescent bacterial reagent was firstly reconstituted in 1 mL of reagent diluent and incubated at 4 °C for at least 30 minutes. Selected PFAS was diluted in 1 mL Milli-Q water with the final concentration of 50 μM. The sample was then adjusted to reach the pH of 7.0 ± 0.2 and the dissolved oxygen (DO) value above 3 mg/L. The adjusted sample with a series of diluted samples (n = 10) and a negative control (sample diluent) with the volume of 150 μL each were

transferred to the white 96-well microplate (Fisher Scientific) labeled as Plate A. Each PFAS chemical had a duplicate test.

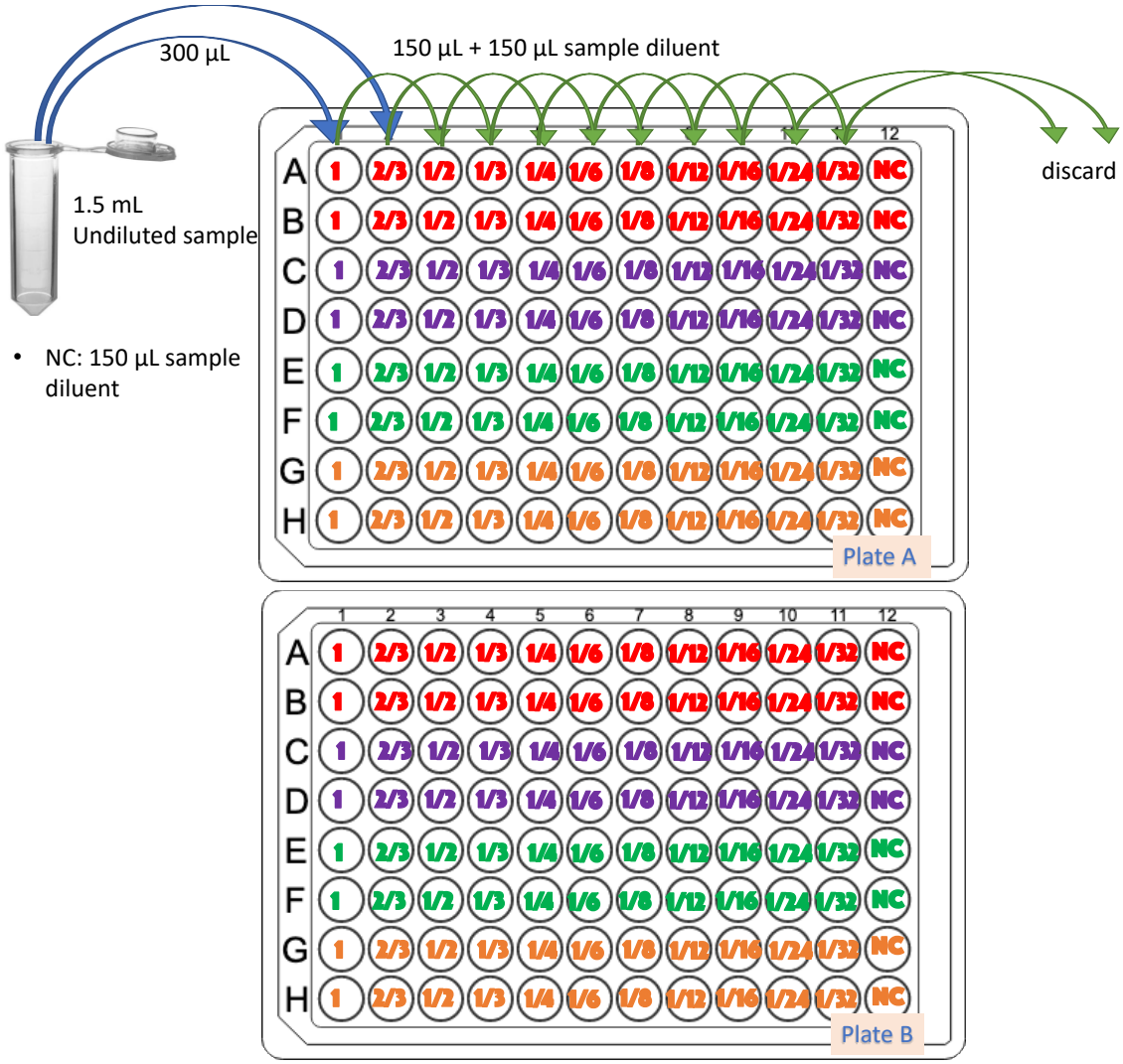
Bacterial suspension (3 µL) and 150 µL sample diluent (20 g/L of NaCl in Milli-Q water) were transferred to the white 96-well microplate (Fisher Scientific) labeled as Plate B. After another incubation at 20 °C ± 1 °C for 15 minutes, the luminescence values (I_0) were detected by the BioTek Synergy H1 Hybrid Multi-Mode Reader (Winooski, VT, USA). Samples in Plate A (150 µL) were then transferred to Plate B. The luminescence values (I_t) were also read after inoculation at 20 °C ± 1 °C for 15 minutes. More details about the toxicity test were shown in the SI. The inhibitory effect (E_t) of a test sample was calculated based on the luminescence strength:

$$E_t\% = \left(1 - \frac{I_t}{I_0 \times \frac{I_{t-NC}}{I_{0-NC}}}\right) \times 100\%$$

where $I_{t\text{-}NC}$ and $I_{0\text{-}NC}$ were the luminescence values of negative control. Inhibitory effects (E_t) versus concentrations (C_i) were plotted in MATLAB (The MathWorks, Natick, MA) and fitted to the adjusted Hill equation to calculate the EC_{50} value of a PFAS chemical:

$$E_t = E_{min} - \frac{E_{max} - E_{min}}{1 + (\frac{EC_{50}}{C_i})^n}$$

where E_{min} and E_{max} were the minimum and maximum effects calculated by the software, and n was the Hill coefficient.

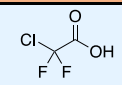
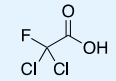
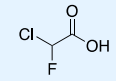
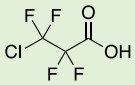
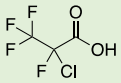
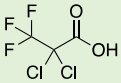
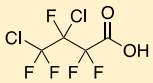
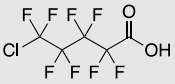
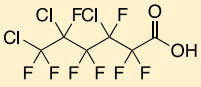


(1) Add 150 µL of sample diluent to each relative well with 3 µL cooled bacterial stock spiked; (2) measure initial light intensity (I_0) after 15-minute incubation; (3) add 150 µL of sample from Plate A; (4) measure light intensity (I_t) after 15-minute incubation.

Density functional theory (DFT) calculation of bond dissociation energies (BDE)

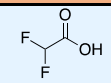
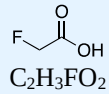
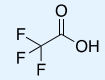
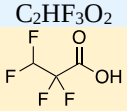
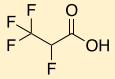
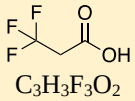
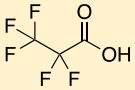
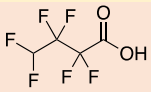
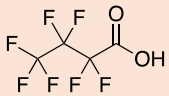
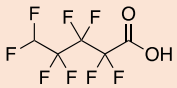
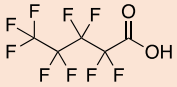
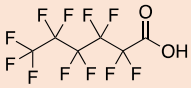
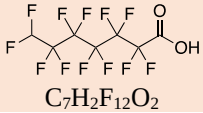
BDE of C–F and C–Cl bonds were calculated for all examined compounds by the GAUSSIAN 09 software package as previously described.[3] All molecular geometries were calculated by the B3LYP/6-311+G(2d,2p) hybrid functional theory. Grimme empirical dispersion correction with the Becke-Johnson damping term (D3-BJ) was added to the calculation for the optimization.

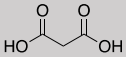
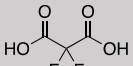
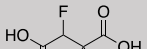
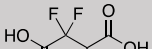
Table S1. Selected Cl-PFCAs and their basic information

Compound*	ID #**	CAS #	Structure and formula	Purity (%)	RT (min)	LOD (μM)	LOQ (μM)
2-chloro-2,2-difluoroacetic acid[(1)]	Cl-C2a	1895-39-2	 $C_2HClF_2O_2$	97	1.34	0.07	0.1
2,2-dichloro-2-fluoroacetic acid[(1)]	Cl-C2b	354-19-8	 $C_2HCl_2FO_2$	≤100	1.72	0.1	0.2
2-chloro-2-fluoroacetic acid[(1)]	Cl-C2c	471-44-3	 $C_2HCl_2FO_2$	≤100	1.02	0.3	0.5
3-chloro-2,2,3,3-tetrafluoropropanoic acid[(1)]	Cl-C3a	661-82-5	 $C_3HClF_4O_2$	97	3.32	0.1	0.2
2-chloro-2,3,3,3-tetrafluoropropanoic acid[(2)]	Cl-C3b	6189-02-2	 $C_3HClF_4O_2$	99	3.47	0.1	0.2
2,2-dichloro-3,3,3-trifluoropropanoic acid[(2)]	Cl-C3c	422-39-9	 $C_3HCl_2F_3O_2$	95	4.62	0.2	0.7
3,4-dichloro-2,2,3,4,4-pentafluorobutanoic acid[(1)]	CTFE2	375-07-5	 $C_4HCl_2F_5O_2$	97	5.62	0.05	0.1
5-chloro-2,2,3,3,4,4,5,5-octafluoropentanoic acid[(3)]	Cl-C5a	66443-79-6	 $C_5HClF_8O_2$	95	6.24	0.005	0.01
3,5,6-trichloro-2,2,3,4,4,5,6,6-octafluorohexanoic acid[(1)]	CTFE3	2106-54-9	 $C_6HCl_3F_8O_2$	95	6.75	0.05	0.1
7-chloro-2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptanoic acid[(1)]	Cl-C7a	1550-24-9	$C_7HClF_{12}O_2$	97	6.97	0.001	0.01
3,5,7,8-tetrachloro-2,2,3,4,4,5,6,6,7,8,8-undecafluorooctanoic acid[(1)]	CTFE4	2923-68-4	$C_8HCl_4F_{11}O_2$	95	7.25	0.1	0.2
9-chloro-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorononanoic acid[(1)]	Cl-C9a	865-79-2	$C_9HClF_{16}O_2$	≤100	7.43	0.01	0.05

*: Cl-PFCA compounds were purchased from three companies: (1) SynQuest Laboratories, Inc (Alachua, FL, USA); (2) Matrix Scientific (Columbia, SC, USA); (3) Manchester Organics, Ltd (Runcorn, UK)
**: ID numbers were created based on the number of carbons. The lowercase "a" represents that the Cl-PFCA only contains one terminal chlorine.

Table S2. Transformation products and compounds structurally similar to the Cl-PFCAs

Compound*	ID #**	CAS #	Structure and formula	Purity (%)	RT (min)	LOD (µM)	LOQ (µM)
2,2-difluoroacetic acid[(1)]	C2a (DFA)	381-73-7	 $C_2H_2F_2O_2$	98	0.91	0.05	0.1
2-fluoroacetic acid[(2)]	C2b (MFA)	144-49-0	 $C_2H_3FO_2$	N/A	0.88	1	1.5
2,2,2-trifluoroacetic acid[(1)]	TFA	76-05-1	 $C_2HF_3O_2$	99	1.1	.01	.05
2,2,3,3-tetrafluoropropanoic acid[(1)]	C3a	756-09-2	 $C_3H_2F_4O_2$	97	1.31	0.001	0.01
2,3,3,3-tetrafluoropropanoic acid[(1)]	C3b	359-49-9	 $C_3H_2F_4O_2$	97	1.23	0.005	0.01
3,3,3-trifluoropropanoic acid[(1)]	C3c	2516-99-6	 $C_3H_3F_3O_2$	97	1.14	0.05	0.5
2,2,3,3,3-pentafluoropropanoic acid[(1)]	PFPrA	422-64-0	 $C_3HF_5O_2$	98	2.22	0.001	0.05
2,2,3,3,4,4-hexafluorobutanoic acid[(1)]	C4a (H-PFBA)	679-12-9	 $C_4H_2F_6O_2$	97	3.01	0.001	0.005
2,2,3,3,4,4,4-heptafluorobutanoic acid[(1)]	PFBA	375-22-4	 $C_4HF_7O_2$	98	5.07	0.001	0.01
2,2,3,3,4,4,5,5-octafluoropentanoic acid[(1)]	C5a (H-PFPeA)	376-72-7	 $C_5H_2F_8O_2$	97	5.25	0.001	0.01
2,2,3,3,4,4,5,5,5-nonafluoropentanoic acid[(1)]	PFPeA	2706-90-3	 $C_5HF_9O_2$	98	6.1	0.001	0.01
2,2,3,3,4,4,5,5,6,6,6-undecafluorohexanoic acid[(1)]	PFHxA	307-24-4	 $C_6HF_{11}O_2$	97	6.59	0.001	0.01
2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptanoic acid[(1)]	C7a (H-PFHpA)	1546-95-8	 $C_7H_2F_{12}O_2$	98	6.38	0.005	0.05

2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptanoic acid[1]	PFHpA	375-85-9	$C_7HF_{13}O_2$	97	6.93	0.005	0.01
2,2,3,3,4,4,5,5,6,6,7,7,8,8-tetradecafluorooctanoic acid[1]	C8a (H-PFOA)	13973-14-3	$C_8H_2F_{14}O_2$	97	6.66	0.001	0.005
2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid[1]	PFOA	335-67-1	$C_8HF_{15}O_2$	98	7.18	0.001	0.005
2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorononanoic acid[1]	C9a (H-PFNA)	76-21-1	$C_9H_2F_{16}O_2$	97	6.92	0.005	0.01
2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluorononanoic acid[1]	PFNA	375-95-1	$C_9HF_{17}O_2$	97	7.4	0.0001	0.001
Propanedioic acid[1]	Malonic acid	141-82-2	 $C_3H_4O_4$	100	0.85	1	2
2,2-difluoromalonic acid[1]	PFPrdiA	1514-85-8	 $C_3H_2F_2O_4$	98	0.82	0.05	0.5
2,2,3-trifluorosuccinic acid[4]	TP170	664-66-4	 $C_4H_3F_3O_4$	N/A	0.84	0.01	0.1
2,2,3,3-tetrafluorosuccinic acid[1]	PFBdiA	377-38-8	 $C_4H_2F_4O_4$	98	0.81	0.005	0.01
2,2,3,3,4,4-hexafluoropentanedioic acid[1]	PFPediA	376-73-8	$C_5H_2F_6O_4$	98	0.86	0.01	0.1
2,2,3,3,4,4,5,5-octafluorohexanedioic acid[1]	PFHxdiA	336-08-3	$C_6H_2F_8O_4$	97	1.12	0.01	0.05
2,2,3,3,4,4,5,5,6,6-decafluoroheptanedioic acid[3]	PFHpdiA	14919-09-6	$C_7H_2F_{14}O_4$	90	NA	NA	NA
2,2,3,3,4,4,5,5,6,6,7,7,8,8-tetradecafluorononanedioic acid[1]	PFNdiA	23453-64-7	$C_9H_2F_{14}O_4$	90	5.36	0.001	0.01

*: (1) SynQuest Laboratories, Inc (Alachua, FL, USA); (2) Actually, the examined chemical is sodium fluoroacetate (CAS#: 62-74-8) from MP Biomedicals LLC (Irvine, CA, USA); (3) Mcule, Inc. (Palo Alto, CA, USA); (4) Oakwood Products, Inc. (Estill, SC, USA)

**: ID numbers were created based on the number of carbons.

Table S3. Parent compound decay half-lives (with 95% confidence bounds) of twelve transformable PFAS calculated with pseudo first-order reaction model by MATLAB.

PFAS	Half-life (Day)	R^2
Cl-C2b	11.5 ± 1.6	0.975
Cl-C2c	24.5 ± 3.5	0.937
MFA	73.2 ± 11.4	0.912
Cl-C3b	15.5 ± 2.3	0.972
Cl-C3c	5.0 ± 1.1	0.973
C3c	637.2 ± 189.5	0.767
Cl-C5a	633.5 ± 247.5	0.738
Cl-C7a	488.6 ± 200.3	0.545
Cl-C9a	53.0 ± 15.5	0.772
CTFE2	18.2 ± 8.5	0.870
CTFE3	3.4 ± 0.4	0.995
CTFE4	3.5 ± 3.0	0.882

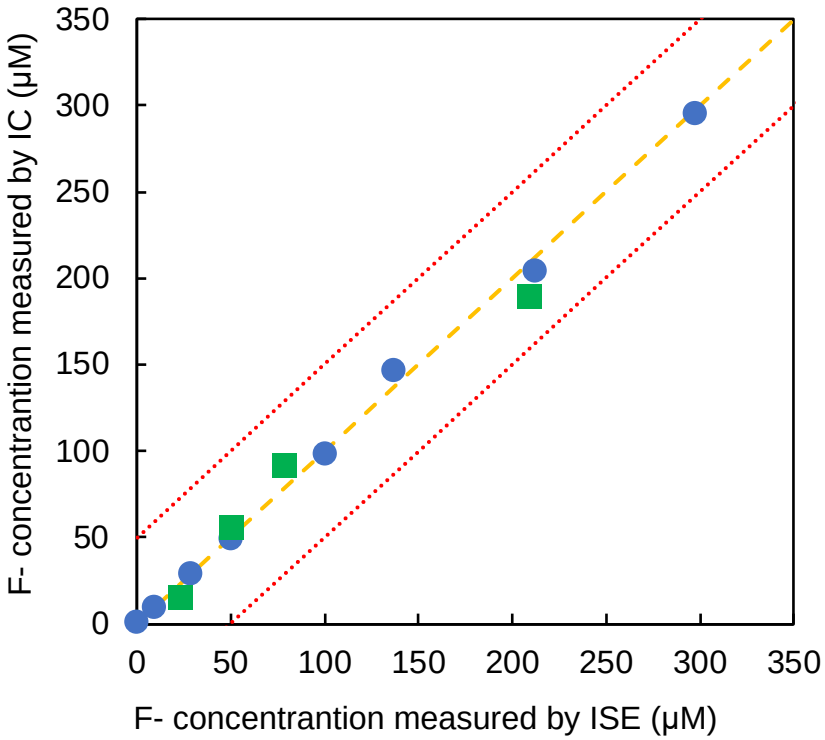


Figure S1. Comparison of F^- measured by ISE and IC using six mock samples (blue) and four real supernatant samples (green). Two red-dashed lines indicate the ±10% variation compared to the 1:1 yellow-dashed line.

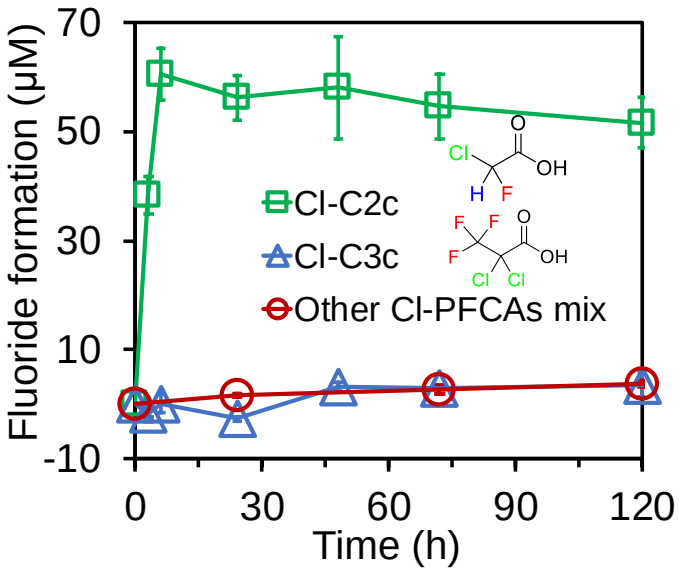


Figure S2. Aerobic defluorination of Cl-PFCAs by the activated sludge community (the Cl-PFCA mix includes all Cl-PFCAs in this study except for **Cl-C2c** and **Cl-C3c**).

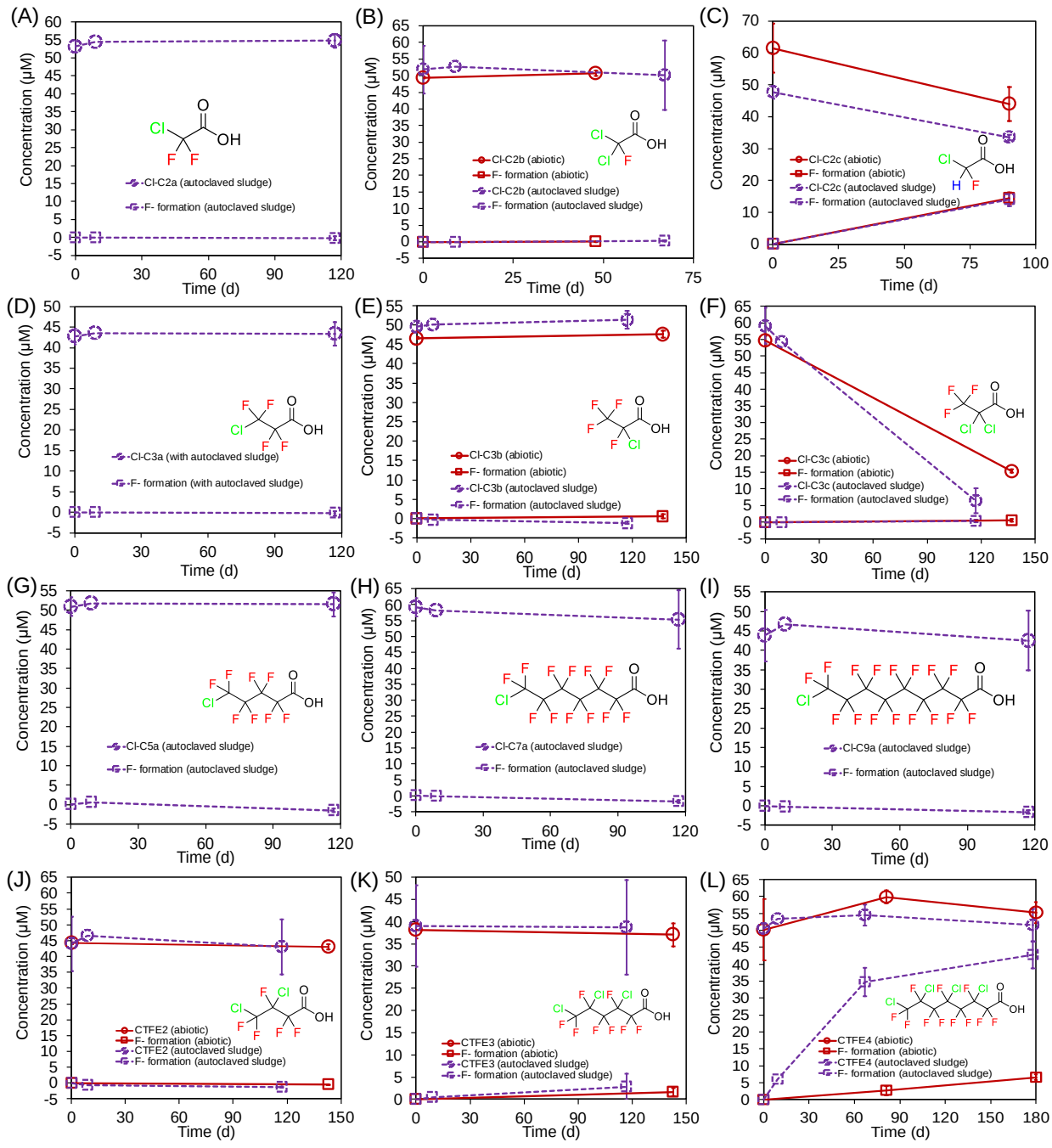


Figure S3. Parent compound decay and fluoride formation in the abiotic and heat-inactivated biomass controls for the investigated Cl-PFCAs.

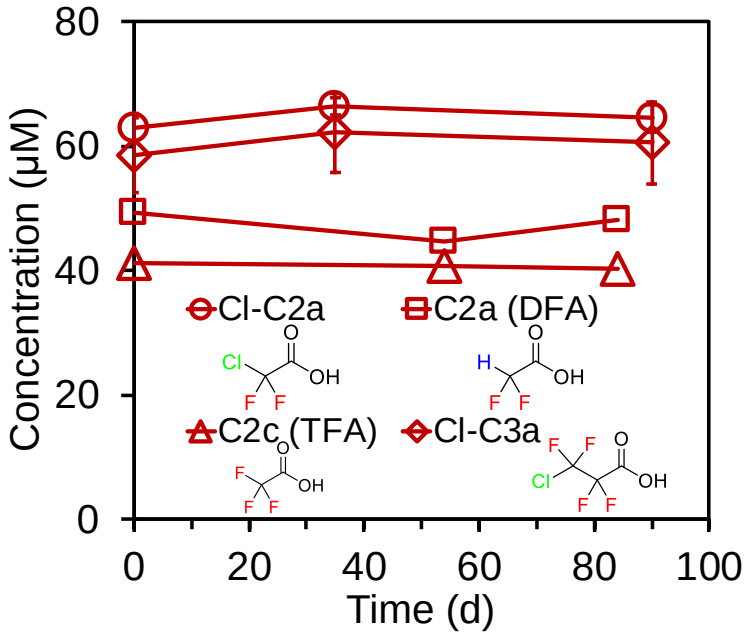


Figure S4. Parent compound decay for **Cl-C2a**, **C2a** (DFA), **C2c** (TFA), and **Cl-C3a** in the activated sludge community under anaerobic condition (the target initial concentration was 50 μM, ±20% variation occurred when adding stock solutions into the anaerobic batch reactors).

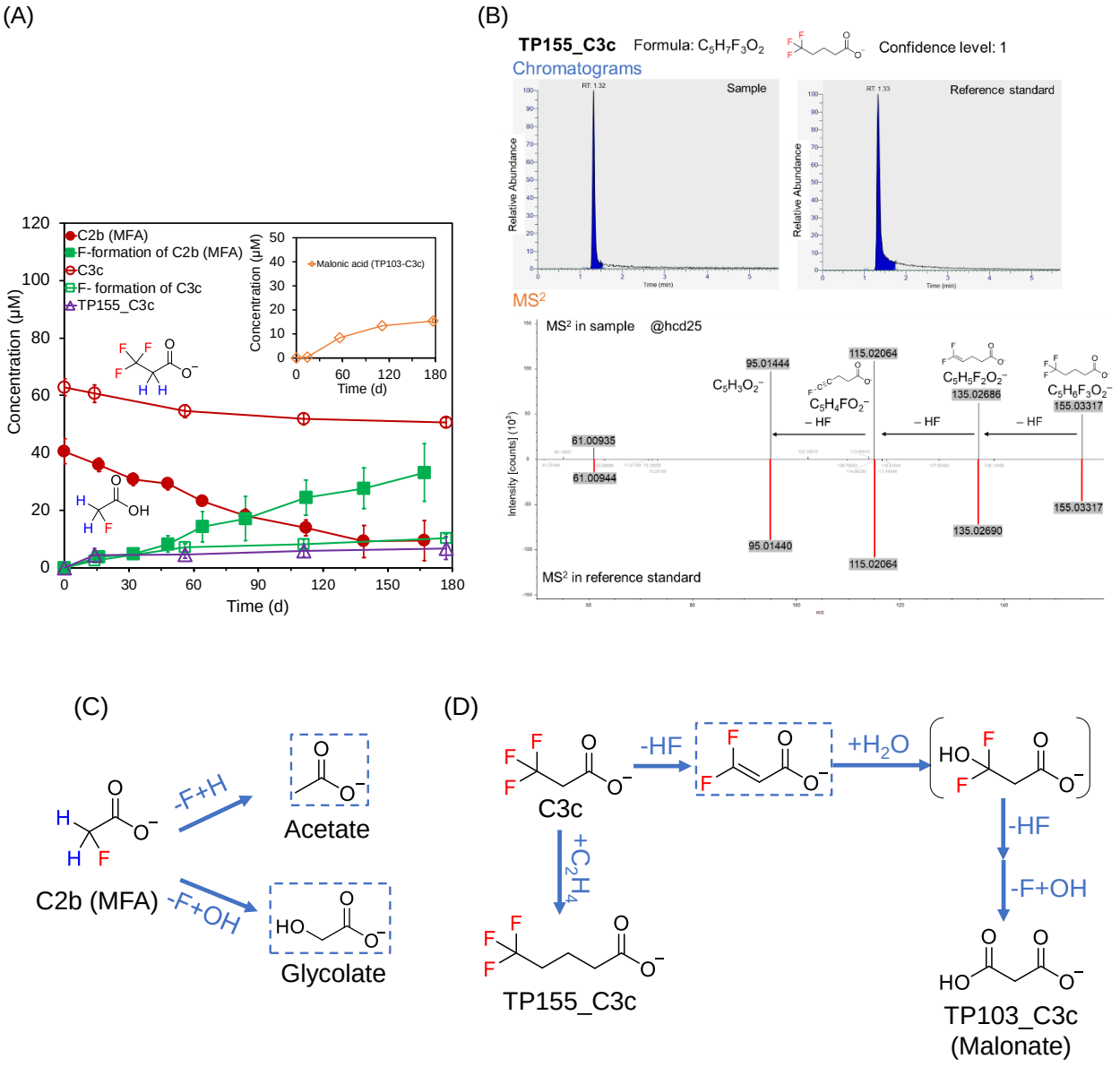


Figure S5. Anaerobic biotransformation and defluorination of **C2b** and **C3c** by the same activated sludge community (A), the confirmation of TP155_C3c (B), and the plausible biotransformation pathway of **C2b** (C) and **C3c** (D) (TPs in brackets were unstable transient intermediates; in dashed boxes were those not detected; other TPs were confirmed using reference compounds).

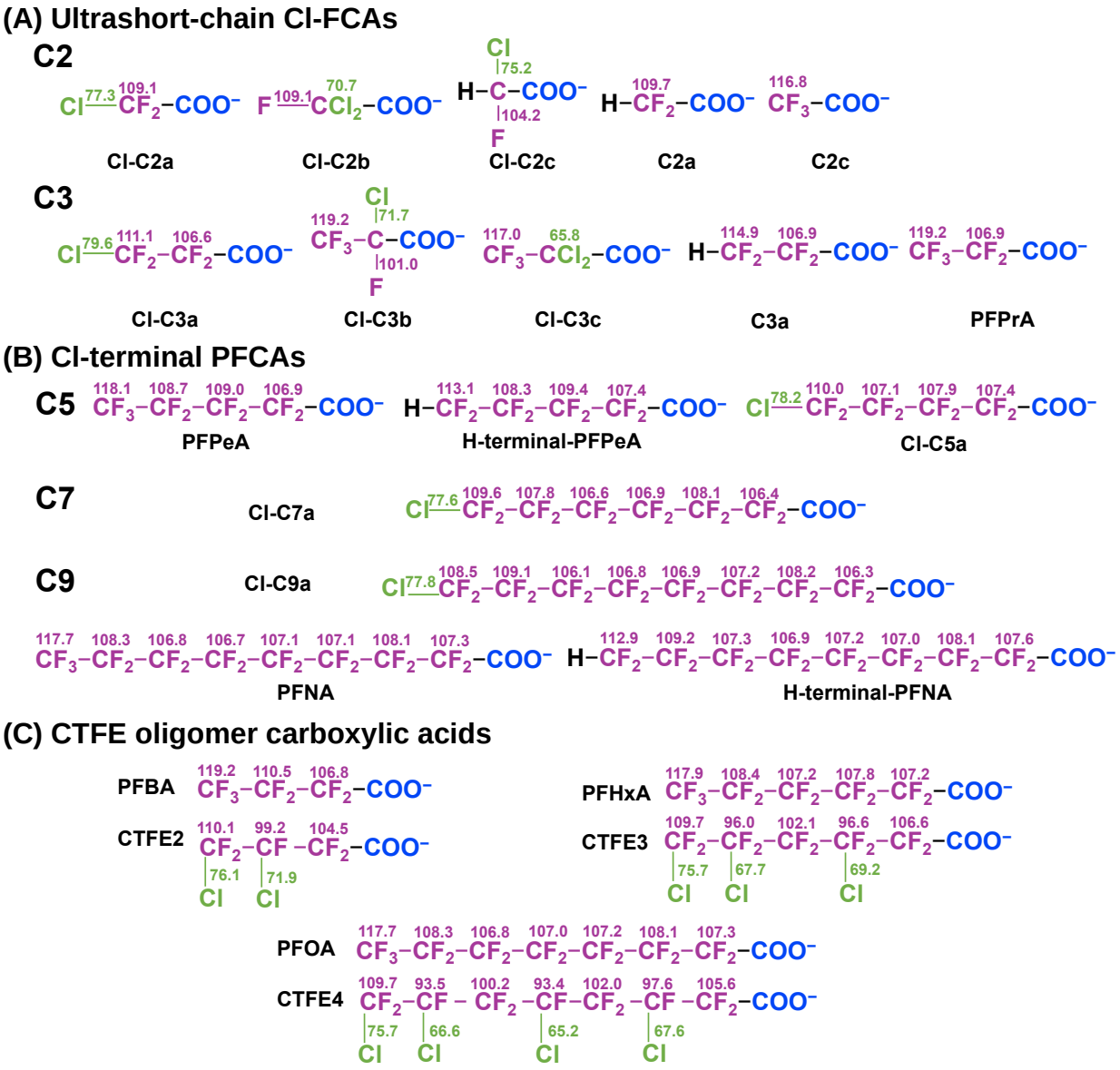
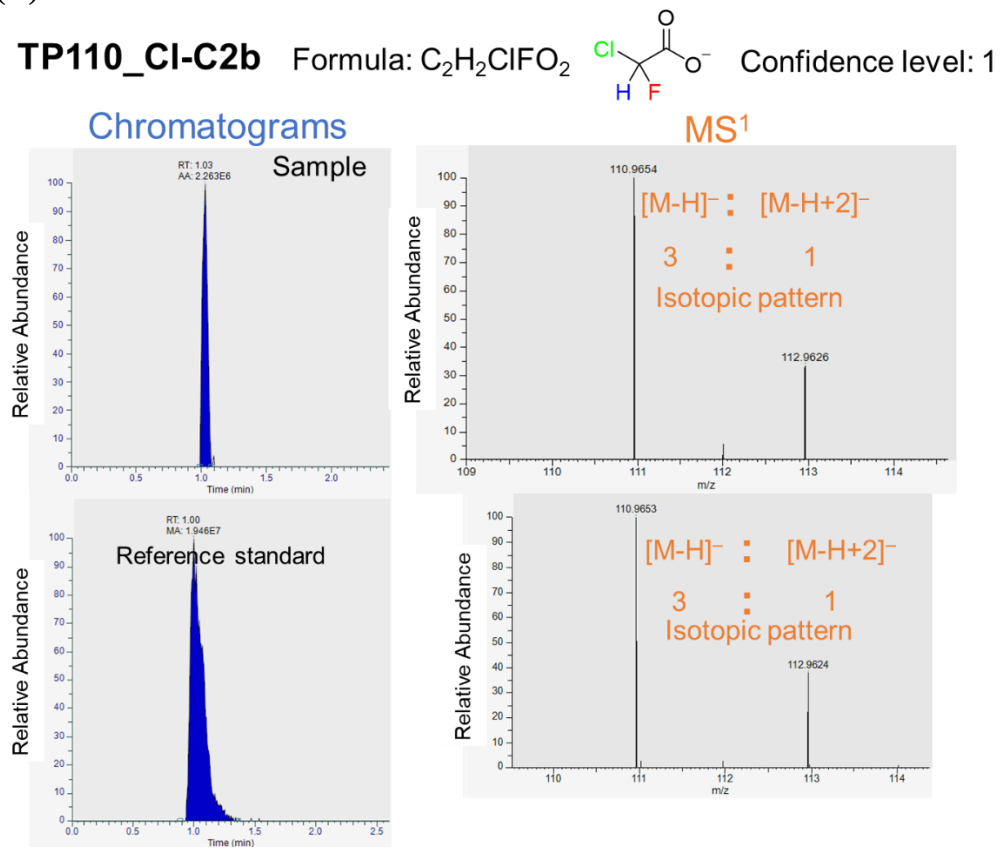


Figure S6. Calculation of bond dissociation energy (BDE) for the investigated Cl-PFCAs.

(A)



(B)

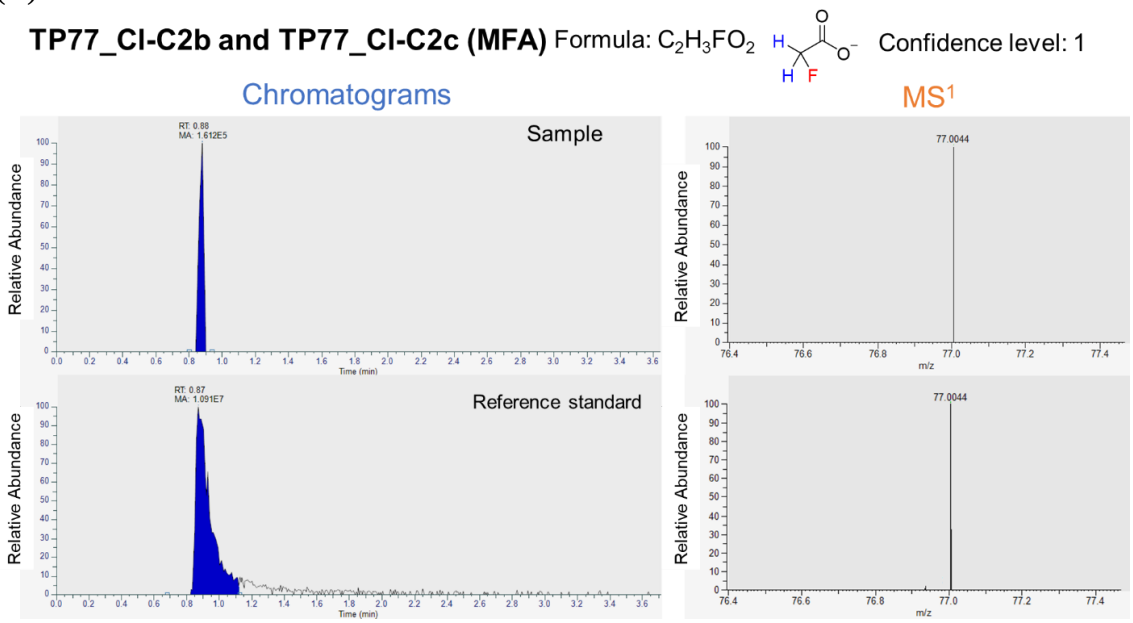


Figure S7. The identification of TP110_Cl-C2b (A) and TP77_Cl-2b/TP77_Cl-C2c (B) (Note: MS^2 was not available for the reference standards, the MS^1 spectrum and the isotopic pattern of Cl (^{35}Cl:^{37}Cl = 3:1 in nature[4, 5]) were used for the structure confirmation, instead).

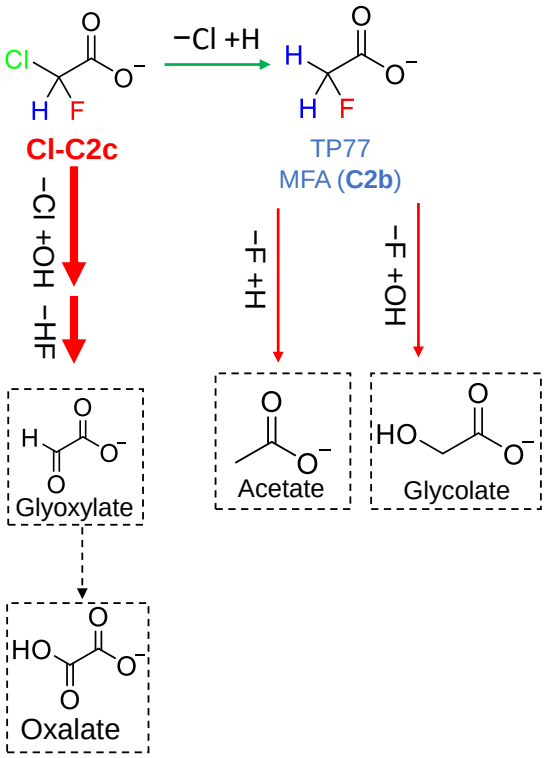
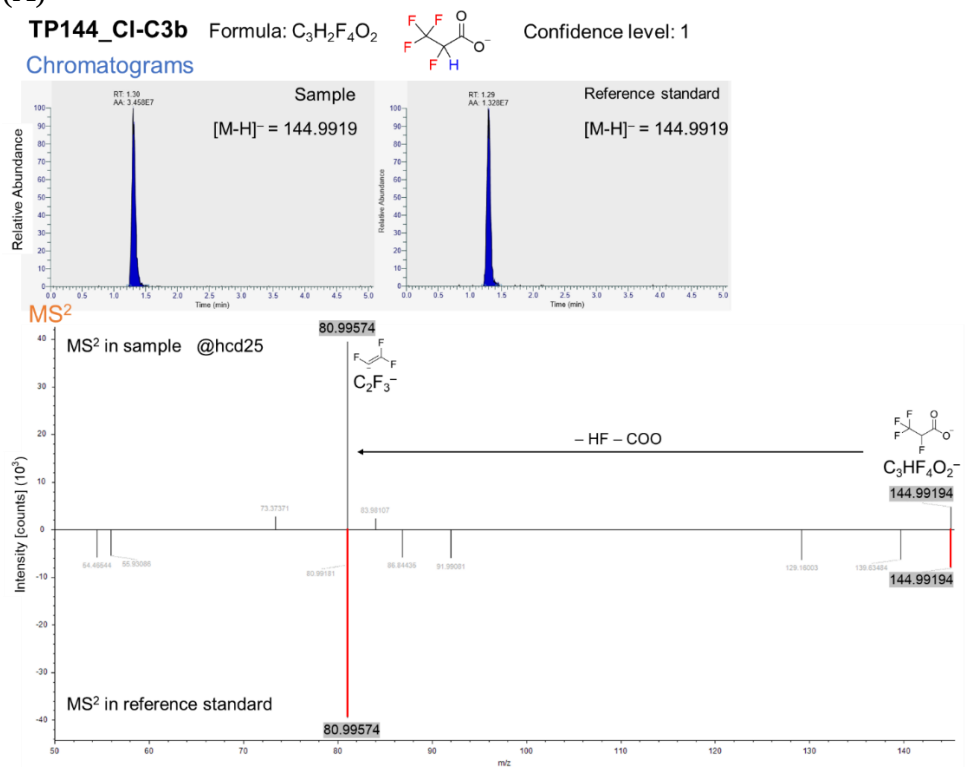


Figure S8. Proposed biodefluorination pathways of **Cl-C2c.**

(A)



(B)

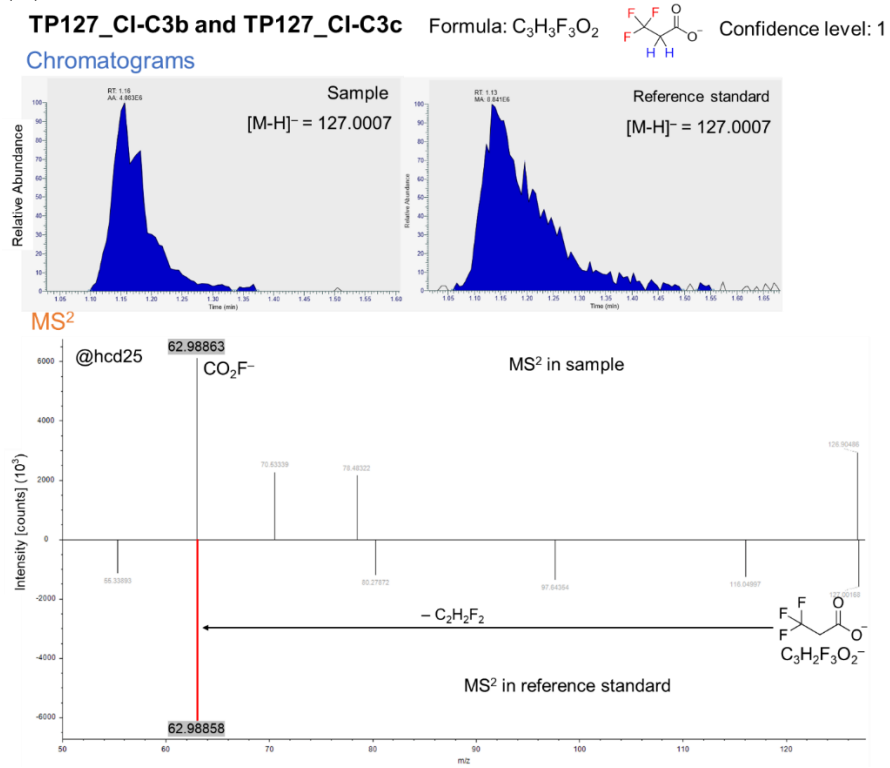


Figure S9. The identification of TP144_Cl-C3b (A) and TP127_Cl-C3b/TP127_Cl-C3c (B).

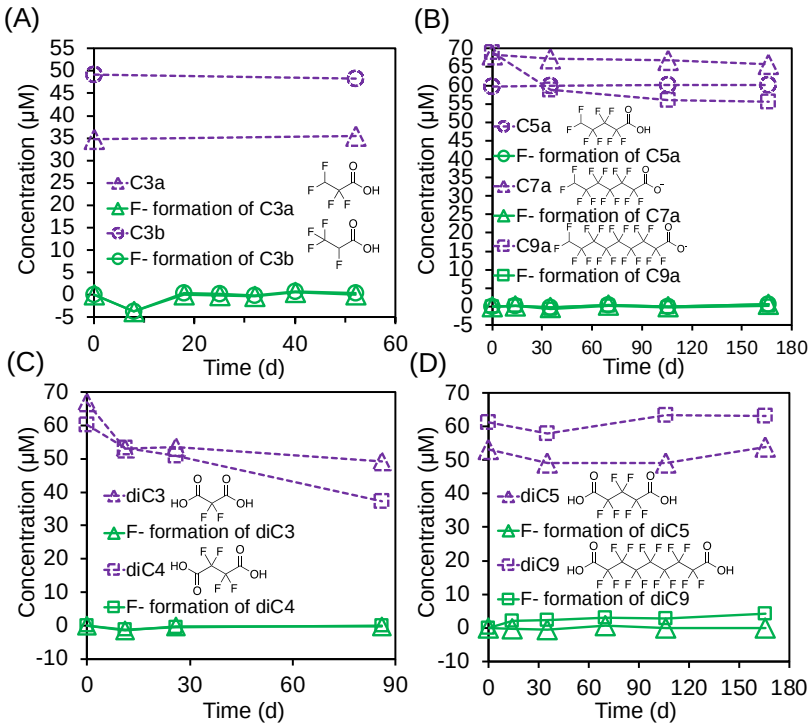


Figure S10. Biotransformation and fluoride formation of H-PFCAs (A & B) and PFdiCAs (C & D) under anaerobic condition.

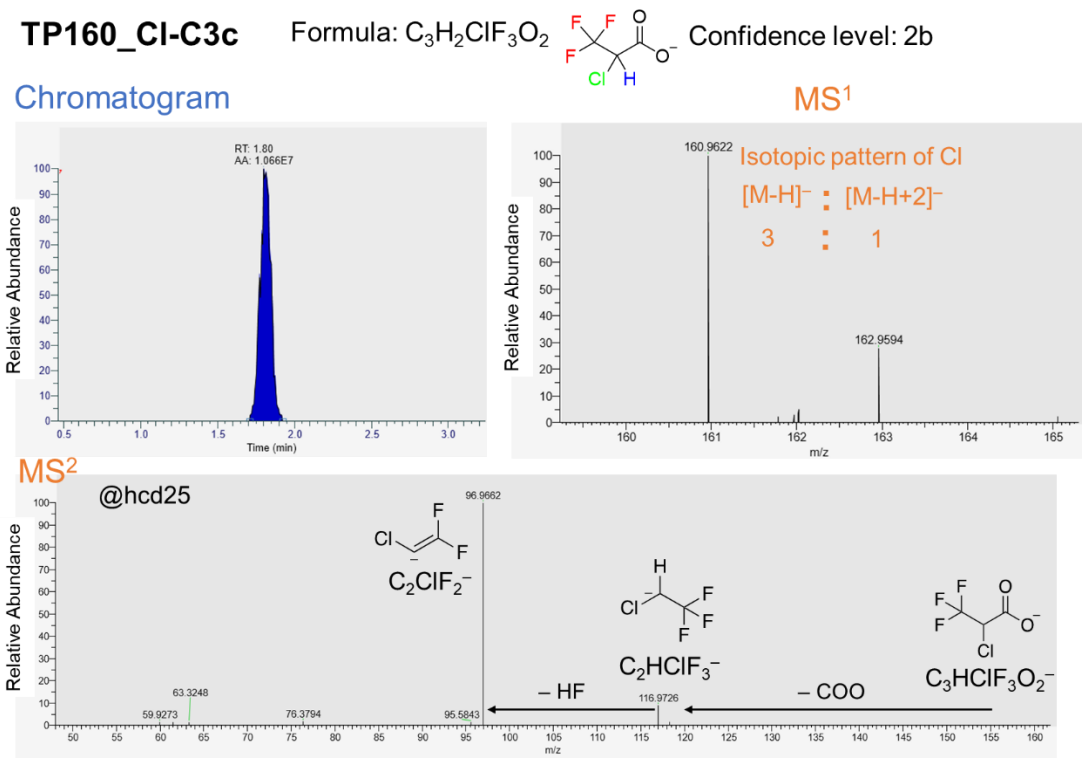


Figure S11. The identification of TP160_Cl-C3c (Note: the one Cl-substitution in the structure was indicated by the isotopic pattern of Cl, ^{35}Cl:^{37}Cl = 3:1[4, 5]).

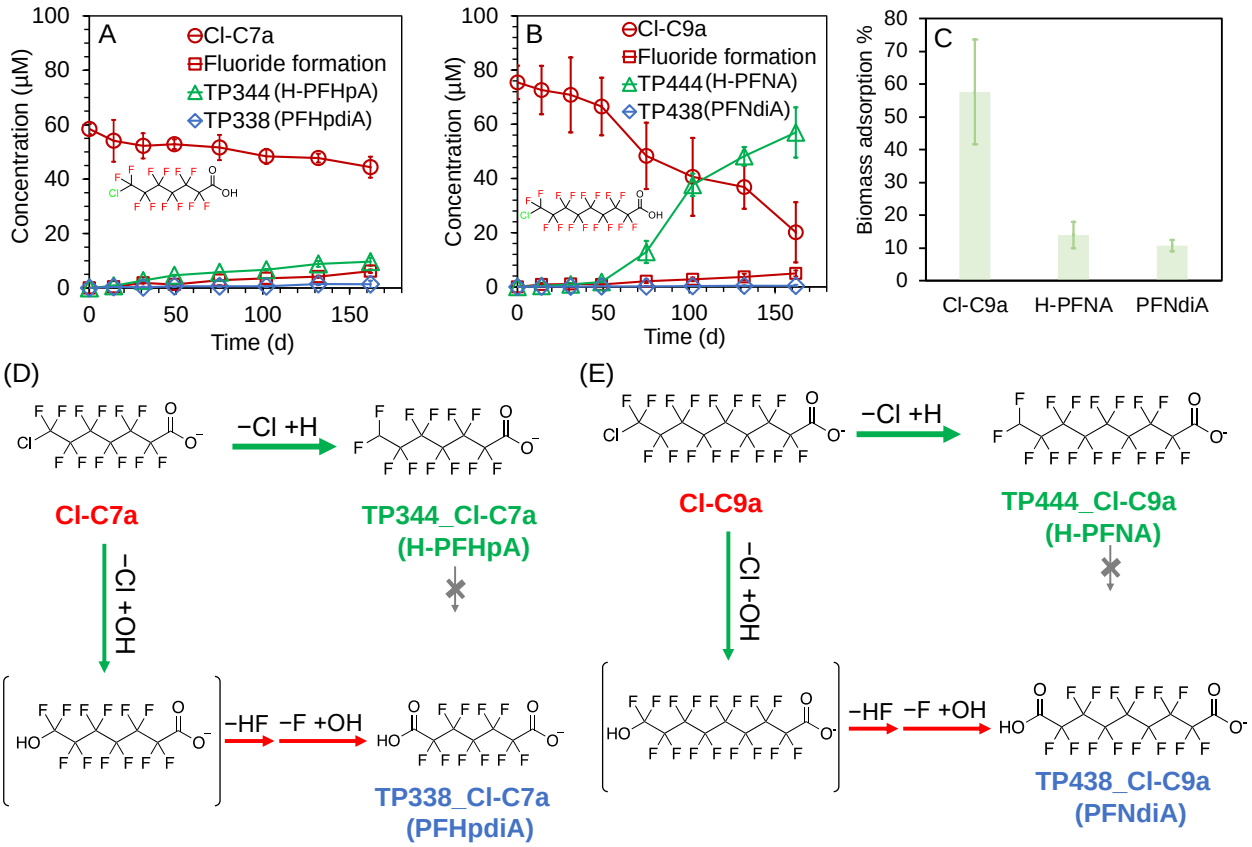
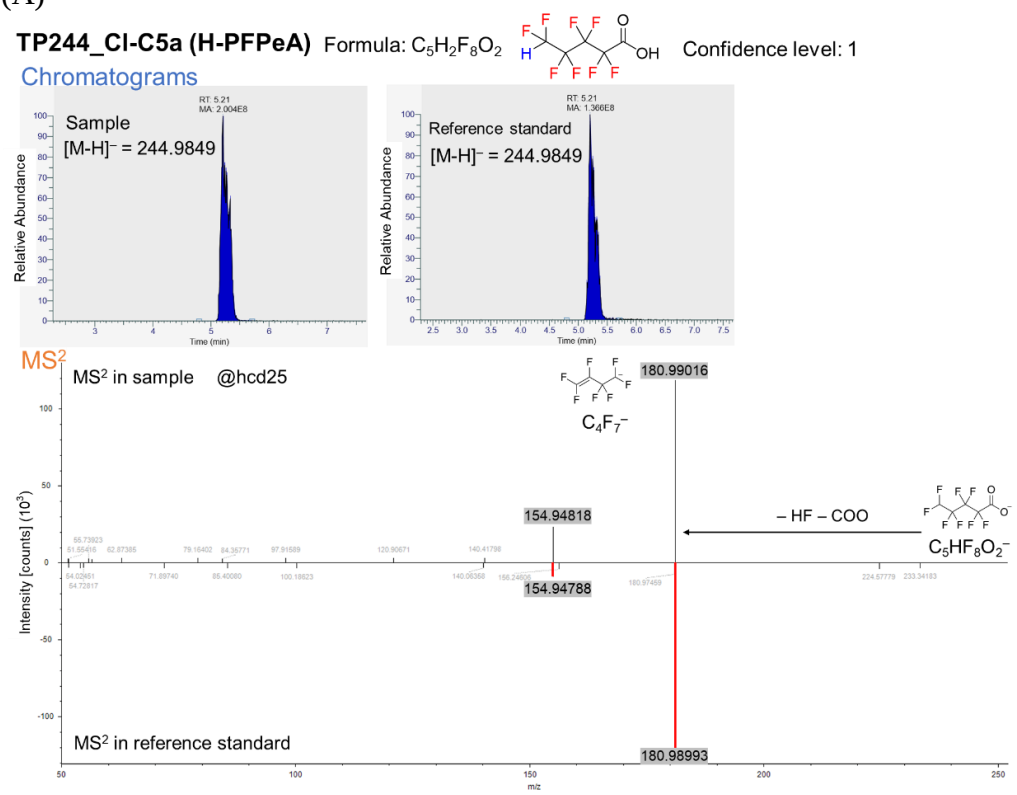
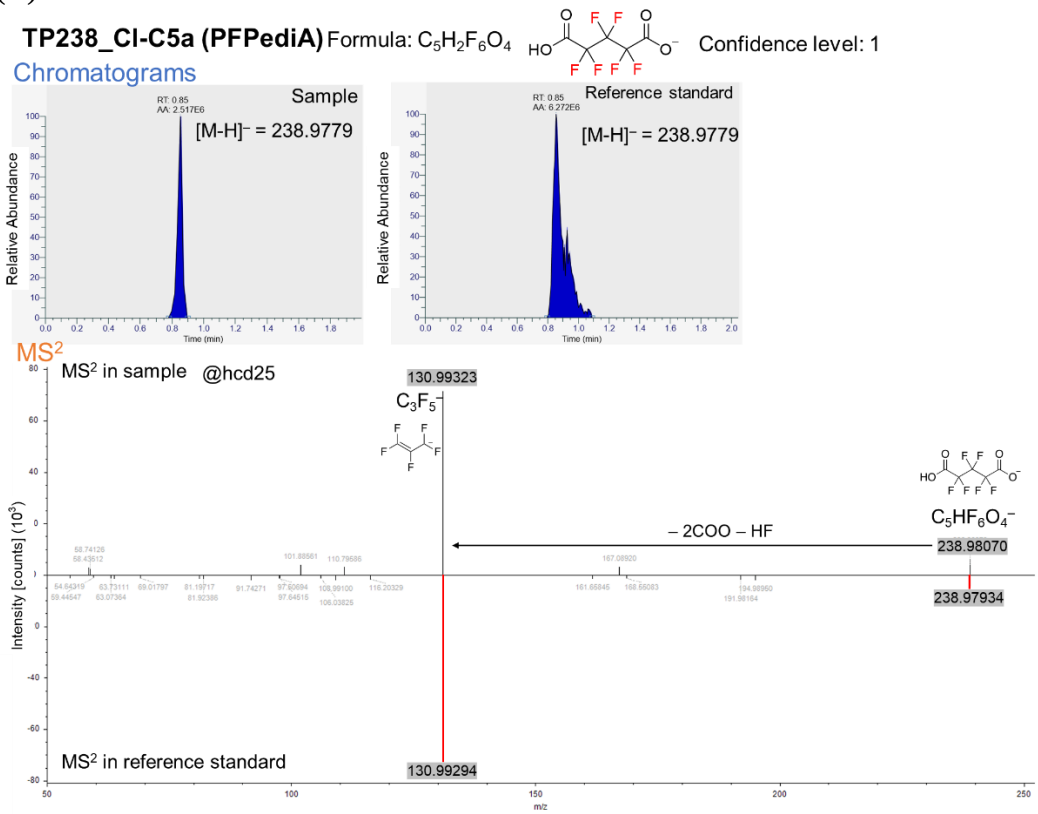


Figure S12. Anaerobic biotransformation and fluoride formation of **Cl-C7a** (A) and **Cl-C9a** (B), the sorption of **Cl-C9a** and its TPs on the active biomass at the end of incubation (C), and the plausible anaerobic biotransformation pathway of **Cl-C7a** (D) and **Cl-C9a** (E) (Note: no significant adsorption shown in autoclaved samples, see **Figure S3I**; the total concentrations of extracellular and biomass-associated **Cl-C9a** and its TPs were shown in panel B due to significant bioadsorption).

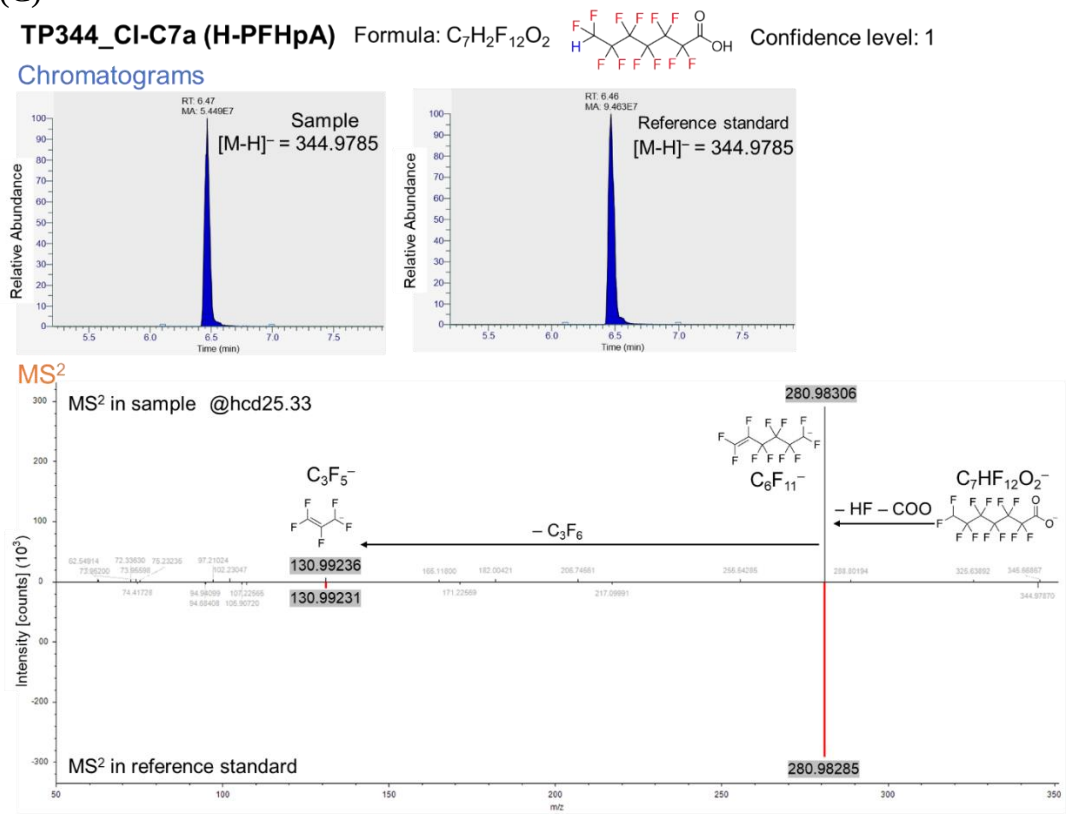
(A)


TP244_Cl-C5a (H-PFPeA) Formula: C5H2F8O2
Confidence level: 1
Chromatograms
Sample
[M-H]⁻ = 244.9849
Reference standard
[M-H]⁻ = 244.9849
Relative Abundance
Time (min)
MS2
MS2 in sample @hcd25
180.99016
C4F7⁻
154.94818
– HF – COO
C5HF8O2⁻
154.94788
MS2 in reference standard
180.98993
Intensity [counts] (10³)
m/z

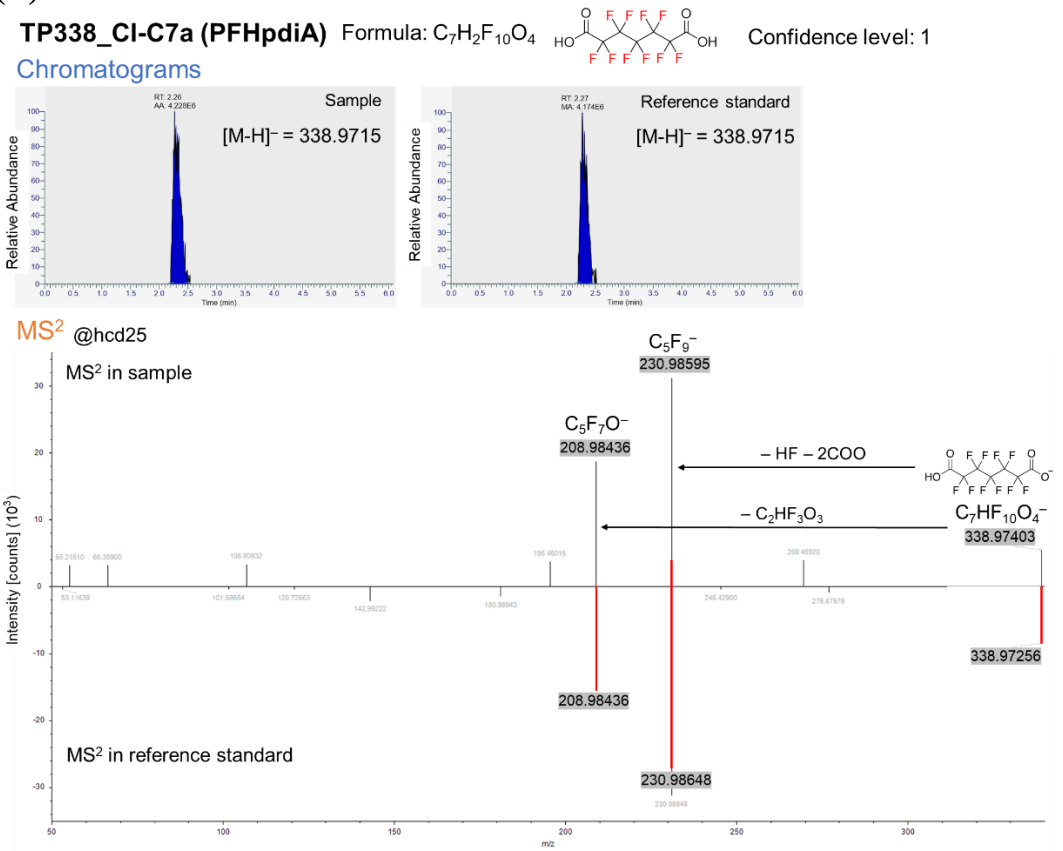
(B)


TP238_Cl-C5a (PFPediA) Formula: C5H2F6O4
Confidence level: 1
Chromatograms
Sample
[M-H]⁻ = 238.9779
Reference standard
[M-H]⁻ = 238.9779
Relative Abundance
Time (min)
MS2
MS2 in sample @hcd25
130.99323
C3F5⁻
– 2COO – HF
C5HF6O4⁻
238.98070
238.97934
MS2 in reference standard
130.99294
Intensity [counts] (10³)
m/z

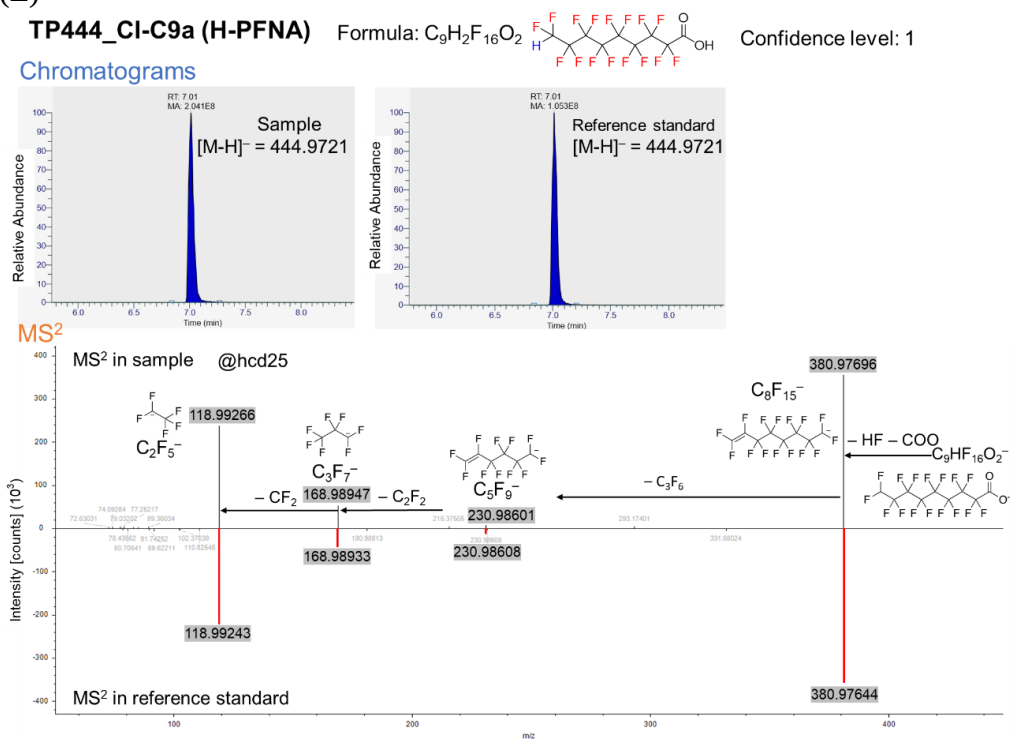
(C)


TP344_Cl-C7a (H-PFHpA) Formula: $C_7H_2F_{12}O_2$
Confidence level: 1
Chromatograms
Sample
[M-H]⁻ = 344.9785
Reference standard
[M-H]⁻ = 344.9785
Relative Abundance
Time (min)
MS²
MS² in sample @hcd25.33
280.98306
$C_6F_{11}^-$
$C_3F_5^-$
$C_7HF_{12}O_2^-$
– HF – COO
– C_3F_6
130.99236
130.99231
MS² in reference standard
280.98285
Intensity [counts] (10^3)
m/z

(D)


TP338_Cl-C7a (PFHpdiA) Formula: $C_7H_2F_{10}O_4$
Confidence level: 1
Chromatograms
Sample
[M-H]⁻ = 338.9715
Reference standard
[M-H]⁻ = 338.9715
Relative Abundance
Time (min)
MS² @hcd25
MS² in sample
$C_5F_9^-$
230.98595
$C_5F_7O^-$
208.98436
– HF – 2COO
– $C_2HF_3O_3$
$C_7HF_{10}O_4^-$
338.97403
338.97256
208.98436
MS² in reference standard
230.98648
Intensity [counts] (10^3)
m/z

(E)



(F)

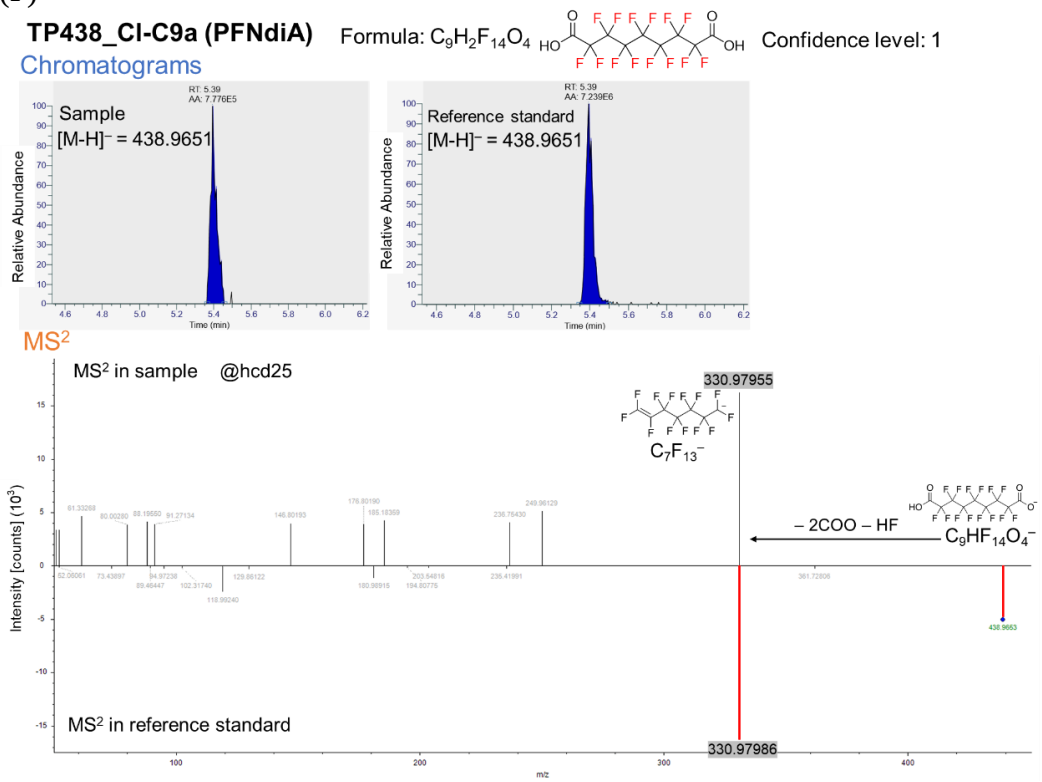
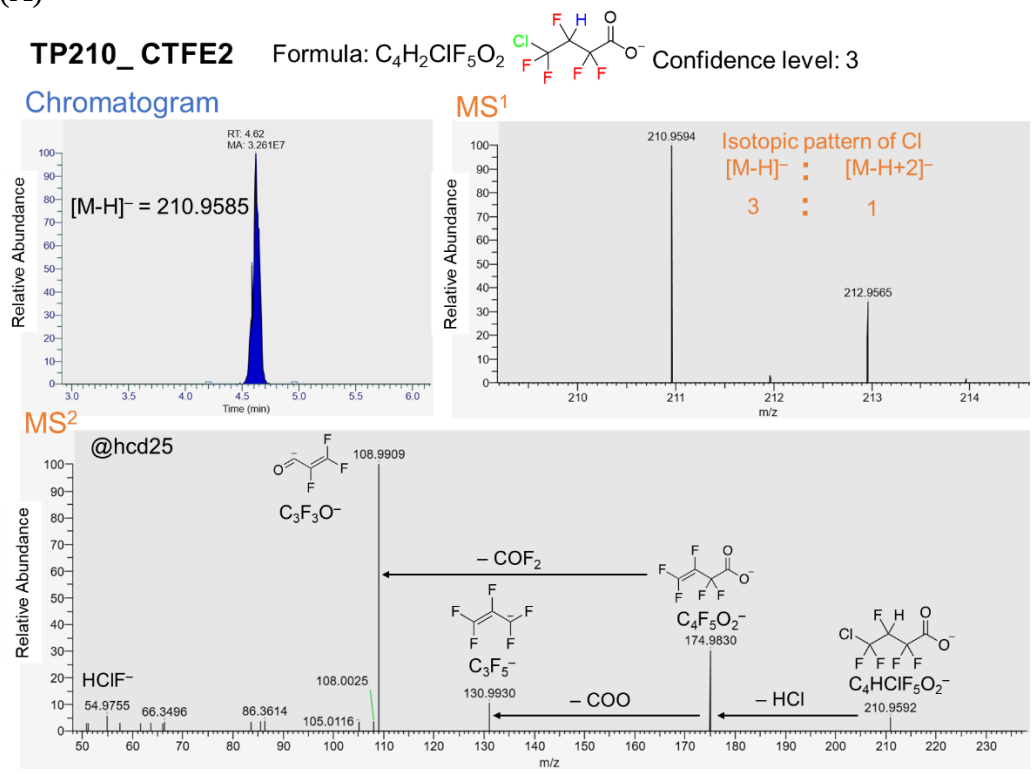
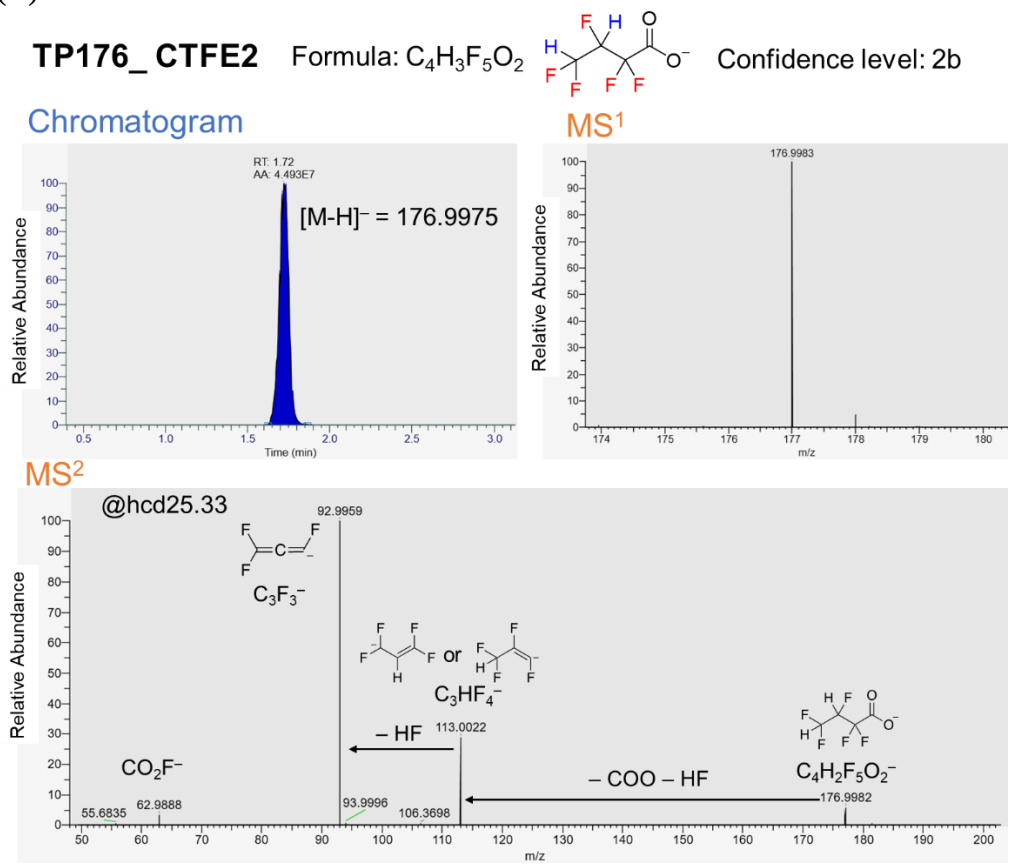


Figure S13. TP identification for **Cl-C5a** (A-B), **Cl-C7a** (C-D), and **Cl-C9a** (E-F).

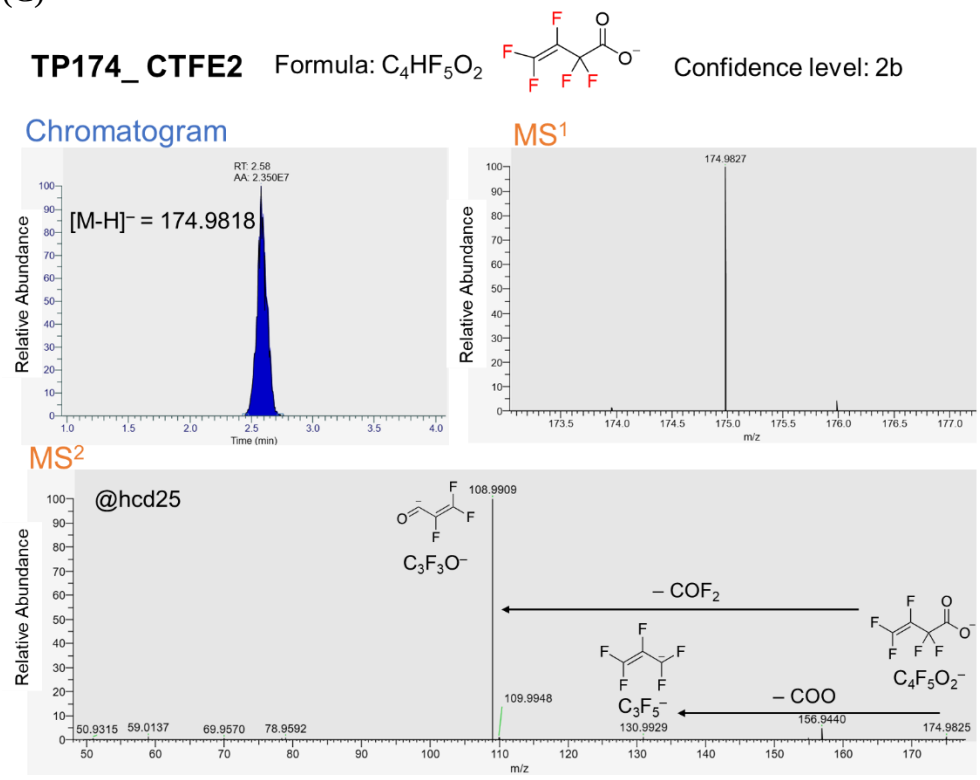
(A)


TP210_ CTFE2
Formula: $C_4H_2ClF_5O_2$
Confidence level: 3
Chromatogram
RT: 4.62
MA: 3.261E7
[M-H]⁻ = 210.9585
Relative Abundance
Time (min)
MS¹
210.9594
Isotopic pattern of Cl
[M-H]⁻ : [M-H+2]⁻
3 : 1
212.9565
m/z
MS²
@hcd25
108.9909
$C_3F_3O^-$
– COF_2
$C_4F_5O_2^-$
174.9830
$C_3F_5^-$
130.9930
$C_4HClF_5O_2^-$
210.9592
HClF⁻
54.9755
66.3496
86.3614
108.0025
105.0116
– COO
– HCl

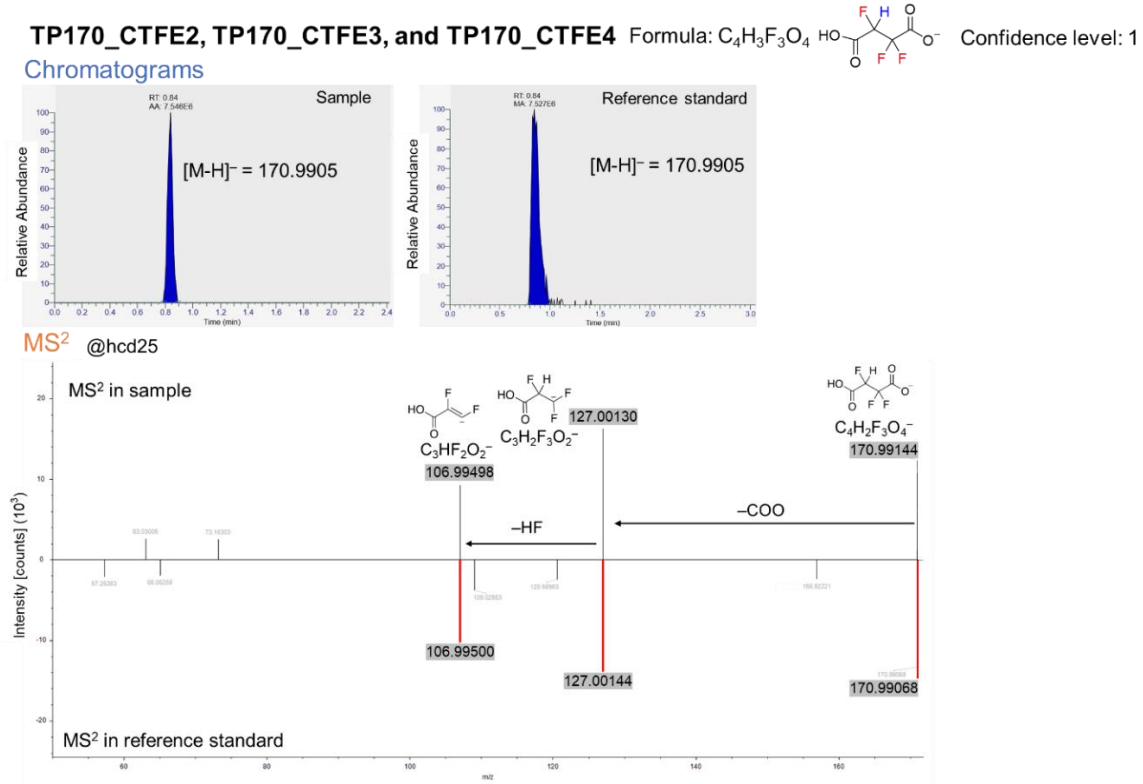
(B)


TP176_ CTFE2
Formula: $C_4H_3F_5O_2$
Confidence level: 2b
Chromatogram
RT: 1.72
AA: 4.493E7
[M-H]⁻ = 176.9975
Relative Abundance
Time (min)
MS¹
176.9983
m/z
MS²
@hcd25.33
92.9959
$C_3F_3^-$
or
$C_3HF_4^-$
– HF
113.0022
CO_2F^-
$C_4H_2F_5O_2^-$
– COO – HF
176.9982
55.6835
62.9888
93.9996
106.3698

(C)

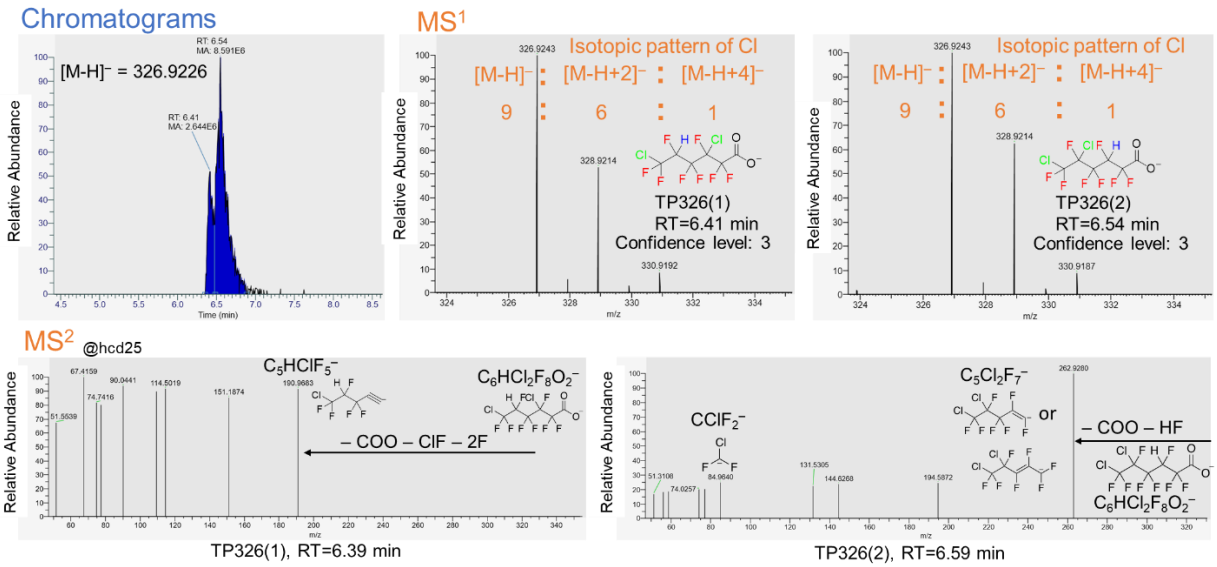

TP174_ CTFE2
Formula: C4HF5O2
Confidence level: 2b
Chromatogram
MS1
MS2
[M-H]⁻ = 174.9818
RT: 2.58
AA: 2.350E7
Relative Abundance
Time (min)
m/z
174.9827
@hcd25
108.9909
C3F3O⁻
– COF2
C4F5O2⁻
C3F5⁻
130.9929
– COO
156.9440
109.9948
174.9825
50.9315
59.0137
69.9570
78.9592

(D)


TP170_CTFE2, TP170_CTFE3, and TP170_CTFE4
Formula: C4H3F3O4
Confidence level: 1
Chromatograms
Sample
Reference standard
[M-H]⁻ = 170.9905
Relative Abundance
Time (min)
MS2
@hcd25
MS2 in sample
C3HF2O2⁻
106.99498
C3H2F3O2⁻
127.00130
C4H2F3O4⁻
170.99144
–HF
–COO
Intensity [counts] (10³)
106.99500
127.00144
170.99068
MS2 in reference standard
m/z

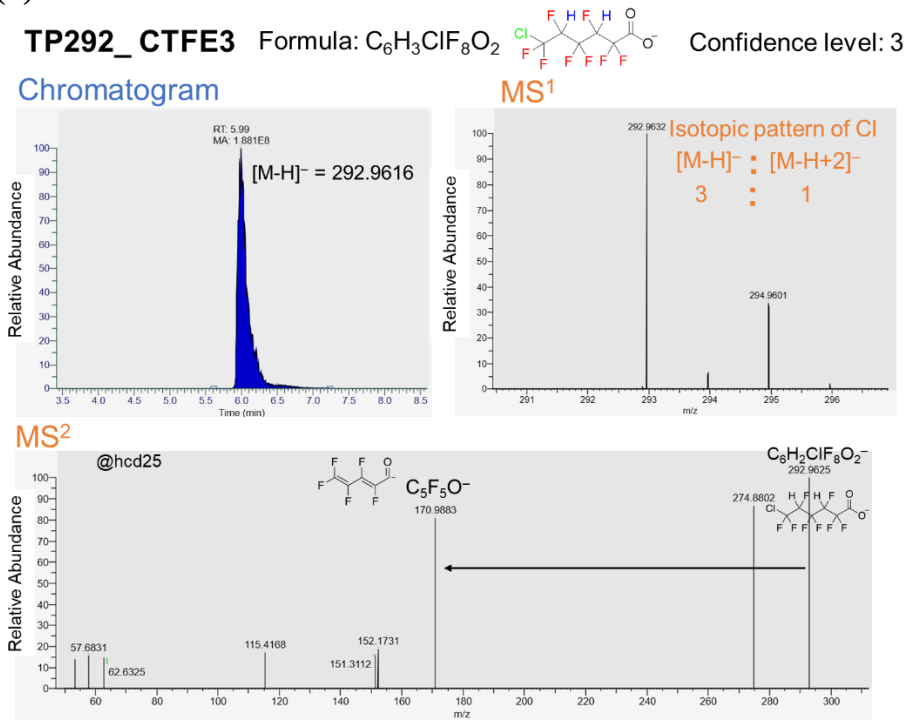
(E)

TP326(1)_ CTFE3 and TP326(2)_ CTFE3 Formula: $C_6H_2Cl_2F_8O_2$

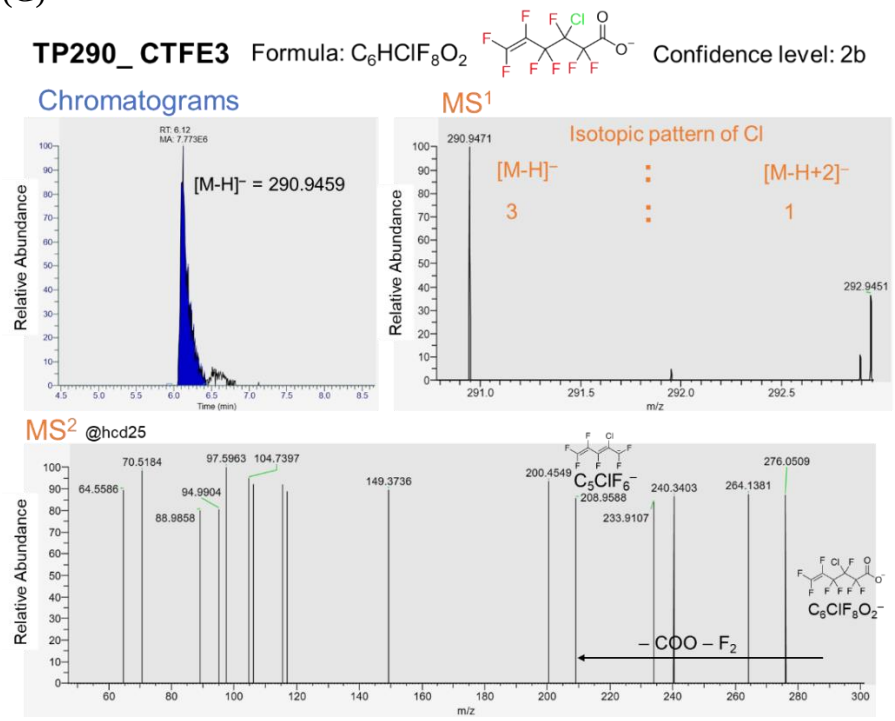


(F)

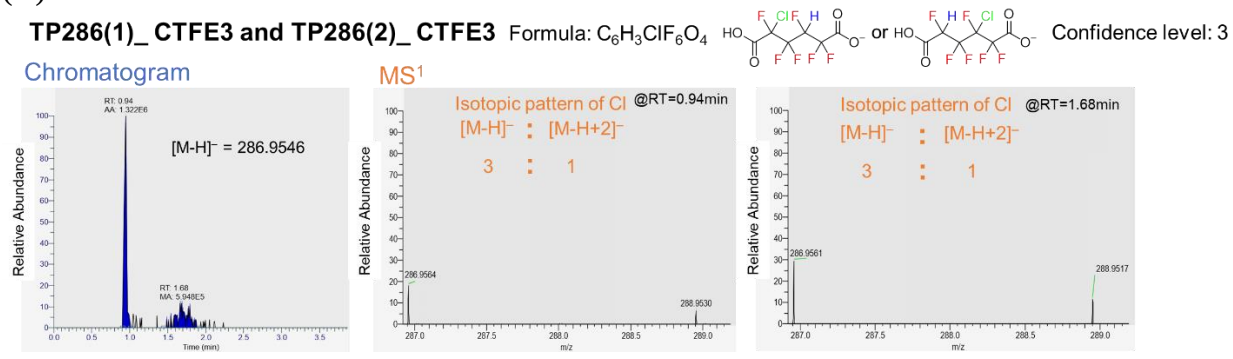
TP292_ CTFE3 Formula: $C_6H_3ClF_8O_2$ Confidence level: 3



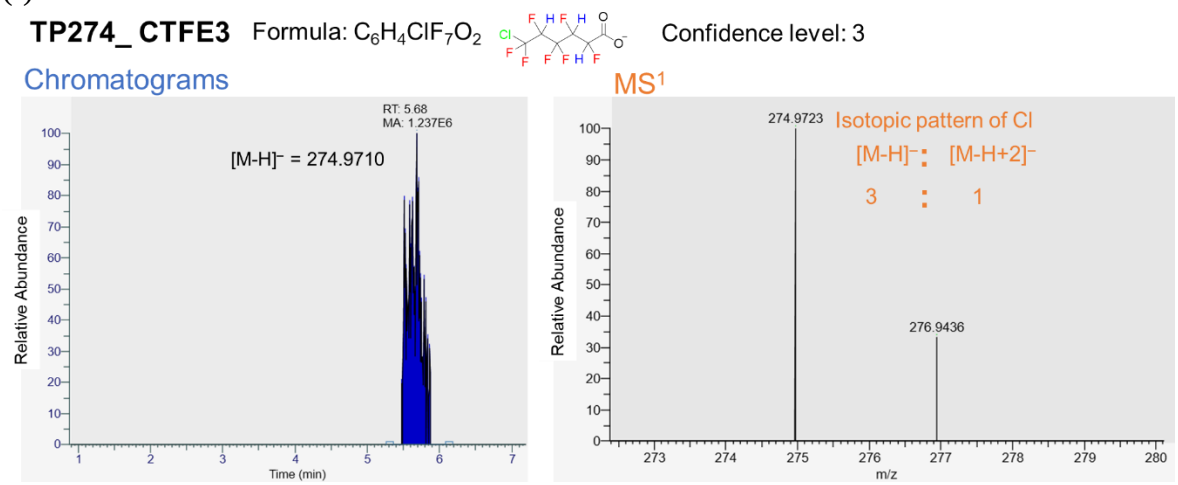
(G)


TP290_ CTFE3 Formula: $C_6HClF_8O_2$
Confidence level: 2b
Chromatograms
MS1
Isotopic pattern of Cl
[M-H]⁻ : [M-H+2]⁻
3 : 1
[M-H]⁻ = 290.9459
290.9471
292.9451
MS2 @hcd25
70.5184
64.5586
94.9904
88.9858
97.5963
104.7397
149.3736
200.4549
$C_5ClF_6^-$
208.9588
233.9107
240.3403
264.1381
276.0509
$C_6ClF_8O_2^-$
– COO – F_2
Relative Abundance
m/z
Time (min)

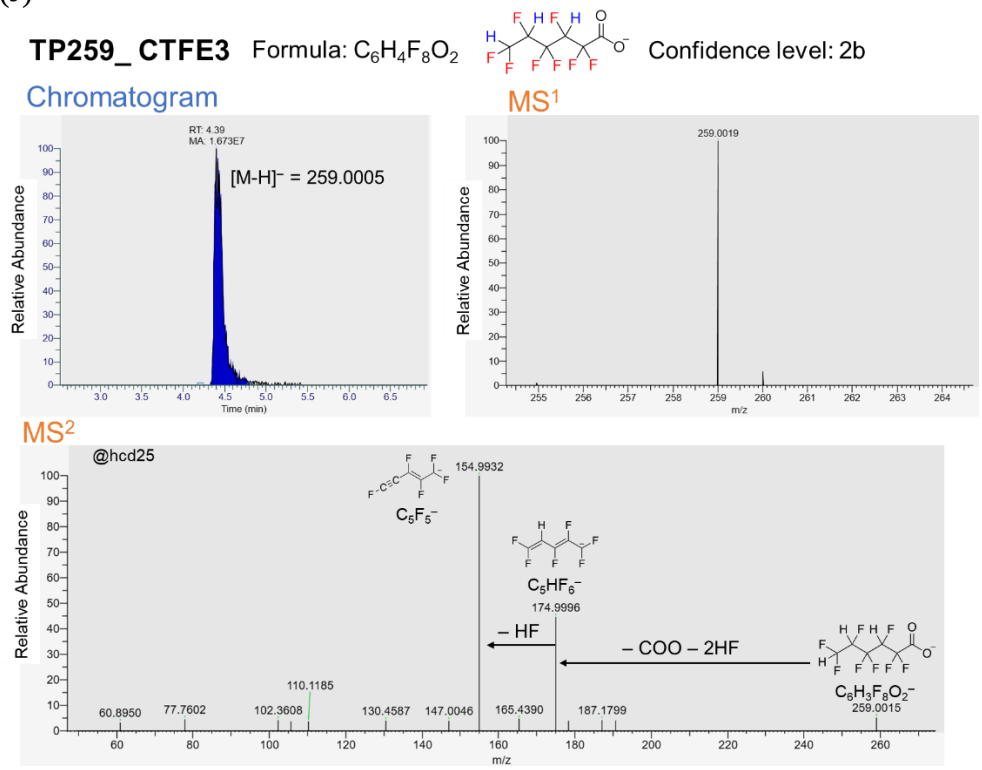
(H)


TP286(1)_ CTFE3 and TP286(2)_ CTFE3 Formula: $C_6H_3ClF_6O_4$
or
Confidence level: 3
Chromatogram
MS1
[M-H]⁻ = 286.9546
Isotopic pattern of Cl @RT=0.94min
[M-H]⁻ : [M-H+2]⁻
3 : 1
286.9564
288.9530
Isotopic pattern of Cl @RT=1.68min
[M-H]⁻ : [M-H+2]⁻
3 : 1
286.9561
288.9517
Relative Abundance
m/z
Time (min)

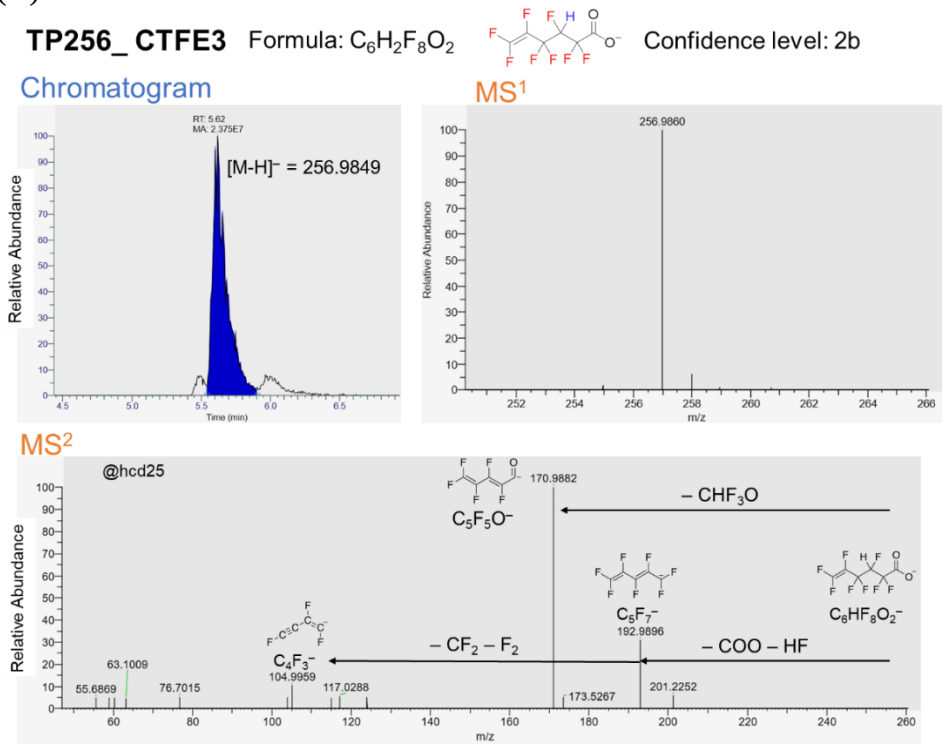
(I)


TP274_ CTFE3 Formula: $C_6H_4ClF_7O_2$
Confidence level: 3
Chromatograms
MS1
[M-H]⁻ = 274.9710
Isotopic pattern of Cl
[M-H]⁻ : [M-H+2]⁻
3 : 1
274.9723
276.9436
Relative Abundance
Time (min)
m/z

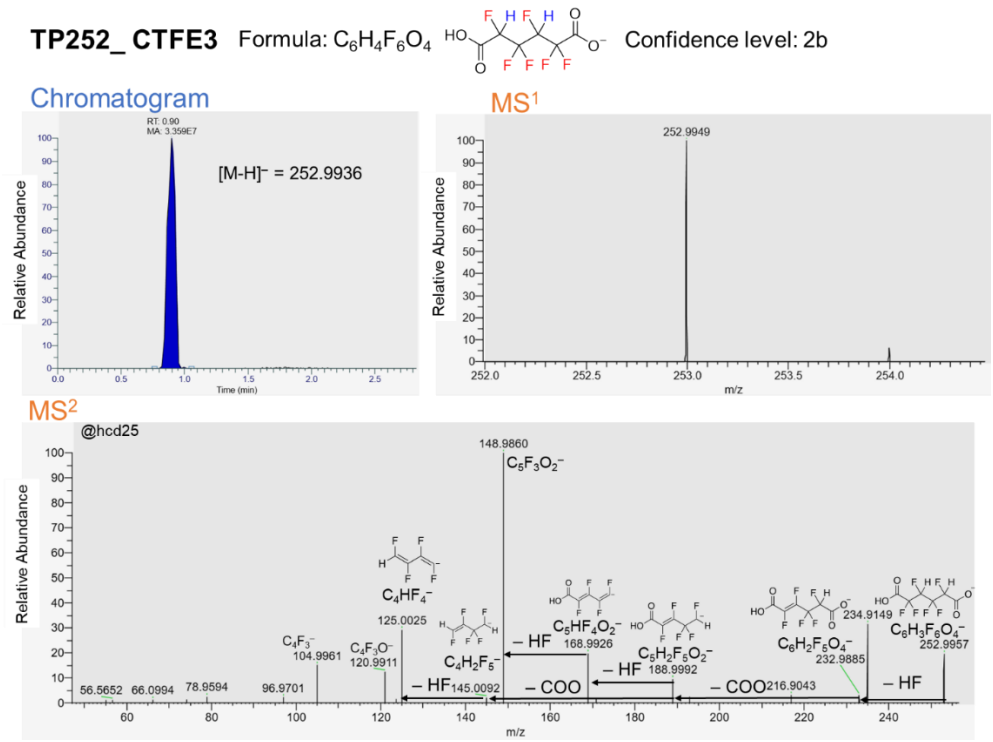
(J)


TP259_ CTFE3 Formula: $C_6H_4F_8O_2$
Confidence level: 2b
Chromatogram
RT: 4.39
MA: 1.673E7
[M-H]⁻ = 259.0005
Relative Abundance
Time (min)
MS1
259.0019
m/z
MS2
@hcd25
154.9932
$C_5F_5^-$
$C_5HF_6^-$
174.9996
– HF
– COO – 2HF
$C_6H_3F_8O_2^-$
259.0015
110.1185
60.8950
77.7602
102.3608
130.4587
147.0046
165.4390
187.1799

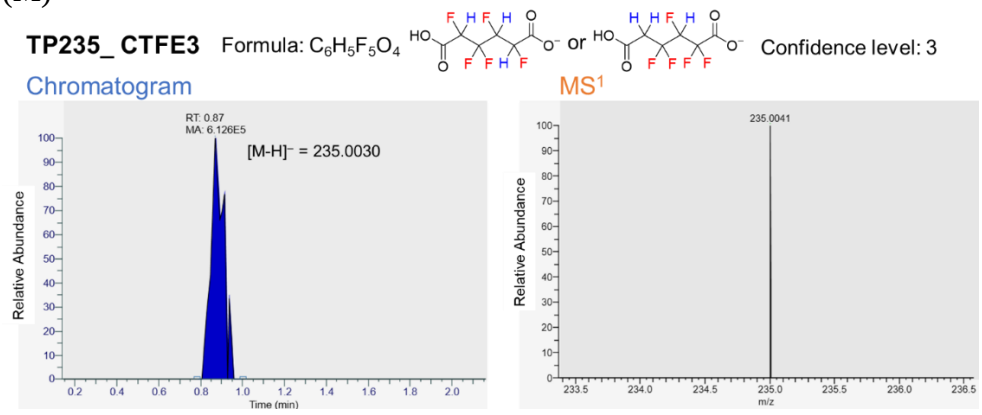
(K)


TP256_ CTFE3 Formula: $C_6H_2F_8O_2$
Confidence level: 2b
Chromatogram
RT: 5.62
MA: 2.375E7
[M-H]⁻ = 256.9849
Relative Abundance
Time (min)
MS1
256.9860
m/z
MS2
@hcd25
170.9882
$C_5F_5O^-$
– CHF_3O
$C_5F_7^-$
192.9896
$C_6HF_8O_2^-$
$C_4F_3^-$
104.9959
– CF_2 – F_2
– COO – HF
63.1009
55.6869
76.7015
117.0288
173.5267
201.2252

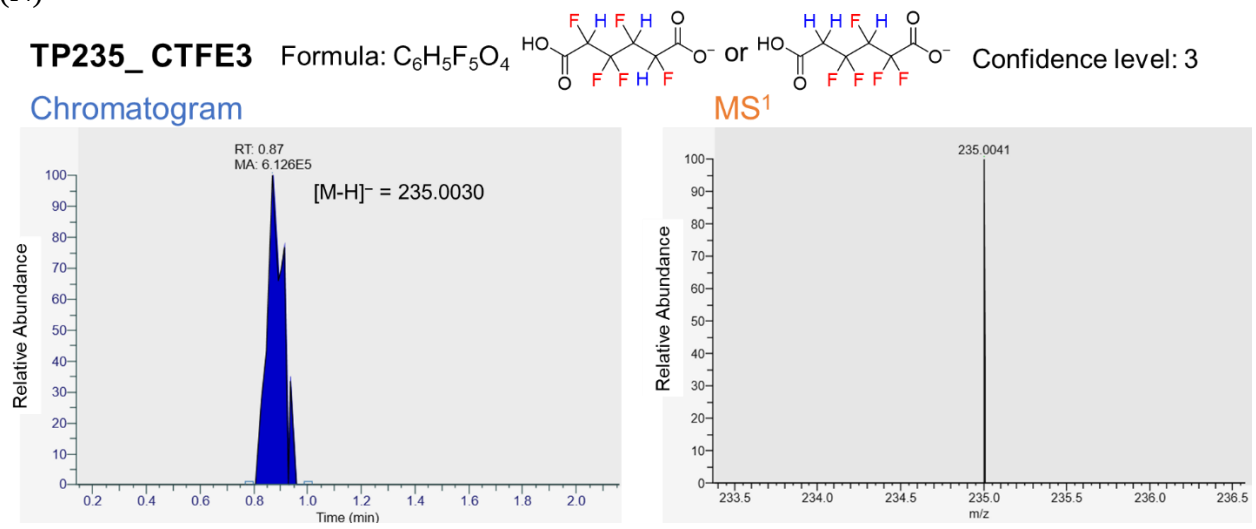
(L)


TP252_ CTFE3 Formula: C6H4F6O4
Confidence level: 2b
Chromatogram
RT: 0.90
MA: 3.359E7
[M-H]⁻ = 252.9936
Relative Abundance
Time (min)
MS1
252.9949
m/z
MS2
@hcd25
148.9860
C5F3O2⁻
C4HF4⁻
125.0025
C4F3⁻
104.9961
C4F3O⁻
120.9911
C4H2F5⁻
C5HF4O2⁻
168.9926
C5H2F5O2⁻
188.9992
C6H2F5O4⁻
232.9885
C6H3F6O4⁻
252.9957
234.9149
– HF
– HF145.0092
– COO
– HF
– COO216.9043
– HF
56.5652
66.0994
78.9594
96.9701

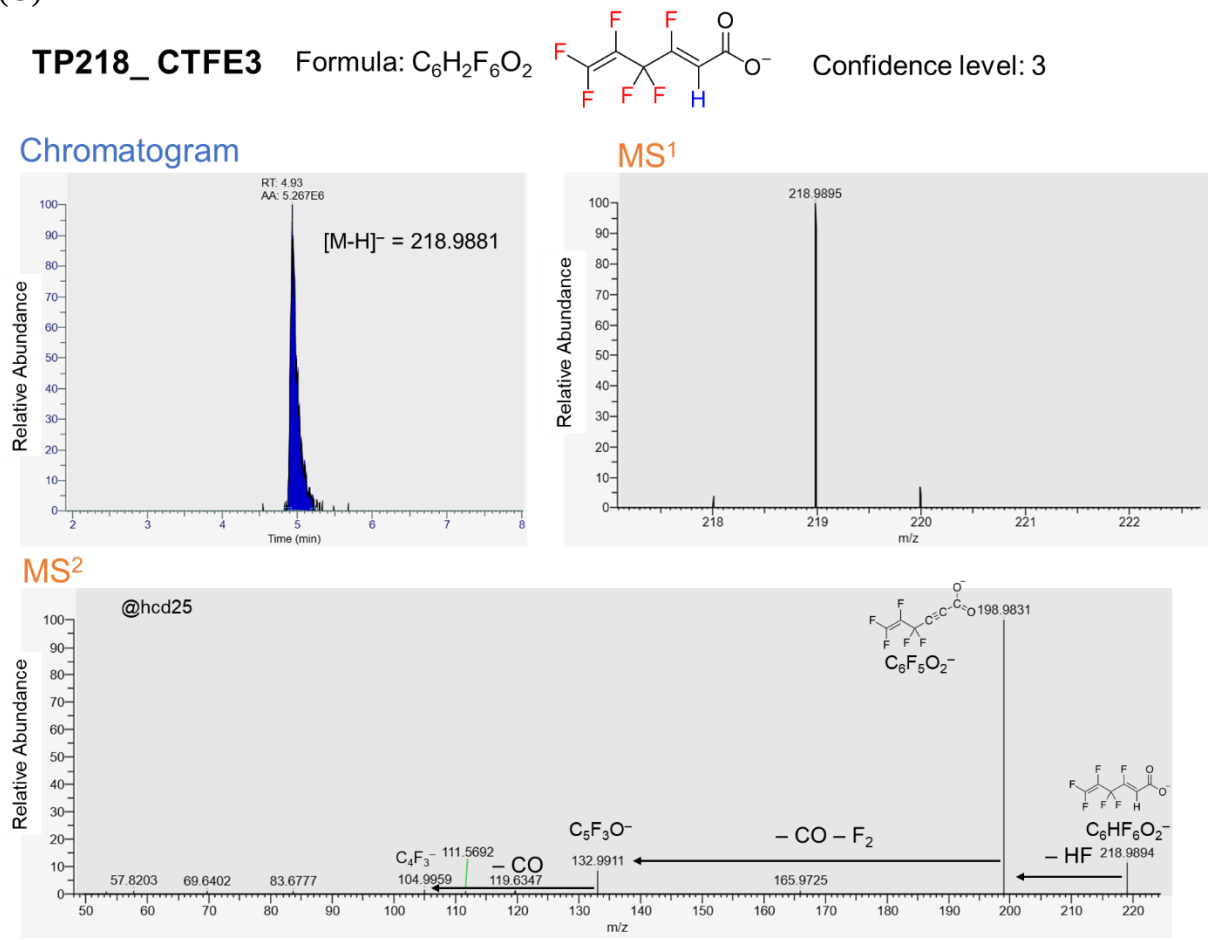
(M)


TP235_ CTFE3 Formula: C6H5F5O4
or
Confidence level: 3
Chromatogram
RT: 0.87
MA: 6.126E5
[M-H]⁻ = 235.0030
Relative Abundance
Time (min)
MS1
235.0041
m/z

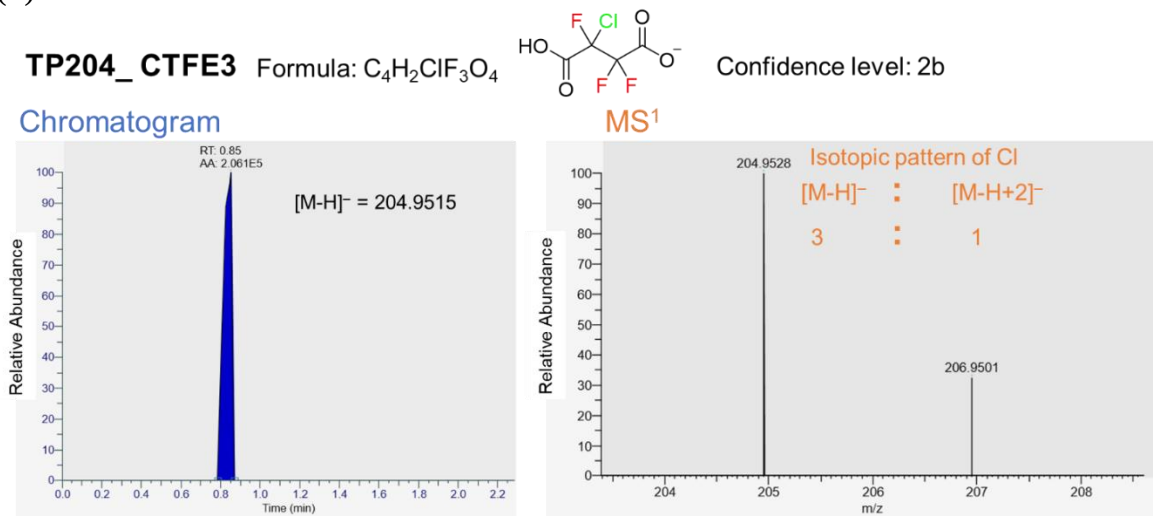
(N)


TP235_ CTFE3
Formula: $C_6H_5F_5O_4$
or
Confidence level: 3
Chromatogram
MS1
RT: 0.87
MA: 6.126E5
[M-H]⁻ = 235.0030
Relative Abundance
Time (min)
235.0041
m/z

(O)


TP218_ CTFE3
Formula: $C_6H_2F_6O_2$
Confidence level: 3
Chromatogram
MS1
RT: 4.93
AA: 5.267E6
[M-H]⁻ = 218.9881
Relative Abundance
Time (min)
218.9895
m/z
MS2
@hcd25
198.9831
$C_6F_5O_2^-$
$C_5F_3O^-$
132.9911
– CO – F_2
$C_6HF_6O_2^-$
– HF 218.9894
$C_4F_3^-$ 111.5692
104.9959
– CO
119.6347
165.9725
57.8203
69.6402
83.6777

(P)



(Q)

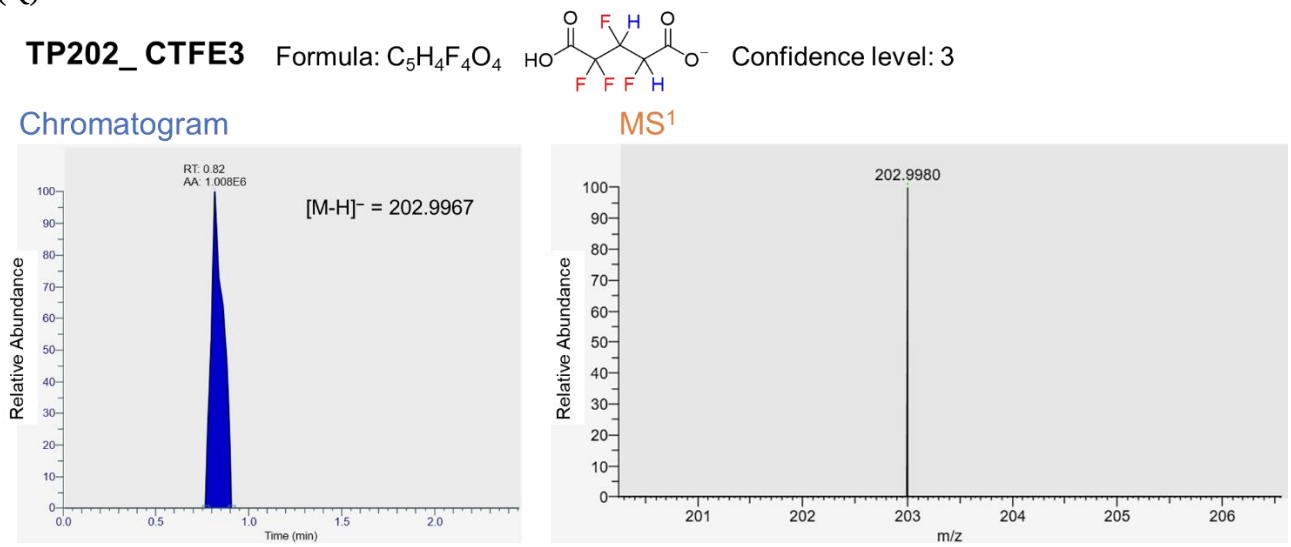
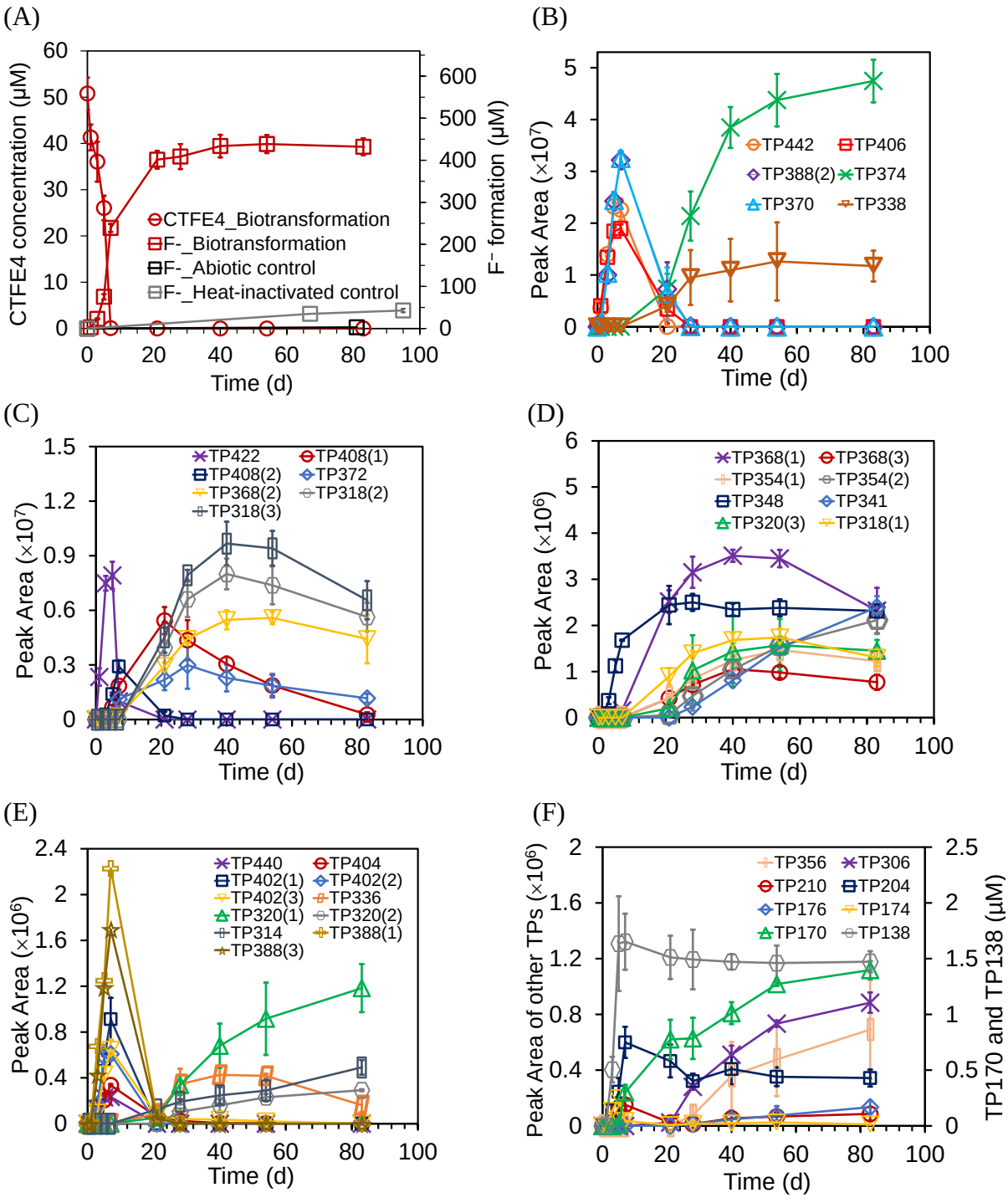
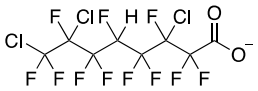
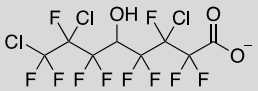
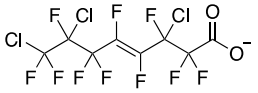
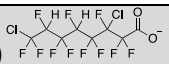
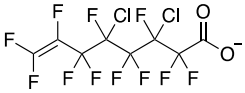
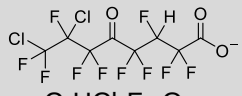
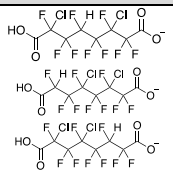
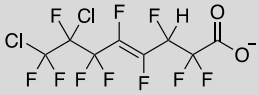
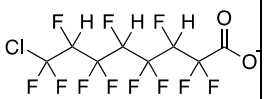
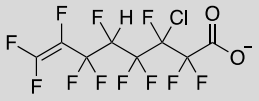
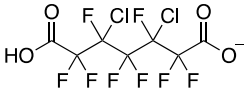
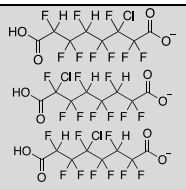
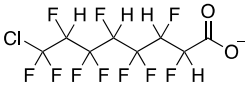
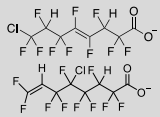
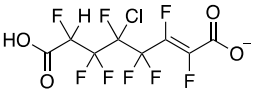
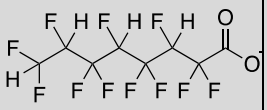
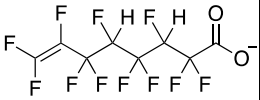
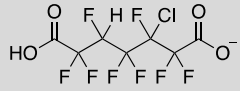
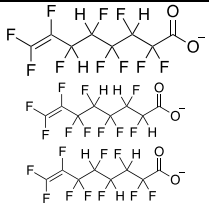


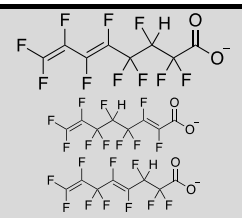
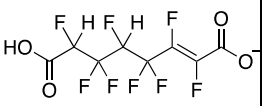
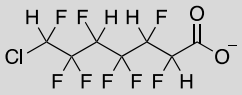
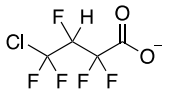
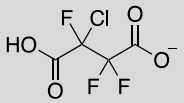
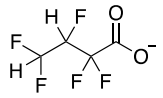
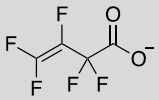
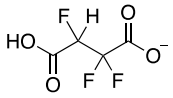
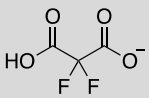
Figure S14. TP identification of **CTFE2** (A – D) and **CTFE3** (E – Q) (Note: for TPs with Cl-substitution, the isotopic pattern of Cl (^{35}Cl:^{37}Cl = 3:1 in nature[4, 5]) in MS^1 was used for the structure elucidation).


(A)
CTFE4 concentration (μM)
F⁻ formation (μM)
Time (d)
CTFE4_Biotransformation
F-_Biotransformation
F-_Abiotic control
F-_Heat-inactivated control
(B)
Peak Area (×10⁷)
Time (d)
TP442
TP406
TP388(2)
TP374
TP370
TP338
(C)
Peak Area (×10⁷)
Time (d)
TP422
TP408(1)
TP408(2)
TP372
TP368(2)
TP318(2)
TP318(3)
(D)
Peak Area (×10⁶)
Time (d)
TP368(1)
TP368(3)
TP354(1)
TP354(2)
TP348
TP341
TP320(3)
TP318(1)
(E)
Peak Area (×10⁶)
Time (d)
TP440
TP404
TP402(1)
TP402(2)
TP402(3)
TP336
TP320(1)
TP320(2)
TP314
TP388(1)
TP388(3)
(F)
Peak Area of other TPs (×10⁶)
TP170 and TP138 (μM)
Time (d)
TP356
TP306
TP210
TP204
TP176
TP174
TP170
TP138

(G)

TP	Structure Formula ([M-H]⁻)	Actual m/z ([M-H]⁻, error <5ppm)	RT (min)	Confidence level[a]	Change from parent compound ($C_8Cl_4F_{11}O_2^-$)
TP442	 $C_8HCl_3F_{11}O_2^-$	442.8872	7.15	3	–Cl+H
TP440	 $C_8H_2Cl_3F_{10}O_3^-$	440.8915	6.70	3	(–Cl+H) + (–F+OH)
TP422	 $C_8Cl_3F_{10}O_2^-$	422.8810	7.21	3	–ClF
TP408(1) TP408(2)	(1)  (2) $C_8H_2Cl_2F_{11}O_2^-$	408.9262	(1) 6.78 (2) 6.93	3	(–Cl+H) + (–Cl+H)
TP406	 $C_8Cl_2F_{11}O_2^-$	406.9105	7.13	3	–2Cl
TP404	 $C_8HCl_2F_{10}O_3^-$	404.9149	6.38	3	(–Cl+H) + (–Cl+OH) + (–HF)
TP402(1) TP402(2) TP402(3)	 $C_8H_2Cl_2F_9O_4^-$	402.9192	(1) 4.17 (2) 4.24 (3) 4.40	3	(–Cl+H) + (–Cl+OH) + (–HF) + (–F+OH)
TP388(1) TP388(2) TP388(3)	 $C_8HCl_2F_{10}O_2^-$	388.9199	(1) 6.87 (2) 6.99 (3) 7.09	3	(–Cl+H) + (–ClF)
TP374[b]	 $C_8H_3ClF_{11}O_2^-$	374.9651	6.55	3	(–Cl+H) + (–Cl+H) + (–Cl+H)

TP372	 $C_8HClF_{11}O_2^-$	372.9495	6.75	3	(–2Cl) + (–Cl+H)
TP370	 $C_7HCl_2F_8O_4^-$	370.9130	6.99	2b	(–2Cl+2OH) + (–2HF) + (–F+OH) + (–CO)
TP368(1)[b] TP368(2)[b] TP368(3)[b]	 $C_8H_3ClF_9O_4^-$	368.9582	(1) 3.00 (2) 3.49 (3) 3.91	3	(–Cl+H) + (–Cl+OH) + (–HF) + (–F+OH) + (–Cl+H)
TP356[b]	 $C_8H_4ClF_{10}O_2^-$	356.9746	6.39	3	(–Cl+H) +(–Cl+H) + (–ClF) + (+2H)
TP354(1)[b] TP354(2)[b]	 $C_8H_2ClF_{10}O_2^-$	354.9589	(1) 6.73 (2) 6.79	3	(–Cl+H) +(–Cl+H) + (–ClF)
TP348[b]	 $C_8H_2ClF_8O_4^-$	348.9519	3.12	3	(–Cl+H) + (–Cl+OH) + (–HF) + (–F+OH) + (–ClF)
TP341[b]	 $C_8H_4F_{11}O_2^-$	341.0041	5.91	2b	(–Cl+H) +(–Cl+H) + (–Cl+H) + (–Cl+H)
TP338[b]	 $C_8H_2F_{11}O_2^-$	338.9885	6.41	2b	(–Cl+H) + (–Cl+H) + (–2Cl)
TP336	 $C_7H_2ClF_8O_4^-$	336.9519	6.55	2b	(–2Cl+2OH) + (–2HF) + (–F+OH) + (–CO) + (–Cl+H)
TP320(1)[b] TP320(2)[b] TP320(3)[b]	 $C_8H_3F_{10}O_2^-$	320.9979	(1) 5.96 (2) 6.07 (3) 6.16	3	(–Cl+H) + (–ClF) + (–2Cl) + (2H)

TP318(1)[b] TP318(2)[b] TP318(3)[b]	 $C_8HF_{10}O_2^-$	318.9822	(1) 6.32 (2) 6.50 (3) 6.66	3	(–2Cl) + (–ClF) + (–Cl+H)
TP314[b]	 $C_8H_3F_8O_4^-$	314.9909	1.62	3	(–Cl+OH) + (–HF) + (–F+OH) + (–2Cl+2H) + (–ClF)
TP306[b]	 $C_7H_4ClF_8O_2^-$	306.9778	5.87	3	(–Cl+OH) + (–HF) + (–F+OH) + (–ClF) + (2H) + (–Cl+H) + (–COO)
TP210	 $C_4HClF_5O_2^-$	210.9591	4.62	3	(–Cl+OH) + (–HF) + ($–C_4ClF_5O_2$) + O + (–Cl+H)
TP204[b]	 $C_4HClF_3O_4^-$	204.9521	0.85	2b	(–2Cl+2OH) + (–2HF) + (–F+OH) + ($–C_4ClF_5O_2$) + O
TP176[b]	 $C_4H_2F_5O_2^-$	176.9980	1.72	3	(–Cl+OH) + (–HF) + ($–C_4ClF_5O_2$) + O + (–2Cl+2H)
TP174	 $C_4F_5O_2^-$	174.9826	2.59	3	(–Cl+OH) + (–HF) + ($–C_4ClF_5O_2$) + O + (–2Cl)
TP170[b]	 $C_4H_2F_3O_4^-$	170.9911	0.85	1	(–2Cl+2OH) + (–2HF) + (–F+OH) + ($–C_4ClF_5O_2$) + O +(–Cl+H)
TP138[b]	 $C_3HF_2O_4^-$	138.9848	0.83	1	(–Cl+OH) + (–HF) + ($–C_5Cl_3F_8$) + OH

[a]Confidence level 1: confirmed structure using reference compounds based on RT, MS^1, and MS^2 (if available); level 2b: probable structure proposed without available reference compounds or library spectrum data and with no other possible structures according to the experimental information including the parent compound information and the MS2 fragmentation profile; level 3: tentative structures, where insufficient information for one exact structure only (e.g., positional isomers).
[b]Stable or semistable TPs, which were accumulated or very slowly transformed.

(H)

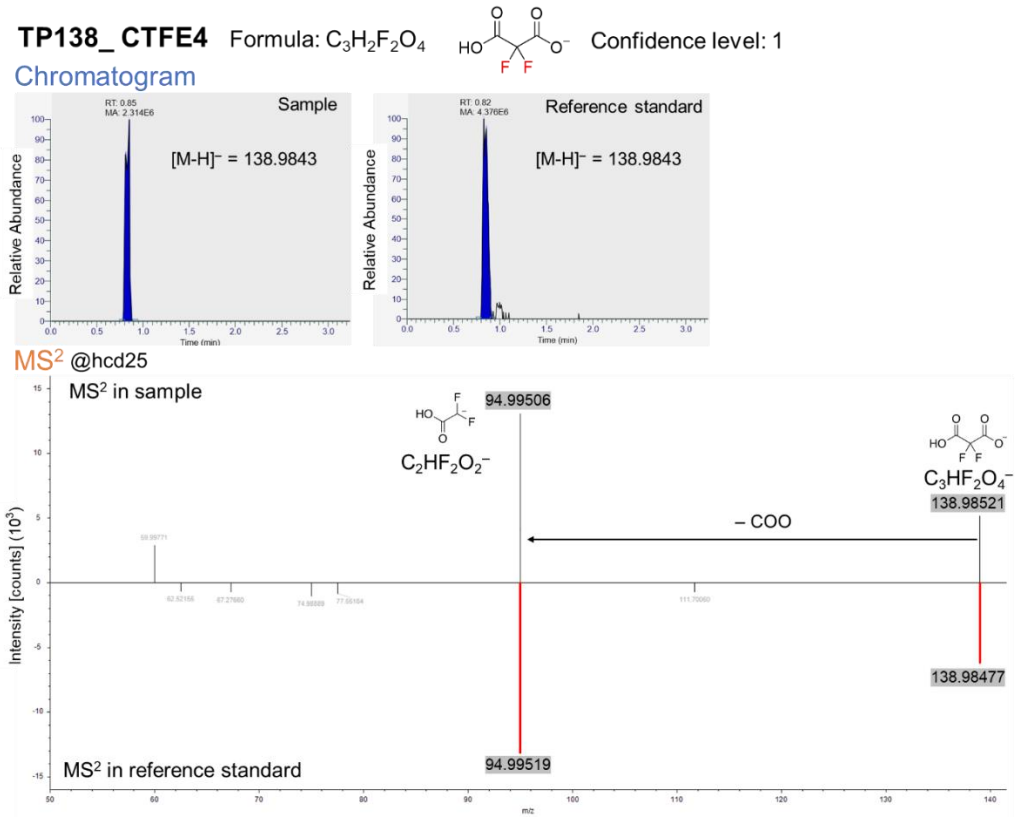


Figure S15. (A) Removal and fluoride formation of **CTFE4** in the biotransformation group, the fresh-medium abiotic control, and the heat-inactivated control; (B – F) the formation of **CTFE4** TPs; (G) the list of **CTFE4** TPs; (H) the confirmation of TP138 of **CTFE4** (See **Figure S14D** for the confirmation of TP170).

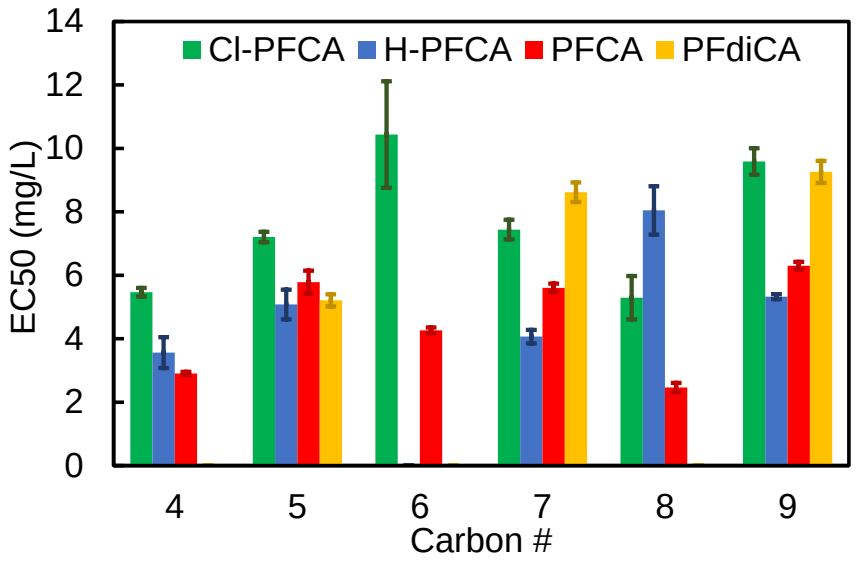


Figure S16. EC_{50} values of Cl-PFCA (for Cl-PFCAs, C# = 4, 6, and 8 represent **CTFE2**, **CTFE3**, and **CTFE4**, respectively, and C# = 5, 7, and 9 represent Cl-terminal PFCA; H-PFCA: H-terminal PFCA).

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