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Natural chlordecone degradation revealed by numerous transformation products characterized in key French West Indies environmental compartments

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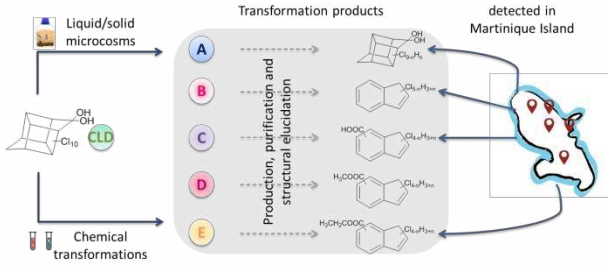
ABSTRACT

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



KEYWORDS. Chlordecone | persistant organic pollutant | pollution | biodegradation | structure elucidation | transformation product

INTRODUCTION

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

To date, chlordecone has already led to two environmental disasters: (1) contamination of the environment nearby the Hopewell chlordecone production plant (U.S.) in 1975 causing massive pollution of the James River over 100 miles for decades and acute exposition of workers[11]. On a wider scale, extensive use of chlordecone 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 chlordecone 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, chlordecone sorbs onto soil and sediments particles, especially organic rich FWI matrices. The commonly admitted paradigm of absolute chlordecone 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 chlordecone, several bacterial degradation experiments suggested two main TP families evidenced by GC-MS [34, 35] [36, 37]. Despite extensive analytical studies on chlordecone contamination in the FWI[6], the only chlordecone derivatives hitherto detected in environmental samples, i.e. chlordecol and 8-monohydrochlordecone, 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 chlordecone TP and (ii) to extend our knowledge on the structural diversity of chlordecone TP possibly formed in laboratory and in the field in order to question the paradigm of chlordecone 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.

96

MATERIAL AND METHODS

Chemicals and Analytics

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

Extraction of natural samples

Field sites and soil sampling

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

Procedure for sample chemical extraction

Each sample was extracted in duplicate. For soils and sediments: to 4 g of crude sample, 15 mL of milli Q water was added, followed by acidification to pH 1 with HCl (1 M) and vortex. After decantation the supernatant was extracted with DCM (12 x 15 mL) and the pellet was washed twice with DCM (15 mL). For river and mangrove water: 0.75 L of water sample was acidified to pH 1 with HCl (1 M) and extracted with DCM (12 x 350 mL). In each case, organic layers were pooled, concentrated *in vacuo* and analyzed in duplicated injection using GC-MS (in hexane/acetone 85:15) and LC-MS (in 10 mM NH_4OAc buffer /MeCN 4:1) protocols.

Production and purification of chlordecone main TP

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 B_{12} (40 mg, 2.9 10^{-5} mol, 0.15
120 eq.). The reaction was carried out under N_2 atmosphere at room temperature (rt) for 30 h. It was
121 then quenched with HCl (6 M) to pH 4.0 and degassed with N_2 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/$(CH_3)_2CO$ (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/$(NH_4)_2CO_3$ 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 B_{12} (60 mg, 5.8 10^{-5} mol,
137 0.15 eq.) in degassed H_2O/MeOH 64:36 (250 mL), was added titanium(III) citrate (50 mL, 3.3
138 10^{-3} mol, 8.4 eq.) basified to pH 12.7 with NaOH (3 M). Reaction mixture was stirred under N_2

atmosphere at room temperature for 80 min and, quenched by contact with O_2. 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/H_2O 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, F_{254}, 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. ^{13}C-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 B_{12} (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 (N_2/H_2, 98:2, V/V) at room temperature for 2 months. After quenching by contact with O_2, 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 NH_4OAc buffer (10 mM ; pH 7 adjusted with NH_4OH) 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 10^{-5} mol; 5%) and C3-C4 (1.5 mg; 5.7 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

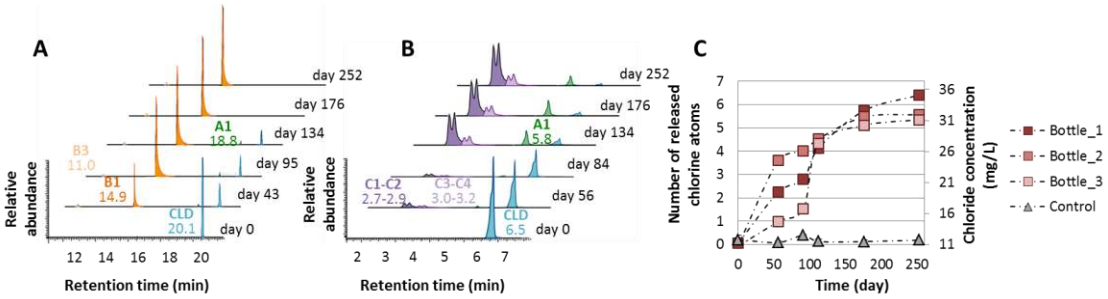
Both early[34, 35] and recent[36, 37] studies of bacterial chlordecone degradation suggested two main TP families: hydrochlordecones from reductive dechlorination reactions (family A), and

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

Further novel chlordecone TP during incubation of chlordecone in presence of consortium 86 and *Citrobacter* sp. 86 were searched for using measurement of chloride concentration, GC-MS, GC-FID, and LC-MS-Orbitrap. After 250 days of incubation, chloride concentrations increased by 18.8 ± 0.9 mg/L (i.e., 5.5 ± 0.3 Cl atoms per chlordecone molecule) in consortium 86 cultures, and by 19.7 ± 1.7 mg/L (i.e., 5.8 ± 0.5 Cl atoms per chlordecone molecule) in *Citrobacter* sp. 86 cultures, corresponding to complete chlordecone transformation (**Figure 1** and Figure S13). Chloride recovery indicated the occurrence of other, so far undetected highly dechlorinated TP, in addition to previously reported main TP B_1 ($C_9Cl_5H_3$) and A_1 ($C_{10}Cl_9H_3O_2$)[36].

While GC-FID analysis confirmed that volatile metabolites A1 and B1 occurred in all microbial experiments (Figure S14), four new polar chlorinated TP (Ci, i=1,2,3,4) of generic formula $C_{10}Cl_{4-n}O_2H_{4+n}$ (n=0,1) were also identified in all chlordecone-degrading microcosms using LC-ESI-MS-Orbitrap in negative mode (**Figure 1** and Figures S24-S25). TLC analysis initially

206 indicated a high match with unknown polar TP[38]. Tandem mass spectrometry using collision
207 induced dissociation (CID) (LC-MS^2 and LC-MS^3) then showed that the four TP followed
208 similar fragmentation patterns with characteristic CO_2 loss. The UV-visible absorption profiles of
209 all TP Ci (i=1,2,3,4) were close to those reported and tentatively assigned to
210 pentachloroindenes[39] (Figure S2).



211

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

220

221 In parallel, laboratory microcosms with contaminated FWI soils (typically from 0.1 to 30 mg
222 chlordecone/kg of dry soil) were incubated for three years under N_2/H_2 atmosphere at room
223 temperature and in the dark to evaluate the capacity of native FWI soil microbiotes to degrade
224 residual chlordecone (Figure S3). For each condition (soil/liquid medium), several replicates
225 were collected over time using a sacrificial approach to evaluate produced chlordecone TP. All
226 analyzed samples contained several chlordecone TP among which 10 new derivatives.

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

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

Production and purification of chlordecone TP

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

249 degradation led to mono- and di-hydrochlordecone derivatives, each with about 1% yield[41].
250 All other conditions under which chlordecone degradation was reported required a reducing
251 agent, either alone[42] or in combination with vitamin B_{12}[37-39, 43-45].

252 Thus, monitoring of chlordecone degradation by GC-MS and LC-MS was used here to determine
253 suitable conditions for selective formation of metabolites from each TP family, including the
254 three novel TP families identified here for the first time. Addition of reducing agents and metal
255 complexes previously applied to enhance mirex degradation[46] were tested (Table S1).
256 Application of degradation conditions (acetoin, vitamin B_{12} and methanol) previously shown to
257 selectively lead to the formation of polychloroindenes[39] also resulted in C and D families of
258 TP. Similarly, two other protocols (vitamin B_{12}, ethanol and either zero-valent zinc or 1,4-
259 dithiothreitol) reported to result in a tentatively assigned pentachloroindene as a unique
260 chlordecone TP[43] also yielded lower chlorinated indenes, as well as several TP of the C and E
261 families (Figure S7).

262 Eventually, we selected 5 protocols of TP production among the 21 tested degradation conditions
263 (Table S1, **Figure 2**). For instance, titanium citrate and zero-valent iron without vitamin B_{12}
264 selectively resulted in selective formation of hydrochlordecones. Most importantly, the
265 combination of sodium sulfide and vitamin B_{12} specifically led to the production of
266 monohydrochlordecone A1 (in up to 50% yield after purification). Polychloroindenes and
267 polychloroindene carboxylic acids were formed at highest levels upon amendment with vitamin
268 B_{12} (**Figure 2** and Table S1). Worthy of note, tested reducing agents did not specifically favor TP
269 from one of these two families, but systematically produced a mixture of B and C TP. Zero-
270 valent iron may slightly enhance production of C family TP, whereas TP of the B family
271 prevailed in assays with titanium citrate. On a preparative scale, addition of methanol

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

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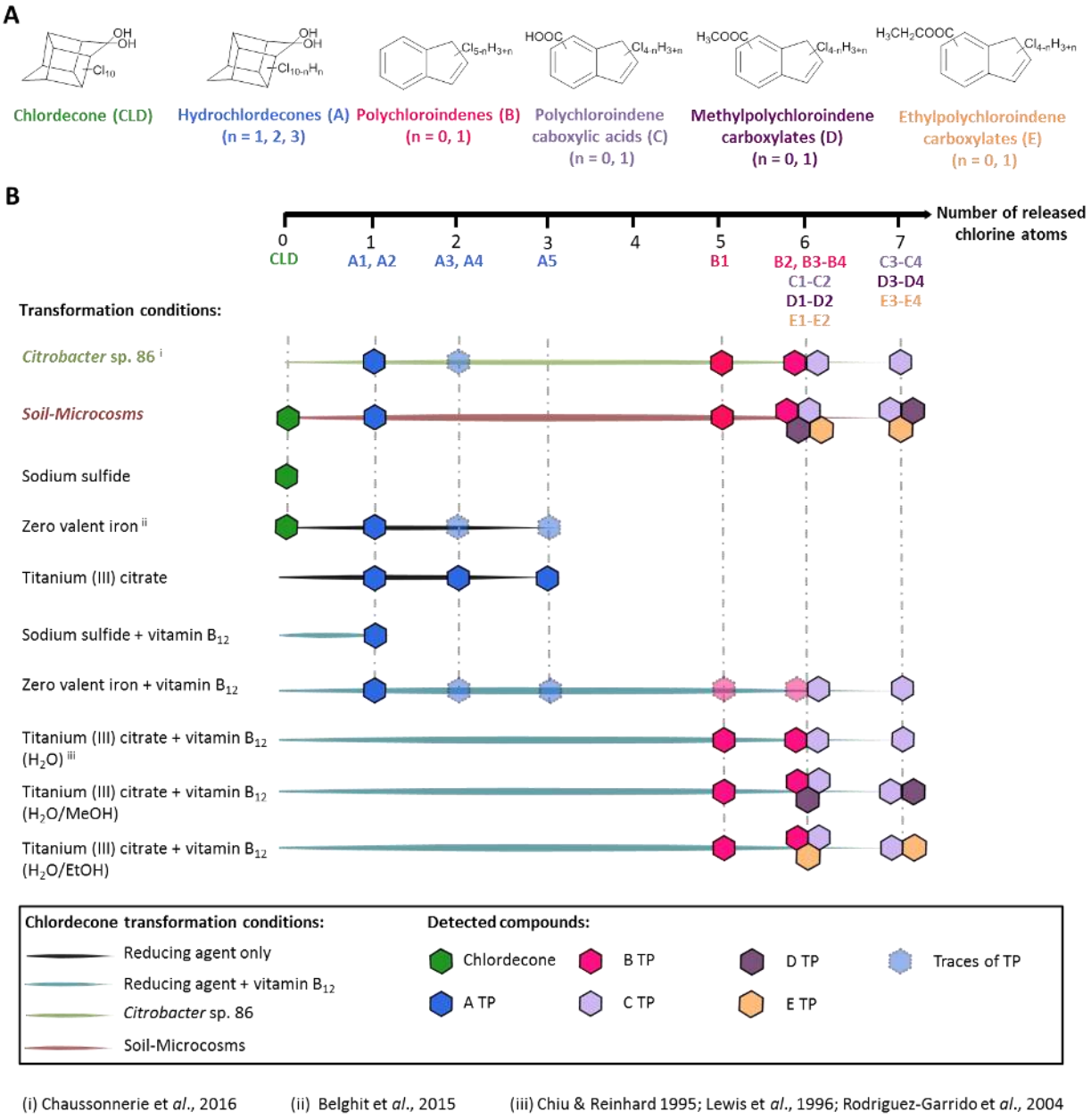


282 (i) Chaussonnerie et *al.*, 2016 (ii) Belghit et *al.*, 2015 (iii) Chiu & Reinhard 1995; Lewis et *al.*, 1996; Rodriguez-Garrido et *al.*, 2004

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

287

Structural elucidation of chlordecone TP

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

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

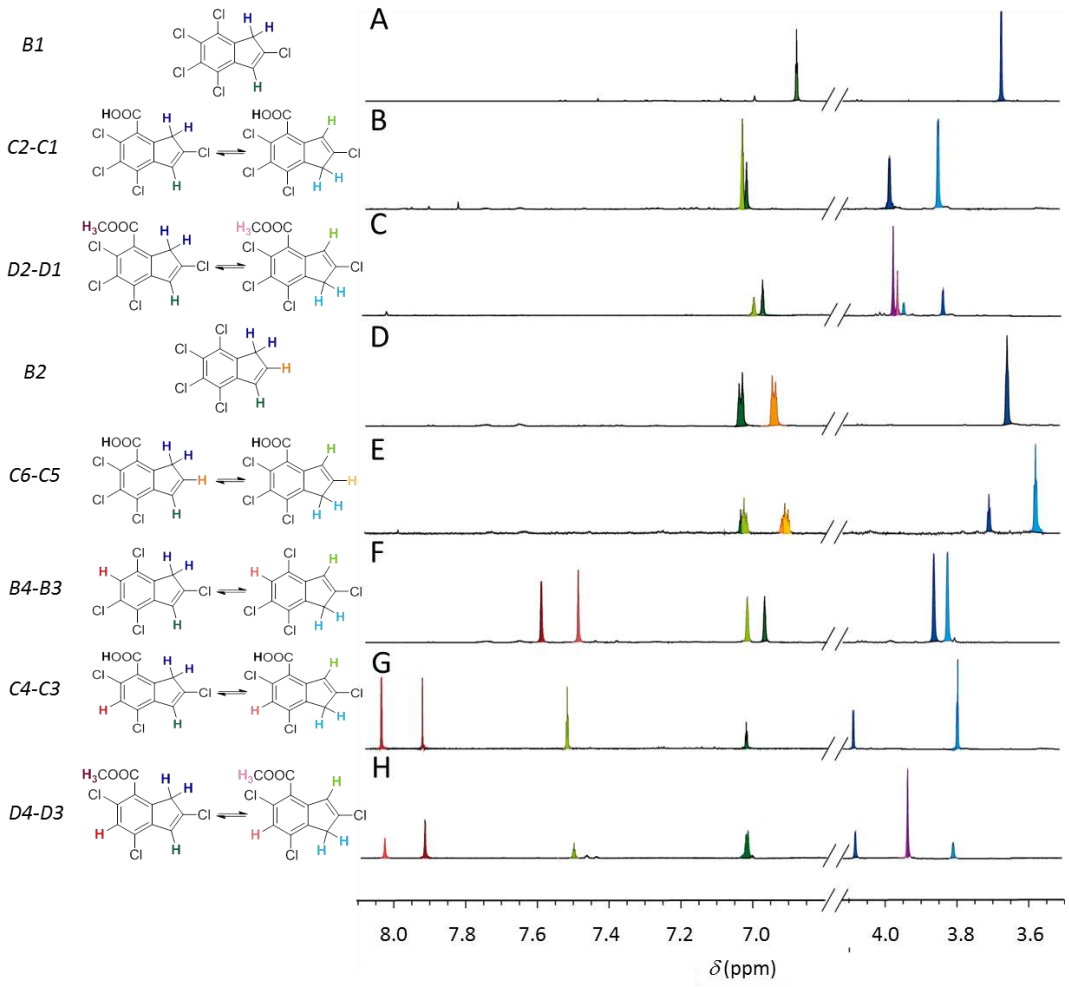


Figure 3. Relevant ^{1}H NMR spectra regions of selected indene-based TP (600 MHz). Data were recorded in $(CD_3)_2CO$ excepted for B1, recorded in $CDCl_3$. The spectral regions δ3.5-4.1 ppm and δ6.8-8.1 ppm are shown for B1 (A), C1-C2 (B), D1-D2 (C), B2 (D), C5-C6 (E), B3-B4 (F), C3-C4 (G), D3-D4 (H).

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. ^{1}H NMR and COSY spectra indicated an allylic domain at δ3.7 ppm (d, J = 1.4 Hz, 2 H) coupled to an aromatic proton at δ 6.87 ppm (t, J = 1.4 Hz, 1 H). For indene

systems, a value of 1.4-Hertz indicates a ^{3}J- or ^{4}J-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 $^{13}C_8$-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 ^{1}H NMR and COSY experiments (**Figure 3** and Figures S48, S57). Comparison of ^{1}H 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 ^{1}H 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 ^{1}H 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 ^{1}H and ^{13}C signals in these TP (**Figure 3** and Figures. S57-S61).

TP B2 and C5-C6 showed the same ^{1}H 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

342 cyclopentadiene part of the indene structure (Figure S26). Structures of B2, C5 and C6 TP were
343 thus identified as 4,5,6,7-tetrachloro-1*H*-indene, 5,6,7-trichloro-1*H*-indene-4-carboxylic acid and
344 4,5,6-trichloro-1*H*-indene-7-carboxylic acid, respectively.

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

364 indene-4-carboxylic acid, 2,4,6-trichloro-1*H*-indene-7-carboxylic acid, methyl 2,5,7-trichloro-
365 1*H*-indene-4-carboxylate, methyl 2,4,6-trichloro-1*H*-indene-7-carboxylate, respectively.

366 As previously shown, Ei (i=1,2,3,4) TP were ethylated forms of Cj (j=1,2,3,4) carboxylates
367 (Figure S6). Consequently, E1-E2 and E3-E4 correspond to ethyl 2,5,6,7-tetrachloro-1*H*-indene-
368 4-carboxylate, ethyl 2,5,6,7-tetrachloro-1*H*-indene-7-carboxylate, ethyl 2,5,7-trichloro-1*H*-
369 indene-4-carboxylate, ethyl 2,4,6-trichloro-1*H*-indene-7-carboxylate, respectively.

370 NMR analyses combined with chemical derivatization work further allowed to identify and
371 structurally elucidate 19 TP for the first time (Figure S15). This significantly expands the list of
372 fully characterized chlordecone TP, hitherto only comprised of chlordecol, 8-
373 monohydrochlordecone and 2,8-dihydrochlordecone[41] and corrects previous findings[39, 42,
374 43].

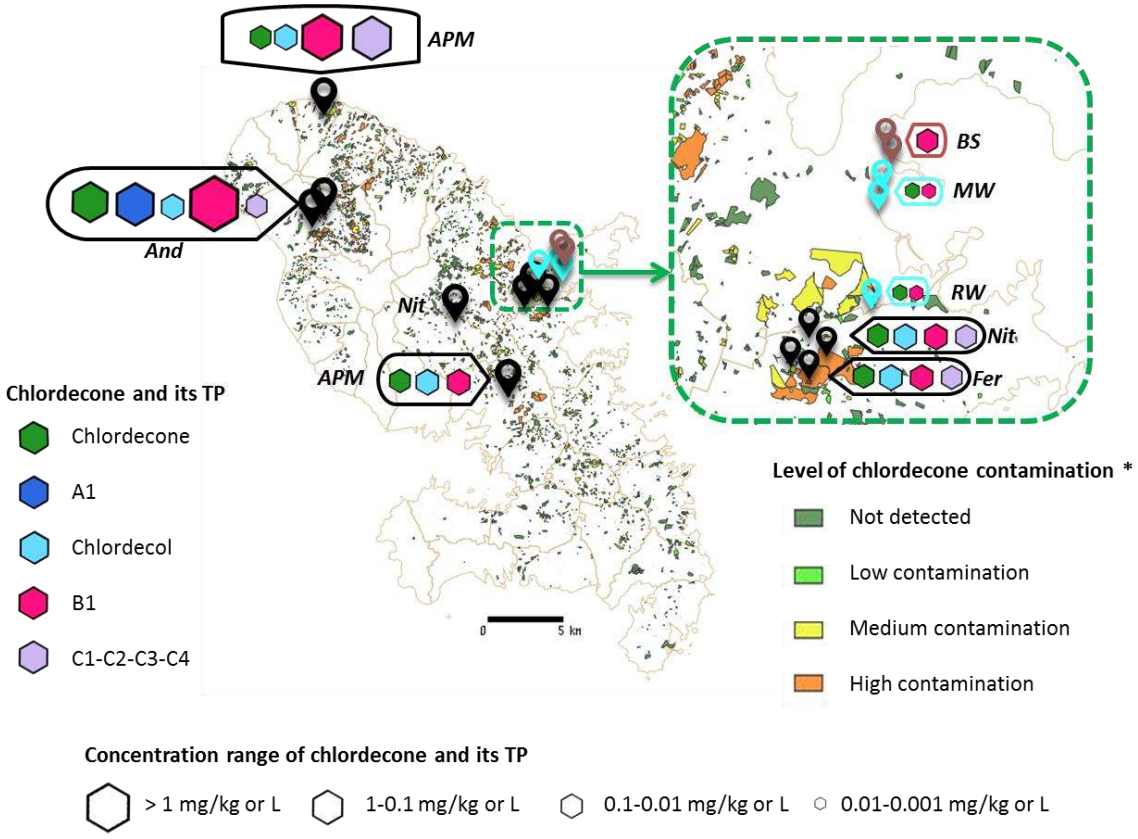
375

376 **Natural chlordecone degradation on the Martinique Island**

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

386 of TP assignment using isotopic patterns, and also allows detection of other chlorinated
387 analogues. Concentrations of the most abundant TP (A1, chlordecol, B1, C1-C2 and C3-C4)
388 were estimated using external calibration with purified TP standards (Figure S11). Significant
389 variability of extraction efficiency in soils was observed, as a function of both TP structures and
390 matrix nature, as shown for chlordecone and metabolite B1 in andosol and nitisol soils (Figure
391 S10), so non-corrected concentration ranges were provided here. Chlordecone concentration in
392 Galion River was in the range found in previous studies (0.1-2 μg/L in Galion River[29]).
393 Worthy of note, the concentration of B1 was in the same order of magnitude as chlordecone
394 itself (0.1-2 μg/L) in both Galion River and nearby mangrove (**Figure 4** and Table S7).
395 Moreover, B1 could be detected in bed sediments where chlordecone was absent. In soils,
396 chlordecone concentrations were roughly 10–fold lower than previously reported typical values
397 of dried samples (0.01-1 mg/kg compared to 0.8-5 mg/kg (dry weight) in Martinique[47, 50]).
398 Chlordecone TP were amply found in solid matrices, with B1 and chlordecol being detected in
399 all soil samples excepted in chlordecone-free nitisol 926. TP B1 fluctuated in the range between
400 0.05 to 5 mg/kg. In contrast, chlordecol was systematically around 0.05 mg/kg, in agreement
401 with previous studies[40]. TP A1 was only observed at significant levels in the two andosol soils
402 (0.05-1 mg/kg). C3-C4 TP, less chlorinated than C1-C2 TP, appeared more frequently and at a
403 higher level, especially in one nitisol soil (above 1 mg/kg). The low concentration of 8-
404 monohydrochlordecone (< 0.01 mg/kg) agreed with previous studies[33]. While methyl
405 polychloroindenecarboxylates were absent of all samples, many other TP were detected at low
406 level, including di-, tri-hydrochlordecones, tetrachloroindenes (B2, B3-B4), ethyl
407 polychloroindenecarboxylates (E1-E2)(**Figure 4** and Table S7). The untargeted analytical
408 approach also led to the discovery of a monohydrochlordecol derivative and two dichloroindene

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



411

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

422

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

430 Altogether, this study shows that four of the five identified TP families occur in natural soil and
431 water samples from Martinique, and include a total of 17 TP initially absent in chlordecone
432 commercial formulations. Pentachloroindene B1 was the most prominent TP, with levels similar
433 to those of chlordecone (**Figure 4**). Bacteria known to transform chlordecone into A, B and C
434 families[36] were searched in the studied soil samples using Illumina sequencing of the 16S
435 rRNA gene (V4-V5 amplicons) following DNA extraction. Only a very low frequency (1.4 to
436 2.10^{-4}) of *Citrobacter* affiliated sequences was found, and this in only three of the investigated
437 samples (918, 919 & 920). Thus, based on current knowledge of bacterial diversity associated
438 with chlordecone degradation, the overall bacterial contribution to chlordecone degradation in
439 Martinique native soils may not be identified yet.

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

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

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

461

462 ASSOCIATED CONTENT

463 **Supporting Information**.

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

465

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Notes

The authors declare no competing financial interest.

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REFERENCES

1. Matolcsy, C.; Nadasy, M.; Andriska, V., Pesticide chemistry. *Studies in environnemental sciences* **1988,** *32*, 1-809.
2. NRC, Kepone/Mirex/Hexachlorocyclopentadiene An environment assessment. *Environmental Studies Board* **1978**.
3. Servien, R.; Mamy, L.; Li, Z.; Rossard, V.; Latrille, E.; Bessac, F.; Patureau, D.; Benoit, P., TyPol – A new methodology for organic compounds clustering based on their molecular characteristics and environmental behavior. *Chemosphere* **2014,** *111*, 613-622.
4. Carlson, D. A.; Konyha, K. D.; Wheeler, W. B.; Marshall, G. P.; Zaylskie, R. G., Mirex in the environment: its degradation to kepone and related compounds. *Science* **1976,** *194*, (4268), 939-41.
5. Figge, K.; Rehm, H., On the behaviour of the insectide "kelevan" and its metabolites in the ecological system "potato field". *Journal of plant disease and protection* **1977,** *84*, 385-409.
6. Le Déaut, J. Y.; Procaccia, C., Les impacts de l'utilisation de la chlordécone et des pesticides aux Antilles: bilan et perspectives d'évolution. *OPECST* **2009,** *487*.
7. Yang, R.; Wei, H.; Guo, J.; McLeod, C.; Li, A.; Sturchio, N. C., Historically and currently used Dechloranes in the sediments of the Great Lakes. *Environmental science & technology* **2011,** *45*, (12), 5156-63.
8. Bell, M. A.; Ewing, R. A.; Lutz Batelle, G. A., Reviews of the environmental effects of pollutants : I Mirex and Kepone. *United States Environmental Protection Agency* **1978**, 1-252.
9. Wang, B.; Iino, F.; Yu, G.; Huang, J.; Wei, Y.; Yamazaki, N.; Chen, J.; Chen, X.; Jiang, W.; Morita, M., HRGC/HRMS analysis of mirex in soil of Liyang and preliminary assessment of mirex pollution in China. *Chemosphere* **2010,** *79*, (3), 299-304.
10. Roche, H.; Salvat, B.; Ramade, F., Study on the contamination of marine organisms by pesticides from French Polynesia coral reef food web. *Rev Ecol* **2011,** *66*, 3-10.
11. Huggett, R. J.; Bender, M. E., Kepone in the James River. *Environmental science & technology* **1980,** *14*, (8), 918-923.
12. Vilardebo, A.; Beugnon, M.; Melin, P.; Lecoq, J.; Aubert, B., Chlordécone et autres insecticides dans la lutte contre le charançon du bananier Cosmopolites sordidus GERM. *Fruits* **1974,** *29*, (4), 267-278.
13. Fournier, A.; Feidt, C.; Lastel, M. L.; Archimede, H.; Thome, J. P.; Mahieu, M.; Rychen, G., Toxicokinetics of chlordecone in goats: Implications for risk management in French West Indies. *Chemosphere* **2017,** *171*, 564-570.
14. Epstein, S. S., Kepone--hazard evaluation. *The Science of the total environment* **1978,** *9*, (1), 1-62.
15. Multigner, L.; Ndong, J. R.; Giusti, A.; Romana, M.; Delacroix-Maillard, H.; Cordier, S.; Jegou, B.; Thome, J. P.; Blanchet, P., Chlordecone exposure and risk of prostate cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **2010,** *28*, (21), 3457-62.
16. Dallaire, R.; Muckle, G.; Rouget, F.; Kadhel, P.; Bataille, H.; Guldner, L.; Seurin, S.; Chajes, V.; Monfort, C.; Boucher, O.; Thome, J. P.; Jacobson, S. W.; Multigner, L.; Cordier, S.,

528 Cognitive, visual, and motor development of 7-month-old Guadeloupean infants exposed to
529 chlordecone. *Environmental research* **2012,** *118*, 79-85.
530 17. Seurin, S.; Rouget, F.; Reninger, J. C.; Gillot, N.; Loynet, C.; Cordier, S.; Multigner, L.;
531 Leblanc, J. C.; Volatier, J. L.; Heraud, F., Dietary exposure of 18-month-old Guadeloupian
532 toddlers to chlordecone. *Regulatory toxicology and pharmacology : RTP* **2012,** *63*, (3), 471-9.
533 18. Boucher, O.; Simard, M. N.; Muckle, G.; Rouget, F.; Kadhel, P.; Bataille, H.; Chajes, V.;
534 Dallaire, R.; Monfort, C.; Thome, J. P.; Multigner, L.; Cordier, S., Exposure to an
535 organochlorine pesticide (chlordecone) and development of 18-month-old infants.
536 *Neurotoxicology* **2013,** *35*, 162-8.
537 19. Kadhel, P.; Monfort, C.; Costet, N.; Rouget, F.; Thome, J. P.; Multigner, L.; Cordier, S.,
538 Chlordecone exposure, length of gestation, and risk of preterm birth. *American journal of*
539 *epidemiology* **2014,** *179*, (5), 536-44.
540 20. Saunders, L.; Kadhel, P.; Costet, N.; Rouget, F.; Monfort, C.; Thome, J. P.; Guldner, L.;
541 Cordier, S.; Multigner, L., Hypertensive disorders of pregnancy and gestational diabetes mellitus
542 among French Caribbean women chronically exposed to chlordecone. *Environment international*
543 **2014,** *68*, 171-6.
544 21. Cordier, S.; Bouquet, E.; Warembourg, C.; Massart, C.; Rouget, F.; Kadhel, P.; Bataille,
545 H.; Monfort, C.; Boucher, O.; Muckle, G.; Multigner, L., Perinatal exposure to chlordecone,
546 thyroid hormone status and neurodevelopment in infants: the Timoun cohort study in
547 Guadeloupe (French West Indies). *Environmental research* **2015,** *138*, 271-8.
548 22. Herve, D.; Costet, N.; Kadhel, P.; Rouget, F.; Monfort, C.; Thome, J. P.; Multigner, L.;
549 Cordier, S., Prenatal exposure to chlordecone, gestational weight gain, and birth weight in a
550 Guadeloupean birth cohort. *Environmental research* **2016,** *151*, 436-444.
551 23. Multigner, L.; Kadhel, P.; Rouget, F.; Blanchet, P.; Cordier, S., Chlordecone exposure
552 and adverse effects in French West Indies populations. *Environmental science and pollution*
553 *research international* **2016,** *23*, (1), 3-8.
554 24. Yang, Y. F.; Chen, P. J.; Liao, V. H., Nanoscale zerovalent iron (nZVI) at
555 environmentally relevant concentrations induced multigenerational reproductive toxicity in
556 Caenorhabditis elegans. *Chemosphere* **2016,** *150*, 615-23.
557 25. Deloumeaux, J.; Bhakkan, B.; Eyraud, R.; Braud, F.; Manip M'Ebobisse, N.; Blanchet,
558 P.; Brureau, L., Prostate cancer clinical presentation, incidence, mortality and survival in
559 Guadeloupe over the period 2008-2013 from a population-based cancer registry. *Cancer causes*
560 *& control : CCC* **2017,** *28*, (11), 1265-1273.
561 26. Gely-Pernot, A.; Hao, C.; Legoff, L.; Multigner, L.; D'Cruz, S. C.; Kervarrec, C.; Jegou,
562 B.; Tevosian, S.; Smagulova, F., Gestational exposure to chlordecone promotes transgenerational
563 changes in the murine reproductive system of males. *Scientific reports* **2018,** *8*, (1), 10274.
564 27. Clostre, F.; Cattan, P.; Gaude, J. M.; Carles, C.; Letourmy, P.; Lesueur-Jannoyer, M.,
565 Comparative fate of an organochlorine, chlordecone, and a related compound, chlordecone-5b-
566 hydro, in soils and plants. *The Science of the total environment* **2015,** *532*, 292-300.
567 28. Clostre, F.; Letourmy, P.; Lesueur-Jannoyer, M., Soil thresholds and a decision tool to
568 manage food safety of crops grown in chlordecone polluted soil in the French West Indies.
569 *Environ Pollut* **2017,** *223*, 357-366.
570 29. Della Rossa, P.; Jannoyer, M.; Mottes, C.; Plet, J.; Bazizi, A.; Arnaud, L.; Jestin, A.;
571 Woignier, T.; Gaude, J. M.; Cattan, P., Linking current river pollution to historical pesticide use:
572 Insights for territorial management? *The Science of the total environment* **2017,** *574*, 1232-1242.

30. Unger, M. A.; Vadas, G. G., Kepone in the James River Estuary: Past, Current and Future Trends. . *Virginia Institute of Marine Science, College of William and Mary*. **2017**, 1-15.
31. Cabidoche, Y. M.; Achard, R.; Cattan, P.; Clermont-Dauphin, C.; Massat, F.; Sansoulet, J., Long-term pollution by chlordecone of tropical volcanic soils in the French West Indies: a simple leaching model accounts for current residue. *Environ Pollut* **2009,** *157*, (5), 1697-705.
32. Fernández-Bayo, J. D.; Saison, C.; Voltz, M.; Disko, U.; Hofmann, D.; Berns, A. E., Chlordecone fate and mineralisation in a tropical soil (andosol) microcosm under aerobic conditions. *The Science of the total environment* **2013,** *463-464*, 395-403.
33. Devault, D. A.; Laplanche, C.; Pascaline, H.; Bristeau, S.; Mouvet, C.; Macarie, H., Natural transformation of chlordecone into 5b-hydrochlordecone in French West Indies soils: statistical evidence for investigating long-term persistence of organic pollutants. *Environmental science and pollution research international* **2016,** *23*, (1), 81-97.
34. Orndorff, S. A.; Colwell, R. R., Distribution and characterization of kepone-resistant bacteria in the aquatic environment. *Applied and environmental microbiology* **1980,** *39*, (3), 611-22.
35. George, S. E.; Claxton, L. D., Biotransformation of chlordecone by Pseudomonas species. *Xenobiotica; the fate of foreign compounds in biological systems* **1988,** *18*, (4), 407-16.
36. Chaussonnerie, S.; Saaidi, P. L.; Ugarte, E.; Barbance, A.; Fossey, A.; Barbe, V.; Gyapay, G.; Bruls, T.; Chevallier, M.; Couturat, L.; Fouteau, S.; Muselet, D.; Pateau, E.; Cohen, G. N.; Fonknechten, N.; Weissenbach, J.; Le Paslier, D., Microbial Degradation of a Recalcitrant Pesticide: Chlordecone. *Frontiers in microbiology* **2016,** *7*, (2025), 2025.
37. Chevallier, M. L.; Cooper, M.; Kummel, S.; Barbance, A.; Le Paslier, D.; Richnow, H. H.; Saaidi, P. L.; Adrian, L., Distinct Carbon Isotope Fractionation Signatures during Biotic and Abiotic Reductive Transformation of Chlordecone. *Environmental science & technology* **2018,** *52*, (6), 3615-3624.
38. Jablonski, P. E.; Pheasant, D. J.; Ferry, J. G., Conversion of Kepone by Methanosarcina thermophila. *FEMS microbiology letters* **1996,** *139*, (2-3), 169-173.
39. Schrauzer, G. N.; Katz, R. N., Reductive dechlorination and degradation of mirex and kepone with Vitamin B12. *Bioinorganic chemistry* **1978,** *9*, (2), 123-43.
40. Almaric, L.; Bristeau, S. *Essai d'intercomparaison de l'analyse de la chlordécone et de ses métabolites dans les sols*; BRGM/RP-63113-FR; BRGM: 2014; p 87.
41. Wilson, N. K.; Zehr, R. D., Structures of some Kepone photoproducts and related chlorinated pentacyclodecanes by carbon-13 and proton nuclear magnetic resonance. *The Journal of organic chemistry* **1979,** *44*, (8), 1278-1282.
42. Belghit, H.; Colas, C.; Bristeau, S.; Mouvet, C.; Maunit, B., Liquid chromatography-high-resolution mass spectrometry for identifying aqueous chlordecone hydrate dechlorinated transformation products formed by reaction with zero-valent iron. *International Journal of Environmental Analytical Chemistry* **2015,** *95*, (2), 93-105.
43. Ranguin, R.; Durimel, A.; Karioua, R.; Gaspard, S., Study of chlordecone desorption from activated carbons and subsequent dechlorination by reduced cobalamin. *Environmental science and pollution research international* **2017,** *24*, (33), 25488-25499.
44. Lewis, T. A.; Morra, M. J.; Brown, P. D., Comparative product analysis of carbon tetrachloride dehalogenation catalyzed by cobalt corrins in the presence of thiol or titanium (III) reducing agents. *Environmental science & technology* **1996,** *30*, (1), 292-300.
45. Rodríguez-Garrido, B.; Camps Arbestain, M.; Monterroso, M. C.; Macías, F., Reductive Dechlorination of α-, β-, δ-, and γ-Hexachlorocyclohexane Isomers by Hydroxocobalamin in the

619 Presence of Either Dithiothreitol or Titanium(III) Citrate as Reducing Agents. *Environmental*
620 *science & technology* **2004,** *38*, (19), 5046-5052.
621 46. Holmstead, R. L., Studies of the degradation of Mirex with an iron(II) porphyrin model
622 system. *Journal of agricultural and food chemistry* **1976,** *24*, (3), 620-4.
623 47. Mouvet, C.; Dictor, M. C.; Bristeau, S.; Breeze, D.; Mercier, A., Remediation by
624 chemical reduction in laboratory mesocosms of three chlordecone-contaminated tropical soils.
625 *Environmental science and pollution research international* **2017,** *24*, (33), 25500-25512.
626 48. Andraos, J.; Kresge, A. J.; Popik, V. V., Kinetics and Mechanism of the Isomerization of
627 1H-Indene-1-carboxylic Acid to 1H-Indene-3-carboxylic Acid in Aqueous Solution and
628 Determination of Their Keto-Enol Equilibrium Constants and Acid Dissociation Constants of the
629 Keto and Enol Forms. Implication for the Photolysis of Diazonaphthoquinones. *Journal of the*
630 *American Chemical Society* **1994,** *116*, (3), 961-967.
631 49. Robert, S. *Historique de la contamination des sédiments littoraux des Antilles françaises*
632 *par la chlordécone (ChloSed). Rapport final de convention MAAP-Ifremer.*; Ifremer:
633 L'Houmeau, 2012; p 92.
634 50. Brunet, D.; Woignier, T.; Lesueur-Jannoyer, M.; Achard, R.; Rangon, L.; Barthes, B. G.,
635 Determination of soil content in chlordecone (organochlorine pesticide) using near infrared
636 reflectance spectroscopy (NIRS). *Environ Pollut* **2009,** *157*, (11), 3120-5.
637 51. Bristeau, S.; Amalric, L.; Mouvet, C., Validation of chlordecone analysis for native and
638 remediated French West Indies soils with high organic matter content. *Analytical and*
639 *bioanalytical chemistry* **2014,** *406*, (4), 1073-80.

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