



Natural Chlordecone Degradation Revealed by Numerous Transformation Products Characterized in Key French West Indies Environmental Compartments

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Complete List of Authors:	<p>Chevallier, Marion; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Della-Negra, Oriane; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Chaussonnerie, Sébastien; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Barbance, Agnès; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Muselet, Delphine; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Lagarde, Florian; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Darii, Ekaterina; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Ugarte, Edgardo; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Lescop, Ewen; Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Sud Fonknechten, Nuria; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Weissenbach, Jean; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Woignier, Thierry; Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille Université, CNRS, IRD, Avignon Université and IRD, UMR IMBE, Campus Agro Environnemental Caraïbes Gallard, Jean-François; Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Sud Vuilleumier, Stéphane; Génétique Moléculaire, Génomique, Microbiologie (GMGM), Université de Strasbourg, UMR 7156 CNRS Imfeld, Gwenaél; Laboratory of Hydrology and Geochemistry of Strasbourg (LHyGeS), Université de Strasbourg, UMR 7517 CNRS/EOST Le Paslier, Denis; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay Saaïdi, Pierre-Loïc; Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry, Université Paris-Saclay</p>



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5 *Marion L. Chevallier^{a,1}, Oriane Della-Negra^{a,1}, Sébastien Chaussonnerie^{a,2}, Agnès Barbance^{a,2},*
6 *Delphine Muselet^{a,2}, Florian Lagarde^a, Ekaterina Darii^a, Edgardo Ugarte^a, Ewen Lescop^b,*
7 *Nuria Fonknechten^a, Jean Weissenbach^a, Thierry Woignier^c, Jean-François Gallard^b, Stéphane*
8 *Vuilleumier^d, Gwenaél Imfeld^e, Denis Le Paslier^{a,3}, Pierre-Loïc Saaidi^{a,3}.*

9

10 ^aGénomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université d'Évry,
11 Université Paris-Saclay, Evry, France.

12 ^bInstitut de Chimie des Substances Naturelles. CNRS - UPR 2301 Bâtiment 27, 1 avenue de la
13 Terrasse 91198 Gif-sur-Yvette Cedex, France.

14 ^cInstitut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix
15 Marseille Université, CNRS, IRD, Avignon Université and IRD, UMR IMBE, Campus Agro
16 Environnemental Caraïbes B. P. 214 Petit Morne, 97235, Le Lamentin, Martinique, France.

17 ^dGénétique Moléculaire, Génomique, Microbiologie (GMGM), Université de Strasbourg, UMR
18 7156 CNRS, 28 rue Goethe, 67000 Strasbourg Cedex, France.

19 ^eLaboratory of Hydrology and Geochemistry of Strasbourg (LHyGeS), Université de Strasbourg,
20 UMR 7517 CNRS/EOST, 1 Rue Blessig, 67084 Strasbourg Cedex, France.

21 ¹M.C. and O.D.N. contributed equally

22 ²S.C., A.B. and D.M. also contributed equally

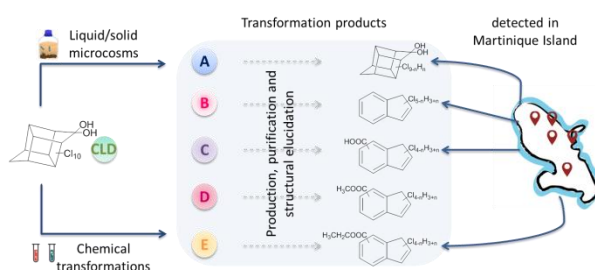
23 ³To whom correspondence may be addressed: Pierre-Loïc Saaïdi, or Denis Le Paslier, E-Mail:
24 plsaaïdi@genoscope.cns.fr or denis@genoscope.cns.fr.

25

26

27 ABSTRACT

28 A large part of the well-established Persistent Organic Pollutants is composed of organochlorine
29 pesticides that are still poisoning our environment. Among them, the insecticide chlordecone
30 shares with mirex a particularly recalcitrant perchlorinated bishomocubane structure. Its
31 production and application has caused long-term environmental pollutions in the James River
32 area (US) and in the French West Indies (FWI) that resulted in acute human health issues and
33 social crisis. Chlordecone is considered virtually non-biodegradable in the environment mainly
34 due to absence of transformation products (TP) and high levels of chlordecone concentration
35 even after decades of prohibition. Here, 19 TP were identified by untargeted GC-MS and LC-
36 HRMS analyses of a series of anoxic degradation experiments, and classified into five distinct
37 families. Chemical synthesis and NMR spectroscopy allowed structural elucidation of 19 so far
38 unidentified TP. Among the 19 TP detected in key FWI compartments (soils, river, mangrove
39 and sediments), 2,4,5,6,7-pentachloroindene showed similar concentration levels as chlordecone.
40 Microcosm experiments on three FWI soils confirmed intrinsic potential for chlordecone natural
41 degradation. These results not only challenge the paradigm of chlordecone persistence over
42 periods of centuries, but also raise the overlooked issue of extensive pollution of soil and aquatic
43 ecosystems by chlordecone TP worldwide.



47 KEYWORDS. Chlordane | persistent organic pollutant | pollution | biodegradation |
48 structure elucidation | transformation product

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50

51 INTRODUCTION

52 The organochlorine (OC) insecticides have been applied extensively for pest control in
53 agriculture worldwide for decades. Their use has gradually been prohibited since the 1970s
54 because of their biological biomagnification, high toxicity and long persistence in the
55 environment. Many OC synthesized from hexachlorocyclopentadiene[1] belong to the
56 Stockholm convention list of Persistent Organic Pollutants (POPs), ie aldrin, dieldrin, endrin,
57 chlordan, heptachlor, mirex, chlordane and endosulfans. Among them, the insecticides
58 chlordane and mirex, that has been commonly used as fire-retardant, represent a particular
59 class of OC as they both arise from hexachlorocyclopentadiene dimerization[1, 2] and share a
60 number of characteristics such as low value of molecular orbital energy, high hydrophobicity and
61 low K_{ow} [3]. Chlordane is also known as a TP of mirex[4] and kelevan, another insecticide
62 mainly used in Europe[5]. The resulting highly stable perchlorinated bishomocubane structure
63 renders such OC extremely recalcitrant to environmental conditions. While chlordane
64 formulations produced in the US (as Kepone) and Brazil (as Curlone) have been mainly used in
65 the Caribbean area, in Central America and Africa[6], mirex utilization has been reported in
66 North America[7, 8], Latin America, Europe[6] and more recently in China[9]. Finally, by its
67 direct use or its in situ formation, chlordane is virtually present all over the world and has been
68 even detected in the coral reefs of the French Polynesia[10], we thus chose it as a pertinent and
69 challenging pollutant from the hexachlorocyclopentadiene-based OC.

70 To date, chlordane has already led to two environmental disasters: (1) contamination of the
71 environment nearby the Hopewell chlordane production plant (U.S.) in 1975 causing massive
72 pollution of the James River over 100 miles for decades and acute exposition of workers[11]. On
73 a wider scale, extensive use of chlordane from 1972 to 1993 in banana plantations of the

French West Indies (FWI) has resulted in a long-term pollution of environmental compartments and the local food chain (soils, water resources, farmed animals, fish)[6, 12, 13]. Chronic exposure to chlordane has resulted in human health problems [13-26] and subsequent socio-economic issues for the FWI and the James River area [27-30].

Following application, chlordane sorbs onto soil and sediments particles, especially organic rich FWI matrices. The commonly admitted paradigm of absolute chlordane persistence for decades/centuries in the FWI based on a leaching model[31]has been recently confirmed by two studies suggesting only marginal degradation in tropical soils, if any [32, 33]. Generally, POPs pollution is assessed and followed by environmental monitoring of the parent molecule, with restricted efforts dedicated to the possible TP in situ formed and their consequences as exemplified for aldrin. In the case of chlordane, several bacterial degradation experiments suggested two main TP families evidenced by GC-MS [34, 35] [36, 37]. Despite extensive analytical studies on chlordane contamination in the FWI[6], the only chlordane derivatives hitherto detected in environmental samples, i.e. chlordaneol and 8-monohydrochlordane, turn to be contaminants from former commercial formulations. However, as only targeted analyses were applied, presence of other TP cannot be excluded.

In this context, we intended (i) to clear the exact structure of the previously reported chlordane TP and (ii) to extend our knowledge on the structural diversity of chlordane TP possibly formed in laboratory and in the field in order to question the paradigm of chlordane environmental non-biodegradability. To address these issues, we applied a dual GC-MS- and LC-HRMS-based approach on both microbiological and chemical degradation processes and also on FWI soil, water and sediments samples.

97 MATERIAL AND METHODS

98 Chemicals and Analytics

99 All used chemicals, analytical and purification methods are described in detail in Supporting
100 Information (SI) (Supporting Methods).

101 Extraction of natural samples

102 Field sites and soil sampling

103 Each sample was collected in Martinique Island. Soils (andosol, nitisol, ferralsol) coming from
104 the vicinity of the “Montagne Pelée” volcano and bed sediments were sampled from the 0–30 cm
105 layer and conserved in glass box; whereas river and mangrove water were collected from the 0–
106 30 cm above the surface with glass bottles (Table S4).

107 Procedure for sample chemical extraction

108 Each sample was extracted in duplicate. For soils and sediments: to 4 g of crude sample, 15 mL
109 of milli Q water was added, followed by acidification to pH 1 with HCl (1 M) and vortex. After
110 decantation the supernatant was extracted with DCM (12 x 15 mL) and the pellet was washed
111 twice with DCM (15 mL). For river and mangrove water: 0.75 L of water sample was acidified to
112 pH 1 with HCl (1 M) and extracted with DCM (12 x 350 mL). In each case, organic layers were
113 pooled, concentrated *in vacuo* and analyzed in duplicated injection using GC-MS (in
114 hexane/acetone 85:15) and LC-MS (in 10 mM NH₄OAc buffer /MeCN 4:1) protocols.

115 Production and purification of chlordecone main TP

116 TP productions were monitored by GC-MS and LC-MS.

117 TP A1

118 To a solution of chlordecone (100 mg, 2.0×10^{-4} mol, 1 eq) in degassed water (300 mL) were
119 added sodium sulfide (2.2 g, 2.8×10^{-2} mol, 140 eq.) and vitamin B₁₂ (40 mg, 2.9×10^{-5} mol, 0.15
120 eq.). The reaction was carried out under N₂ atmosphere at room temperature (rt) for 30 h. It was
121 then quenched with HCl (6 M) to pH 4.0 and degassed with N₂ for one hour to evacuate
122 hydrogen sulfide. The aqueous reaction mixture was extracted with DCM (3 x 200 mL) and the
123 combined organic phases were concentrated *in vacuo* to give a brown crude oil. A first
124 purification step was performed using a Combi Flash® Companion® Elution at a flowrate of 40
125 mL/min using heptane as solvent A and a mixture of DCM/(CH₃)₂CO (1:1; V/V) as solvent B.
126 Elution started at 0% B for 7 min, followed by a linear gradient reaching 50% B within 5 min, a
127 second linear gradient reaching 100% B within 3 min and remained 15 min at 100% B. Fractions
128 containing A1 (from 8 to 29 min) were pooled and concentrated under reduced pressure. A
129 second purification step was performed using a preparative HPLC system. Isocratic elution made
130 of tetrahydrofuran/MeCN/(NH₄)₂CO₃ buffer (10 mM, pH 9.5) (29:29:42; V/V/V) was applied at
131 a flowrate of 20 mL/min. Fractions containing A1 (retention time of 9 min) were pooled,
132 acidified to pH 3 with HCl (6 M), extracted 3 times with DCM, and concentrated under reduced
133 pressure, to give the title compound A1 (46.4 mg; 9.8×10^{-5} mol; 50%) as a white solid. All NMR,
134 GC-MS and LC-HRMS analyses for A1 are available in Figures S16, S23, S27-S28.

135 TP B1, B2 and B3/B4

136 To a solution of chlordecone (200 mg, 3.9×10^{-4} mol, 1 eq.) and vitamin B₁₂ (60 mg, 5.8×10^{-5} mol,
137 0.15 eq.) in degassed H₂O/MeOH 64:36 (250 mL), was added titanium(III) citrate (50 mL, 3.3
138 $\times 10^{-3}$ mol, 8.4 eq.) basified to pH 12.7 with NaOH (3 M). Reaction mixture was stirred under N₂

atmosphere at room temperature for 80 min and, quenched by contact with O₂. Extraction with pentane (5 x 250 mL) followed by concentration under reduced pressure gave rise to a white crude solid. B1, B2 and B3/B4 were purified using a preparative HPLC system. Isocratic elution (MeCN/H₂O 7:3; v/v) was applied at a flowrate of 25 mL/min. Fractions containing B1 (retention time of 42 min), B2 (retention time of 28 min) and B3/B4 (retention time of 32 min) were pooled separately, extracted 3 times with pentane and concentrated under reduced pressure. Each compound was then purified through PLC (Preparative Layer Chromatography; Merck, PLC Silica gel, 1 mm, F₂₅₄, 20 x 20) (cyclohexane/EtOAc 9:1); B1, B2 and B3/B4 retardation factors were respectively 0.78, 0.68 and 0.88. B1 (32.6 mg; 1.2 10⁻⁴ mol; 30%), B2 (3.0 mg; 1.2 10⁻⁵ mol; 3%) and B3/B4 (4.1 mg; 1.3 10⁻⁵ mol; 4%) were finally obtained as white solids. All NMR, GC-MS and LC-HRMS analyses for B1, B2, B3-B4 are available in Figures S17-S18, S23, S29-S45. ¹³C-enriched B1 synthesis and purification protocols are described in SI (Supporting Methods).

TP C1, C2, C3 and C4

To a solution of chlordecone (100 mg, 2.0 10⁻⁴ mol, 1 eq.) and vitamin B₁₂ (40 mg, 2.9 10⁻⁵ mol, 0.3 eq.) in degassed water/acetone 3:1 (300 mL), was added zero-valent iron (2 g, 3.6 10⁻² mol, 182 eq.). The reaction mixture was carried out in a glovebox (N₂/H₂, 98:2, V/V) at room temperature for 2 months. After quenching by contact with O₂, acetone was evaporated under reduced pressure, the resulting aqueous phase was acidified to pH 1 (HCl 6 M) and extracted with DCM (10 x 100 mL). The combined organic layers were finally concentrated under reduced pressure. A first purification step was carried out using preparative HPLC. Elution was performed at a flowrate of 25 mL/min using NH₄OAc buffer (10 mM ; pH 7 adjusted with NH₄OH) as solvent A and MeCN as solvent B. Elution started at 12% B for 4 min, followed by a linear

gradient reaching 29% B within 11 min, followed by a second linear gradient reaching 50% B within 15 min and a third one reaching 100% B within 6 min. Fractions containing respectively C1-C2 and C3-C4 (from 21.5 to 23 and 25 to 28 min respectively) were pooled and MeCN was removed under reduced pressure. The resulting aqueous layers were basified to pH 10 with K_2CO_3 (10 mM, pH 10), washed with 50 ml of hexane/EtOAc 95:5 (V/V), acidified to pH 3 with HCl (12 M) and extracted three times with hexane/EtOAc 95:5 (V/V). The combined final organic layers were concentrated under reduced pressure to give the title compounds C1-C2 (3.0 mg; $1.0 \cdot 10^{-5}$ mol; 5%) and C3-C4 (1.5 mg; $5.7 \cdot 10^{-6}$ mol; 3%) as white solids. All NMR and LC-HRMS analyses for C1-C2 and C3-C4 are available in Figures S24-S25, S46-S56.

Production, and purification protocols of transformation products D1, D2, D3 and D4 are described in SI (Supporting Methods). All NMR and LC-HRMS are available in Figures S19-S20, S23, S57-S66.

Anoxic microbiological experiments

Liquid/solid microcosm experiments and analytical protocols are described in SI (Supporting Methods).

RESULTS AND DISCUSSION

Diversity of chlordecone TP from anoxic biodegradation

Both early[34, 35] and recent[36, 37] studies of bacterial chlordecone degradation suggested two main TP families: hydrochlordecones from reductive dechlorination reactions (family A), and

183 polychloroindenes formed after ring-opening and elimination steps (family B) (**Figure 1**).
184 Another study with the Archaeon *Methanosarcina thermophila* suggested quantitative
185 chlordecone conversion into unknown polar and nonpolar metabolites based on silica gel thin-
186 layer chromatography (TLC)[38]. The technique used for hydrochlordecones and
187 polychloroindenes TP produced by consortium 86[36] was applied here to confirm the
188 uniqueness of TP formed during bacterial and archaeal chlordecone degradation. Retardation
189 factor (Rf) values for polychloroindenes corresponded to those of nonpolar TP in
190 *Methanosarcina thermophila* cultures[38], while hydrochlordecones behaved like chlordecone
191 (Figure S1). Hence, hydrochlordecones cannot represent the previously reported unknown
192 archaeal polar TP and define a novel, so far undescribed, family of chlordecone TP.

193 Further novel chlordecone TP during incubation of chlordecone in presence of consortium 86
194 and *Citrobacter* sp. 86 were searched for using measurement of chloride concentration, GC-MS,
195 GC-FID, and LC-MS-Orbitrap. After 250 days of incubation, chloride concentrations increased
196 by 18.8 ± 0.9 mg/L (i.e., 5.5 ± 0.3 Cl atoms per chlordecone molecule) in consortium 86
197 cultures, and by 19.7 ± 1.7 mg/L (i.e., 5.8 ± 0.5 Cl atoms per chlordecone molecule) in
198 *Citrobacter* sp. 86 cultures, corresponding to complete chlordecone transformation (**Figure 1**
199 and Figure S13). Chloride recovery indicated the occurrence of other, so far undetected highly
200 dechlorinated TP, in addition to previously reported main TP B₁ (C₉Cl₅H₃) and A₁
201 (C₁₀Cl₉H₃O₂)[36].

202 While GC-FID analysis confirmed that volatile metabolites A1 and B1 occurred in all microbial
203 experiments (Figure S14), four new polar chlorinated TP (Ci, i=1,2,3,4) of generic formula
204 C₁₀Cl_{4-n}O₂H_{4+n} (n=0,1) were also identified in all chlordecone-degrading microcosms using LC-
205 ESI-MS-Orbitrap in negative mode (**Figure 1** and Figures S24-S25). TLC analysis initially

indicated a high match with unknown polar TP[38]. Tandem mass spectrometry using collision induced dissociation (CID) (LC-MS² and LC-MS³) then showed that the four TP followed similar fragmentation patterns with characteristic CO₂ loss. The UV-visible absorption profiles of all TP Ci (i=1,2,3,4) were close to those reported and tentatively assigned to pentachloroindenes[39] (Figure S2).

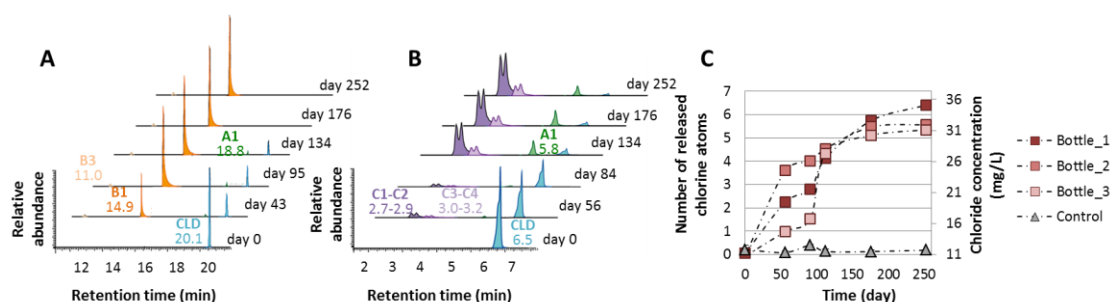


Figure 1. TP production during chlordecone degradation by *Citrobacter* sp. 86 (A) GC-MS full scan analysis during chlordecone degradation by *Citrobacter* sp. 86 and detection of CLD (blue), A1 (green), B1 (orange) and B3 (yellow) TP. (B) TP production during chlordecone degradation by *Citrobacter* sp. 86 using LC-MS-Orbitrap analysis and detection of CLD (blue), A1 (green), C1 (purple), C2 (purple), C3 (magenta) and C4 (magenta) TP Extract ion chromatograms for quasimolecular ion $[M-H]^-$ of $m/z = 506.6797$; 472.7187 ; 296.8852 ; 260.9271 (C) Chloride concentration and average number of released chlorine atoms per consumed chlordecone molecule during chlordecone degradation by *Citrobacter* sp. 86.

In parallel, laboratory microcosms with contaminated FWI soils (typically from 0.1 to 30 mg chlordecone/kg of dry soil) were incubated for three years under N₂/H₂ atmosphere at room temperature and in the dark to evaluate the capacity of native FWI soil microbiotes to degrade residual chlordecone (Figure S3). For each condition (soil/liquid medium), several replicates were collected over time using a sacrificial approach to evaluate produced chlordecone TP. All analyzed samples contained several chlordecone TP among which 10 new derivatives.

227 Chlordecol, a known chlordecone contaminant in former commercial formulations that is present
228 in FWI soils at low level[40], was also systematically found.

229 The GC-FID technique turned out to be inappropriate due to high background from soil samples
230 and low chlordecone concentration. Successive liquid-liquid extractions were thus combined to
231 concentrate chlordecone TP prior to GC-MS and LC-ESI-MS-Orbitrap analyses (Tables S2-S3).
232 Newly identified chlordecone TP were classified into two families (named D and E), with
233 respective generic formula $C_{11}Cl_{4-n}O_2H_{6+n}$ ($n=0, 1$) and $C_{12}Cl_{4-n}O_2H_{8+n}$ ($n=0, 1$). MS spectra
234 from GC-MS analysis revealed the likely occurrence of methyl and ethyl ester moieties for
235 families D and E, respectively. A series of fragments ($C_9Cl_xH_y^+$, $x=5, 4, 3, 2$; $y=4, 3, 2$) were
236 common to compounds Bi ($i=1, 2, 3$), Dj ($j=1, 2, 3, 4$) and Ek ($k=1, 2, 3, 4$), suggesting a
237 shared aromatic polychloroindene core ring system, with methyl- and ethyl-polychloroindene-
238 carboxylate structures for D and E families, respectively (**Figure 2** and Figures S19-S22).
239 Altogether, 19 chlordecone TP were discovered from liquid and solid microcosm experiments
240 using the untargeted analytical approach (Figure S15).

241

242 **Production and purification of chlordecone TP**

243 The major chlordecone TP detected in biodegradation experiments (**Figure 2**) were synthesized
244 and purified on a (sub)milligram scale to achieve complete structural elucidation using NMR
245 spectroscopy. Chlordecone degradation by bacterial consortia and *Citrobacter* sp. 86 takes
246 several weeks to several months[36] and only allowed partial TP elucidation (A1, B1 and Ci
247 ($i=1,2,3,4$)). We reasoned that different chemical degradation conditions may favor sufficient
248 production of each type of TP for subsequent purification. For example, photochemical

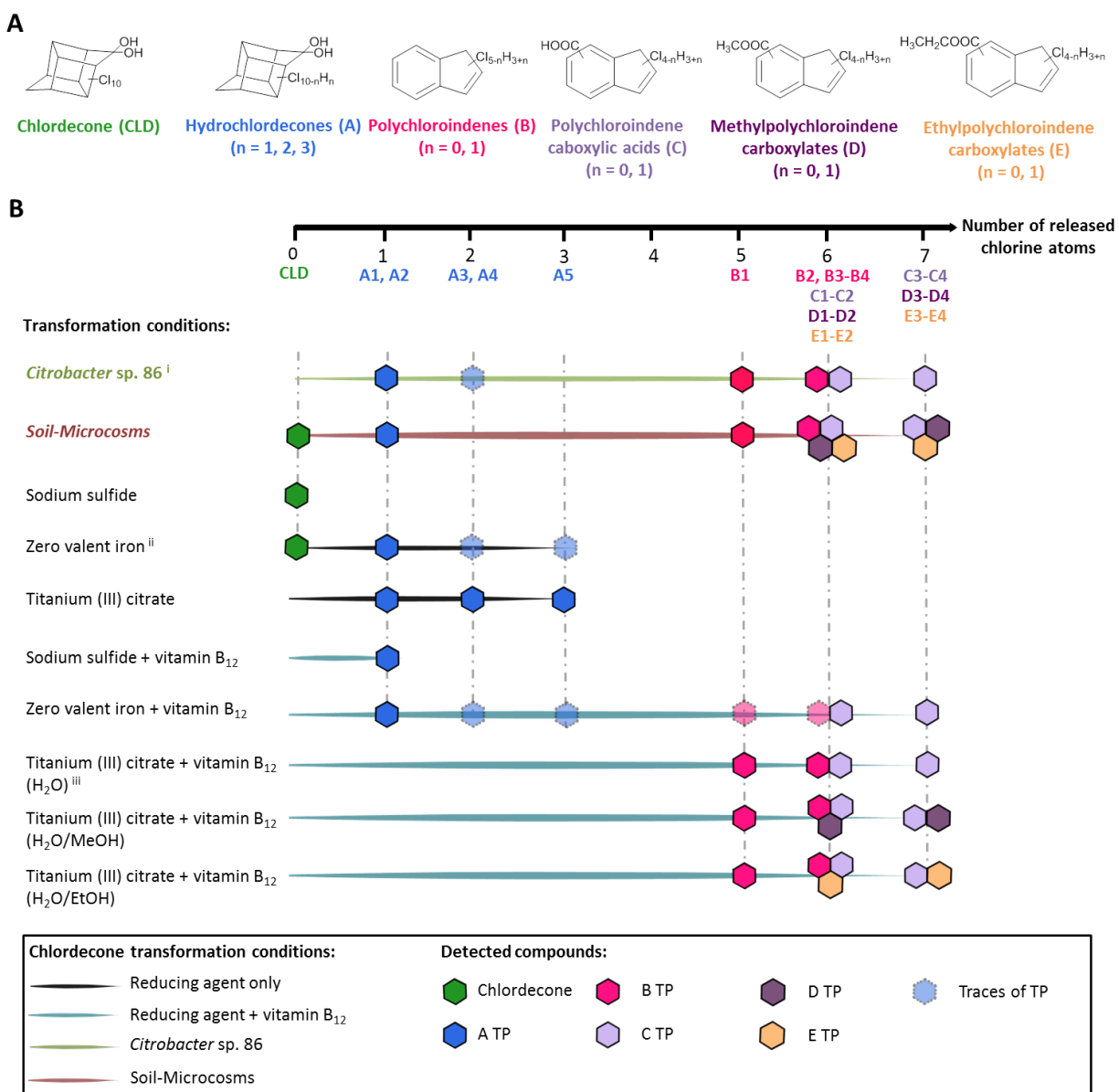
degradation led to mono- and di-hydrochlordecone derivatives, each with about 1% yield[41]. All other conditions under which chlordecone degradation was reported required a reducing agent, either alone[42] or in combination with vitamin B₁₂[37-39, 43-45].

Thus, monitoring of chlordecone degradation by GC-MS and LC-MS was used here to determine suitable conditions for selective formation of metabolites from each TP family, including the three novel TP families identified here for the first time. Addition of reducing agents and metal complexes previously applied to enhance mirex degradation[46] were tested (Table S1). Application of degradation conditions (acetoin, vitamin B₁₂ and methanol) previously shown to selectively lead to the formation of polychloroindenes[39] also resulted in C and D families of TP. Similarly, two other protocols (vitamin B₁₂, ethanol and either zero-valent zinc or 1,4-dithiothreitol) reported to result in a tentatively assigned pentachloroindene as a unique chlordecone TP[43] also yielded lower chlorinated indenenes, as well as several TP of the C and E families (Figure S7).

Eventually, we selected 5 protocols of TP production among the 21 tested degradation conditions (Table S1, **Figure 2**). For instance, titanium citrate and zero-valent iron without vitamin B₁₂ selectively resulted in selective formation of hydrochlordecones. Most importantly, the combination of sodium sulfide and vitamin B₁₂ specifically led to the production of monohydrochlordecone A1 (in up to 50% yield after purification). Polychloroindenes and polychloroindene carboxylic acids were formed at highest levels upon amendment with vitamin B₁₂ (**Figure 2** and Table S1). Worthy of note, tested reducing agents did not specifically favor TP from one of these two families, but systematically produced a mixture of B and C TP. Zero-valent iron may slightly enhance production of C family TP, whereas TP of the B family prevailed in assays with titanium citrate. On a preparative scale, addition of methanol

272 significantly improved the reproducibility of B family TP production. From the same
273 experimental batch, B1, B2 and B3 TP were isolated with yields up to 30%, 3% and 4%,
274 respectively. Compounds C1-C2 (5% yield) and C3-C4 (3% yield) were obtained as non-
275 separable pairs of isomers. Indeed, when a single Ci ($i = 1,2,3,4$) TP was isolated using
276 preparative HPLC, partial interconversion to its isomer was observed after evaporation,
277 demonstrating the existence of an equilibrium between pairs of isomers. Finally,
278 supplementation of reaction mixtures leading to B and C TP with either methanol or ethanol led
279 to production of either D or E TP. Purification of pairs D1-D2 and D3-D4 was achieved in 5%
280 and 3% yield, respectively.

281



282

(i) Chaussonnerie et al., 2016

(ii) Belghit et al., 2015

(iii) Chiu & Reinhard 1995; Lewis et al., 1996; Rodriguez-Garrido et al., 2004

Figure 2. Chlordecone TP (A) Five families of observed chlordecone TP (B) Chemical conditions for chlordecone degradation and released of one to seven chloride atoms. 8-monohydrochlordecone was assigned to A2. A3 and A4 correspond to dihydrochlordecones, and A5 to a trihydrochlordecone.

287

288 **Structural elucidation of chlordecone TP**

289 A1, the major hydrochlordecone observed in recent work[36] previously identified as either 9- or
290 10-monohydrochlordecone[42] and also formed after In Situ Chemical Reduction Daramend®
291 treatment of an FWI field[47], was identified here as 10-monohydrochlordecone. Indeed,
292 replacement of a chlorine atom by a hydrogen in chlordecone can only lead to four different
293 regioisomers, i.e. 6-, 8-, 9- or 10-monohydrochlordecones (Figure S5). The obtained ^{13}C NMR
294 spectrum for this TP showed six signals, indicating the presence of a conserved vertical plane of
295 symmetry in A1. To satisfy this feature, the hydrogen atom can only be positioned on carbon 10.

296 TP belonging to TP families B, C, D and E likely possess an indene core structure as assigned
297 previously to TP B1 following chemical conversion to commercially available indane[37]. The
298 same hydrogenation protocol for TP pairs C1-C2 and C3-C4 unambiguously resulted in the
299 formation of indane-4-carboxylic acid confirming that TP family C features a carboxyindene
300 aromatic ring system (Figure S8). Equilibria between pairs of Ci ($i=1,2,3,4$) corresponded to a
301 keto-enol mediated isomerization (Figure S12), as previously shown for 1*H*-indene-1-carboxylic
302 acid and 1*H*-indene-3-carboxylic acid in water[48]. TP C1, C2, C3 and C4 were assigned to
303 tetrachloroindene-4-carboxylic acid, tetrachloroindene-7-carboxylic acid, trichloroindene-4-
304 carboxylic acid and trichloroindene-7-carboxylic acid, respectively. Esterification of these pairs
305 of TP using methanol led to the formation of Di ($i=1,2,3,4$), while ethanol gave rise to Ej
306 ($j=1,2,3,4$) (Figure S6). This confirmed the presence of methyl- and ethyl-indene-carboxylate
307 structures and ester functions on the indene ring for Di ($i=1,2,3,4$) and Ej ($j=1,2,3,4$) TP, i.e.
308 either on carbon 4 or 7.

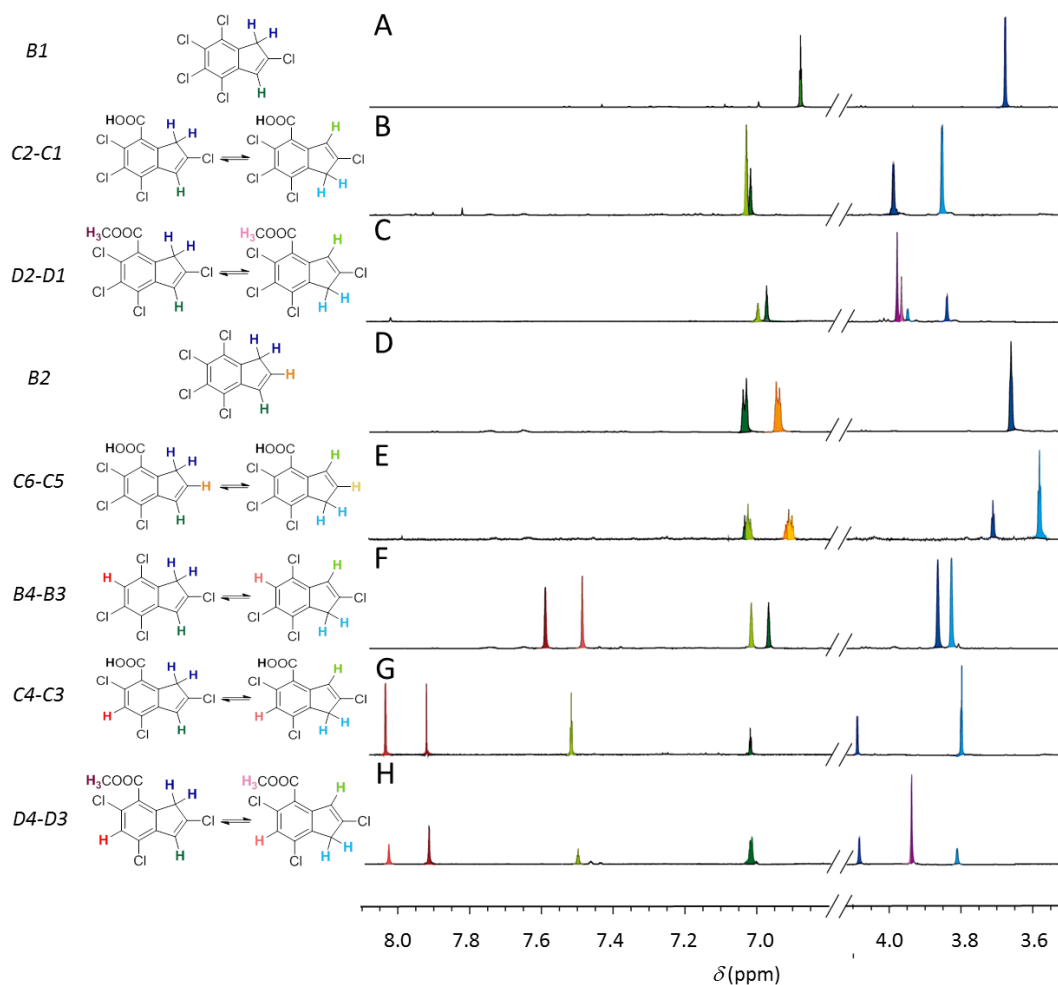


Figure 3. Relevant ^1H NMR spectra regions of selected indene-based TP (600 MHz). Data were recorded in $(\text{CD}_3)_2\text{CO}$ excepted for B1, recorded in CDCl_3 . The spectral regions $\delta 3.5\text{--}4.1$ ppm and $\delta 6.8\text{--}8.1$ ppm are shown for B1 (A), C1-C2 (B), D1-D2 (C), B2 (D), C5-C6 (E), B3-B4 (F), C3-C4 (G), D3-D4 (H).

Pentachloroindene B1 was then used as a reference compound for the 4 identified indene-based TP families (**Figure 3**) in subsequent NMR analyses since it was the first TP to have been elucidated by NMR. ^1H NMR and COSY spectra indicated an allylic domain at $\delta 3.7$ ppm (d, $J = 1.4$ Hz, 2 H) coupled to an aromatic proton at $\delta 6.87$ ppm (t, $J = 1.4$ Hz, 1 H). For indene

systems, a value of 1.4-Hertz indicates a 3J - or 4J -type coupling constant (Figure S26) leaving possibilities for a proton at carbon position 2, 3 or 7 for the structure of B1. Further NMR analyses of B1 (Figures S27-S33) did not allow to elucidate the proton position, so ^{13}C -enriched B1 was synthesized from commercially available $^{13}\text{C}_8$ -CLD to perform a ^{13}C - ^{13}C COSY experiment (Figure S35). Each carbon atom could thereby be linked to its direct carbon neighbors, and B1 could then be eventually unequivocally assigned to 2,4,5,6,7-pentachloro-1*H*-indene by way of a HSQC experiment (Figure S32).

TP C1-C2 and D1-D2 represented mixtures of isomers according to ^1H NMR and COSY experiments (**Figure 3** and Figures S48, S57). Comparison of ^1H and ^{13}C NMR spectra highlighted the similarity between TP B1, C1-C2, and D1-D2 (**Figure 3** and Figures S30, S47, S58), and all protons of C1-C2 and D1-D2 were unambiguously positioned on the five-membered ring moiety of the indene structure. Interestingly, the HMBC experiment of C1-C2 showed only one cross correlation peak between the most deshielded allylic protons and a carboxylate carbon atom from one of the two carboxyl groups (Figure S50). This set of ^1H and ^{13}C signals was thus assigned to 2,5,6,7-tetrachloro-1*H*-indene-7-carboxylic acid, i.e. C2, and the other set to 2,5,6,7-tetrachloro-1*H*-indene-4-carboxylic acid, i.e. C1 (**Figure 3**). The same deshielding effect was responsible for chemical shift differences for both ^1H and ^{13}C spectra between allylic signals of D1-D2, although no difference in HMBC spectrum was observed for D1-D2. This allowed to unequivocally assign the sets of ^1H and ^{13}C signals in these TP (**Figure 3** and Figures. S57-S61).

TP B2 and C5-C6 showed the same ^1H NMR pattern (i.e. one triplet in the allylic domain, and two doublets of triplets in the aromatic domain) that allowed them to be distinguished from B1, C1-C2 and D1-D2 (**Figure 3**). All corresponding NMR signals can be affiliated to the

cyclopentadiene part of the indene structure (Figure S26). Structures of B2, C5 and C6 TP were thus identified as 4,5,6,7-tetrachloro-1*H*-indene, 5,6,7-trichloro-1*H*-indene-4-carboxylic acid and 4,5,6-trichloro-1*H*-indene-7-carboxylic acid, respectively.

Even if tetrachloroindene B3 appeared as a single compound in GC-MS analysis, its ¹H NMR spectrum showed two similar sets of signals indicating two regioisomers, arbitrarily termed B3 and B4. ¹H NMR spectra of C3-C4 and D3-D4 strongly resembled that of B3-B4 (**Figure 3**). By analogy with 2,4,5,6,7-pentachloroindene B1, the methylene groups and the less deshielded aromatic protons of B3-B4, C3-C4 and D3-D4 were positioned on carbons 1 and 3 of the corresponding indene isomers. An additional coupling of 0.6-0.7 Hz was observed for B3, C3 and D3 only, i.e. between methylene protons on carbon position 1 and the most deshielded aromatic proton. In non-substituted indene, such a weak value is observed for methylene protons coupled with aromatic protons placed on carbons 6 and 7 (Figure S26). Therefore, the last aromatic proton of B3, C3 and D3 was positioned on either carbon 6 or 7. Worthy of note, indene isomerization formally transferred substituents from carbon position 4 to position 7, and from position 5 to position 6. The complexity of the ¹³C spectra did not permit complete elucidation. However HMBC and HSQC experiments indicated that both B3 and B4 featured a chlorine substituent at carbon position 4. Hence, B3 possessed a chlorine atom on carbon position 7, leaving only one plausible structure for B4, i.e. 2,4,5,7-tetrachloroindene. In the case of C3 and D3 (and as also observed for C4 and D4), a strong cross correlation peak in HMBC was systematically detected between the unassigned proton and the carbon from the carboxylic and ester functions. This excluded a ⁵J(H-C) coupling and thus discarded carbon position 7 for the remaining aromatic proton. Finally, C3-C4 and D3-D4 were assigned to 2,5,7-trichloro-1*H*-

indene-4-carboxylic acid, 2,4,6-trichloro-1*H*-indene-7-carboxylic acid, methyl 2,5,7-trichloro-1*H*-indene-4-carboxylate, methyl 2,4,6-trichloro-1*H*-indene-7-carboxylate, respectively.

As previously shown, E_i (i=1,2,3,4) TP were ethylated forms of C_j (j=1,2,3,4) carboxylates (Figure S6). Consequently, E1-E2 and E3-E4 correspond to ethyl 2,5,6,7-tetrachloro-1*H*-indene-4-carboxylate, ethyl 2,5,6,7-tetrachloro-1*H*-indene-7-carboxylate, ethyl 2,5,7-trichloro-1*H*-indene-4-carboxylate, ethyl 2,4,6-trichloro-1*H*-indene-7-carboxylate, respectively.

NMR analyses combined with chemical derivatization work further allowed to identify and structurally elucidate 19 TP for the first time (Figure S15). This significantly expands the list of fully characterized chlordecone TP, hitherto only comprised of chlordecol, 8-monohydrochlordecone and 2,8-dihydrochlordecone[41] and corrects previous findings[39, 42, 43].

Natural chlordecone degradation on the Martinique Island

In order to validate laboratory results and the discovery of novel chlordecone TP, several representative environmental compartments contaminated with chlordecone were sampled on Martinique Island, including Galion River basin (water, bed sediments and mangrove)[29, 49], andosol, nitisol and ferralsol soils[47, 50], as well as ashes and pumice stones (Table S4). Samples were analyzed using a different extraction protocol than the recommended ISO 17025 standard[31, 50, 51] in order to preserve their initial chemical composition. Specifically, the initial drying step was omitted for soil analysis to limit volatilization of B, D and E TP. All extracted samples were concentrated and analyzed using GC-MS and LC-HRMS in full-scan mode. Even if full-scan mode reduces intrinsic analytical sensitivity, it enhances the robustness

of TP assignment using isotopic patterns, and also allows detection of other chlorinated analogues. Concentrations of the most abundant TP (A1, chlordecol, B1, C1-C2 and C3-C4) were estimated using external calibration with purified TP standards (Figure S11). Significant variability of extraction efficiency in soils was observed, as a function of both TP structures and matrix nature, as shown for chlordecone and metabolite B1 in andosol and nitisol soils (Figure S10), so non-corrected concentration ranges were provided here. Chlordecone concentration in Galion River was in the range found in previous studies (0.1-2 $\mu\text{g/L}$ in Galion River[29]). Worthy of note, the concentration of B1 was in the same order of magnitude as chlordecone itself (0.1-2 $\mu\text{g/L}$) in both Galion River and nearby mangrove (**Figure 4** and Table S7). Moreover, B1 could be detected in bed sediments where chlordecone was absent. In soils, chlordecone concentrations were roughly 10-fold lower than previously reported typical values of dried samples (0.01-1 mg/kg compared to 0.8-5 mg/kg (dry weight) in Martinique[47, 50]). Chlordecone TP were amply found in solid matrices, with B1 and chlordecol being detected in all soil samples excepted in chlordecone-free nitisol 926. TP B1 fluctuated in the range between 0.05 to 5 mg/kg. In contrast, chlordecol was systematically around 0.05 mg/kg, in agreement with previous studies[40]. TP A1 was only observed at significant levels in the two andosol soils (0.05-1 mg/kg). C3-C4 TP, less chlorinated than C1-C2 TP, appeared more frequently and at a higher level, especially in one nitisol soil (above 1 mg/kg). The low concentration of 8-monohydrochlordecone (< 0.01 mg/kg) agreed with previous studies[33]. While methyl polychloroindenecarboxylates were absent of all samples, many other TP were detected at low level, including di-, tri-hydrochlordecones, tetrachloroindenes (B2, B3-B4), ethyl polychloroindenecarboxylates (E1-E2)(**Figure 4** and Table S7). The untargeted analytical approach also led to the discovery of a monohydrochlordecol derivative and two dichloroindene

carboxylic acids, which had never been observed before in laboratory biodegradation experiments (Table S7).

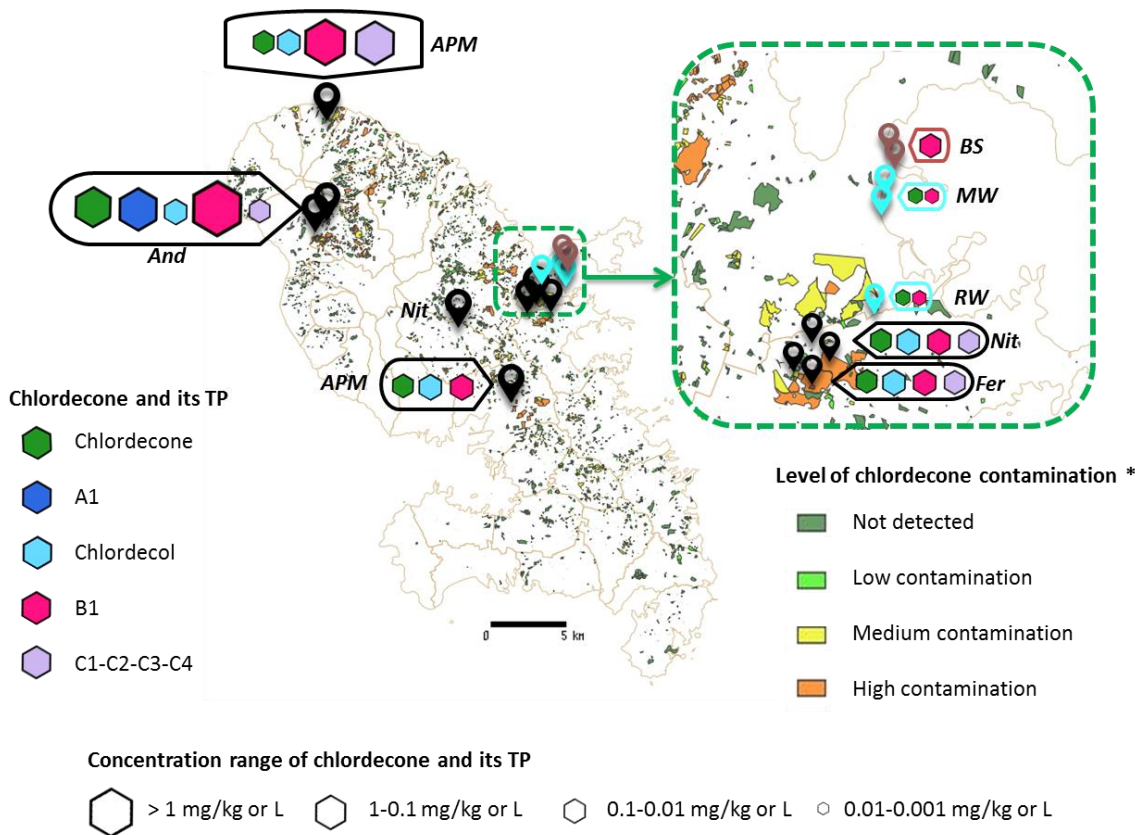


Figure 4. Distribution of samplings in Martinique and estimation of chlordecone and TP concentrations. Martinique map that was taken from http://carto.geomartinique.fr/1/layers/pref_chlordecone_analyse_sol_s_972.map, represents chlordecone contamination according to 2018 sampling campaign. *indicates chlordecone contamination level and were established by the previous quoted website. Samples were classified into type: andosol (And), nitisol (Nit), ferralsol (Fer), ashes and pumice stones (APM), bed sediments (BS), mangrove water (MW), river water (RW) and samplings were done in duplicate. If duplicates were closed, they showed the same chlordecone TP distribution. Samplings were done according to location icons (black ones represent soil samplings, brown: bed sediment and blue: water).

The only previously reported chlordecone derivatives were chlordecol and 8-monohydrochlordecone, present in FWI soils at much lower levels than chlordecone (0.03-0.5 mg/kg, and 0.05-0.2 mg/kg respectively)[33, 40]. Chlordecol and 8-monohydrochlordecone were actually known contaminants from commercial chlordecone, and were as such the only chlordecone derivatives included in targeted analyses until today. Even if our dual GC-MS- and LC-HRMS-based approach was intrinsically less sensitive, it allowed us to uncover in environmental samples the presence of numerous hidden chlordecone TP.

Altogether, this study shows that four of the five identified TP families occur in natural soil and water samples from Martinique, and include a total of 17 TP initially absent in chlordecone commercial formulations. Pentachloroindene B1 was the most prominent TP, with levels similar to those of chlordecone (**Figure 4**). Bacteria known to transform chlordecone into A, B and C families[36] were searched in the studied soil samples using Illumina sequencing of the 16S rRNA gene (V4-V5 amplicons) following DNA extraction. Only a very low frequency (1.4 to 2.10^{-4}) of *Citrobacter* affiliated sequences was found, and this in only three of the investigated samples (918, 919 & 920). Thus, based on current knowledge of bacterial diversity associated with chlordecone degradation, the overall bacterial contribution to chlordecone degradation in Martinique native soils may not be identified yet.

Finally, exploratory soil/liquid microcosms were set up under anoxic conditions with three representative FWI soils contaminated with chlordecone, (i.e. one andosol (914), one nitisol (918) and one ferralsol (919)). These experiments were further used to inoculate liquid culture supplemented with 40 mg/L of chlordecone. Degradation in all experiments and the concomitant formation of TP (Figure S9) confirmed the intrinsic widespread natural chlordecone degradation

in natural FWI soils, as well as the production of many novel chlordecone TP that had hitherto remained undetected.

Taken together, our results raise the issue of the real extent of chlordecone pollution in the FWI that goes beyond the parent molecule and includes a significant number of TP. Their structural diversity illustrates the hitherto unsuspected extant complexity of processes and pathways. Further mechanistic investigations will benefit from the large panel of purified and elucidated TP. As phylogenetic analyses suggest strong differences between the FWI bacterial community and the previous reported bacteria associated with chlordecone degradation, it cannot be excluded that other chlordecone TP yet undetected may also be formed under field conditions. The paradigm of absolute chlordecone persistence taken for granted for decades[31] now clearly appears obsolete and calls for setting new monitoring and risk priorities. Synthetic access to this new panel of chlordecone TP opens the doors to accurate environmental quantification protocols, toxicological studies and biodegradation assays. Last but not least, the original untargeted dual GC/MS- and LC/HRMS-based approach we used may also be applied to other POP such as mirex which shares the same perchlorinated bishomocubane structure to assess their global environmental biodegradability.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website at DOI:

466 AUTHOR INFORMATION

467 Corresponding Author

468 * E-mail: plsaaidi@genoscope.cns.fr or denis@genoscope.cns.fr.

469 Author Contributions

470 ¹M.C. and O.D.N. contributed equally471 ²S.C., A.B. and D.M. also contributed equally

472 Notes

473 The authors declare no competing financial interest.

474

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486

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