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► **To cite this version:**

Maria Prieto-Espinoza, Laure Malleret, Sylvain Ravier, Patrick Höhener. A Novel Multi-ion Evaluation Scheme to Determine Stable Chlorine Isotope Ratios ($^{37}\text{Cl}/^{35}\text{Cl}$) of Chlordecone by LC-QTOF. Journal of The American Society for Mass Spectrometry, 2023, 10.1021/jasms.3c00270 . hal-04265054

HAL Id: hal-04265054

<https://amu.hal.science/hal-04265054v1>

Submitted on 29 Jan 2024

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A novel multi-ion evaluation scheme to determine stable chlorine isotope ratios (³⁷Cl/³⁵Cl) of chlordecone by LC-QTOF

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Published in the Journal of the American Society for Mass Spectrometry, 2023

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Abstract

Organochlorinated pesticides are highly persistent organic pollutants having important adverse effects in the environment. To study their fate, compound-specific isotope analysis (CSIA) may be used to investigate their degradation pathways and mechanisms but is currently limited to ¹³C isotope ratios. The assessment of ³⁷Cl isotope ratios from mass spectra is complicated by the large number of isotopologues of polychlorinated compounds. For method development, chlordecone (C₁₀Cl₁₀O₂H₂; hydrate form), an organochlorine insecticide that led to severe contamination of soils and aquatic ecosystems of the French West Indies, was taken as a model analyte. Chlorine isotope analysis of chlordecone hydrate was evaluated using high-resolution liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS), enabling smooth ionization to detect the molecular ion. First, a new evaluation scheme is presented to correct for multiple isotope presence in polychlorinated compounds. The scheme is based on probability calculations of the most frequent isotopologues, distributions by binomial probability functions, and corrections for presence of non-chlorine heavy isotopes. Second, mobile-phase modifiers, ionization energy (sampling cone tension) and scan time were optimized for accurate chlorine isotope ratios. Chlordecone standard samples were measured up to 10-fold and bracketed with a second chlordecone external standard. $\delta^{37}\text{Cl}$ values were obtained after conversion to the SMOC scale by a two-point calibration. The robustness of the analysis method and evaluation scheme were tested and gave satisfactory results with standard errors (σ_m) of $\pm 0.34\text{‰}$ for precision and 0.89‰ for long-term accuracy of chlorine isotope ratios of chlordecone hydrate. This work opens perspectives for applications of the C-Cl CSIA approach to investigate the fate of highly toxic and low reactive polychlorinated compounds in the environment.

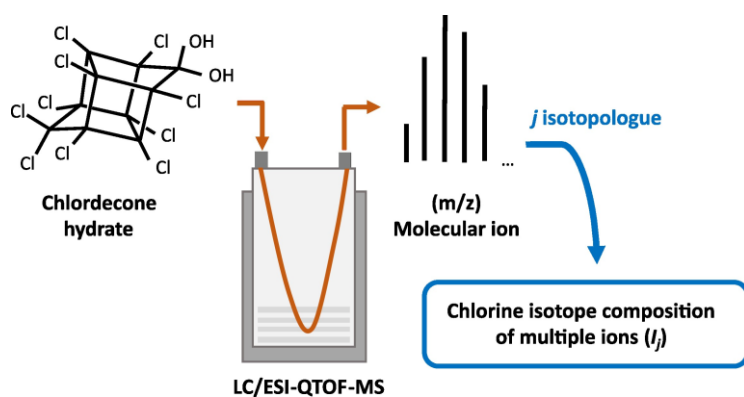
Keywords: Stable isotopes; Chlorine-37; Polychlorinated molecules; Compound-specific Isotope Analysis

25 **Highlights**

- 26
- Chlorine isotope ratios ($^{37}\text{Cl}/^{35}\text{Cl}$) of chlordecone were measured by LC-QTOF-MS
 - The novel evaluation scheme enables to correct for presence of other stable isotopes
 - Optimizing mobile phase and ionization parameters improved the chlorine isotope accuracy
 - Satisfactory precision and long-term accuracy were achieved (<0.89%)
 - Validation of chlorine isotope analysis to track chlordecone degradation in the environment
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33 **Graphical Abstract**

34



36 1. Introduction

37 The past use of highly chlorinated compounds as insecticides and wood-preserving
38 agents has caused a worldwide problem of soil and groundwater pollution. The list of
39 compounds includes γ -hexachlorocyclohexane (lindane), dichlorodiphenyltrichloroethane
40 (DDT), aldrin, endrin, dieldrin, chlordane, chlordecone, mirex, heptachlor and
41 pentachlorophenol (PCP), classified as Persistent Organic Pollutants.^{1,2} These compounds have
42 a high recalcitrance in soils, can bioaccumulate in the food chain and leach to water bodies,
43 further contaminating drinking water resources. Although these substances were banned by the
44 Stockholm convention in the early 2000's^{3,4} or by national restrictions – in part for more than
45 twenty years already – they have persisted in soils and surface waters and still present a risk
46 today.⁵⁻⁷ Appropriate monitoring tools are thus needed to understand the fate and persistence
47 of these polychlorinated compounds in the environment.

48 Compound-specific isotope analysis (CSIA) has become a widely applied tool for the
49 study of the fate of pollutants in the environment.⁸⁻¹¹ The CSIA approach is based on the
50 produced changes of stable isotope ratios in a pollutant during a reactive process. CSIA makes
51 use of kinetic isotope effects in which typically heavy isotopes react more slowly than light
52 isotopes when bond-breaking reactions occur. CSIA is also able to unveil phase-change
53 phenomena like sorption¹² or volatilization.¹³ The use of CSIA thus offers the benefit of
54 distinguishing between degrading and non-degrading processes, and quantitatively evidencing
55 pollutant degradation extent. CSIA targeting carbon isotope ratios (i.e., ¹³C/¹²C) has been
56 successfully applied to study transformation reactions of many low-molecular weight
57 chlorinated solvents, such as chlorinated ethenes¹⁴, and few polychlorinated insecticides, such
58 as lindane,^{15,16} DDT¹⁷ and chlordecone.¹⁸ Studies on the fate of chlorinated ethenes also used
59 additional isotope measurements on a second or even third stable isotope ratio (e.g., ³⁷Cl/³⁵Cl
60 or ²H/¹H) to enlarge the diagnostic power of CSIA to 3D-CSIA.^{10,19} The so-called multielement

61 compound-specific isotope analysis (ME-CSIA) allows to understand different reaction
62 mechanisms that act upon pollutant degradation, e.g., abiotic *versus* biotic reduction.^{20,21}
63 Studies on stable chlorine isotope analysis of polychlorinated pesticides, however, remain
64 scarce.

65 Chlorine isotope analysis on isotope ratio mass spectrometers (IRMS) was established
66 by Shouakar-Stash and coworkers in 2003.²² With this technique, only low-molecular weight
67 chlorinated compounds like trichloroethene (TCE) can be followed.²³ Offline conversion of
68 large pesticide molecules to chlorine gases for dual-inlet IRMS is an alternative way for
69 chlorine isotope analysis,^{24,25} but a considerable mass of analyte is required.²⁶ To offer a more
70 widely applicable way, chlorine isotope analysis using conventional mass spectrometers was
71 first developed by Sakaguchi-Söder and co-workers (2007)²⁷ targeting chlorinated ethenes on
72 a quadrupole MS. It was furthermore shown that isotope fractionation can be followed by
73 quantifying chlorine isotopologues of chloroethenes.²⁸ The accuracy of this method was
74 confirmed by an interlaboratory comparison for TCE involving both quadrupole and isotope
75 ratio mass spectrometers.²⁹ Improvements of the evaluation schemes and the bracketing with
76 two chlorine isotope standards of known isotope ratios further validated stable chlorine isotope
77 analysis of low-molecular weight chlorinated compounds on quadrupole MS.³⁰ These studies
78 advanced the use of ME-CSIA of chlorinated compounds with few chlorine atoms, such as
79 tetrachloroethene (PCE), 1,2-dichloroethane (1,2-DCA) and chloroform (CF), to elucidate their
80 degradation pathways and mechanisms.^{31–33}

81 Most recent advances on stable chlorine isotope analysis of pesticides by GC-qMS have
82 focused on herbicides such as atrazine, acetochlor and metolachlor containing only one chlorine
83 atom.^{34 35 36} Aeppli and co-workers (2010)¹⁷ were also able to analyze the $^{37}\text{Cl}/^{35}\text{Cl}$ isotope
84 ratio in highly chlorinated insecticides such as pentachlorophenol and DDT by GC-qMS, thanks
85 to the presence of their molecular ions in the mass spectrum using electron impact ionization.

86 Although highly chlorinated compounds can be analyzed by quadrupole MS, there is the
87 inconvenience that they exist as many isotopologues (i.e., isotopic species with different
88 numbers of heavy isotopes), which complicates the method by which the chlorine isotope ratio
89 is obtained. These isotopologues not only have a different number of ^{37}Cl atoms, but also a
90 different number of heavy carbon, hydrogen, or oxygen isotopes. Appropriate correction of
91 chlorine isotope ratios should thus be included in the evaluation of chlorine isotope signatures.

92 The problem of highly chlorinated compounds is that they also have rather high numbers
93 of carbon atoms, and it is not so rare that two ^{13}C are present in the same molecule at natural
94 abundance ratios. For instance, the probability of having two ^{13}C atoms in a molecule with 10
95 carbon atoms is 5.1 ‰, which is a significant number in isotope geochemistry. One of the
96 drawbacks of low-resolution MS is that the same mass is measured in an isotopologue having
97 two ^{13}C substitutions for ^{12}C and in an isotopologue having one ^{37}Cl substituted for ^{35}Cl . The
98 evaluation of chlorine isotope ratios of polychlorinated compounds may thus benefit with the
99 use of high-resolution mass spectrometers that must be capable of distinguishing such chlorine
100 isotopologues, according to their mass resolution.

101 Among the persistent organochlorinated pesticides, we choose the insecticide
102 chlordecone as model analyte. This organochlorine insecticide was extensively used from 1972
103 to 1993 in the French West Indies for protecting banana trees against a ravaging beetle.³⁷
104 Chlordecone (also known as Kepone) has a bis-homocubane cage of 10 carbon atoms which is
105 fully chlorinated with 10 chlorine atoms and has a ketone oxygen group (Figure 1a). In soils, it
106 is found in the hydrated form. Its high stability and sorption on organic matter reveals a severe
107 contamination of soils even 30 years since its ban.³⁸ The development of a ME-CSIA approach
108 of chlordecone may help improve the understanding of its degradation pathways and
109 mechanisms,¹⁸ and provide another line of evidence for *in situ* degradation in contaminated
110 soils.

111 This work provides a new multiple ion evaluation scheme that can correct for multiple
112 isotope presence in polychlorinated compounds, such as chlordecone hydrate, which are
113 unresolved by low resolution mass spectrometers and current calculation approaches. Chlorine
114 isotope analysis of chlordecone hydrate will be evaluated using high-resolution liquid
115 chromatography coupled to time-of-flight mass spectrometry with electrospray ionization
116 (LC/ESI-QTOF-MS), as it enables the detection of chlordecone's molecular ion, conversely to
117 gas chromatography coupled to high resolution mass spectrometry (GC/HRMS) with electron
118 impact or electron capture negative ionizations. The proposed scheme will be tested on a
119 theoretical mass spectrum and compared to mass spectra from LC/ESI-QTOF-MS. The effect
120 of mobile-phase modifiers, ionization energy (sampling cone tension) and scan time on chlorine
121 isotope ratios will be further analyzed. Finally, the analysis of chlordecone hydrate by LC/ESI-
122 QTOF-MS will be tested to determine the robustness of the evaluation scheme on experimental
123 data and to characterize the performance and potential efficiency in the determination of $\delta^{37}\text{Cl}$
124 values.

125 **2. Materials and experimental methods**

126 **2.1 Chemicals and reagents**

127 Chlordecone (CLD) analytical standards, CLD_{CLUZEAU} and CLD_{SIGMA}, were purchased
128 from Cluzeau Info Labo (CLD_{CLUZEAU}, Dr. Ehrenstorfer, Ref. C11220000) and Sigma Aldrich
129 (CLD_{SIGMA}, Supelco, Ref. LC00470V), respectively. Standard purities were 96.7% and 99.9,
130 respectively. Chlordecone standard stock solutions were prepared at 1 g/L in LC/MS-grade
131 acetonitrile (ACN, >99.9%, Optima LC-MS, Fischer). Standard solutions were further diluted
132 with ACN and kept at -20°C until analysis.

133 **2.2 Chlorine isotope ratios by LC-QTOF-MS**

134 Stable chlorine isotope ratios of chlordecone hydrate were measured using ultra-high
135 performance liquid chromatography (UPLC, Waters) coupled to a hybrid quadrupole-time of
136 flight (TOF) mass spectrometer (SynaptG2 HDMS, Waters), fitted with an electrospray
137 ionization source (ESI). Chromatographic separations were carried out with an UPLC column
138 (BEH C18, 2.1 mm × 100 mm, 1.8 μm; Waters). The column and autosampler temperatures
139 were 30°C and 8°C, respectively, and the injection volume was 5 μL.

140 First, chlordecone solutions were infused directly into the MS to evaluate chlordecone
141 signal, optimize source parameters and establish the mobile phase. Fresh chlordecone standard
142 solutions were prepared with LC/MS-grade water (Optima, Fischer) and ACN 30:70 (v:v)
143 acidified with 0.1% formic acid (FAc) and with or without addition of 5 mM of ammonium
144 formate (AF), at 1000 and 5000 μg/L. FAc and AF were initially tested as mobile phase
145 modifiers and allowed to compare the measured and theoretical mass spectra of chlordecone
146 hydrate for chlorine isotope analysis. A series of ten infusions were performed for two minutes
147 for each of the standard chlordecone solutions. Optimization of mobile phase modifiers, scan
148 time and cone voltage are discussed in Section 4.

149 For chlordecone standard injections, LC/MS-grade water (A) and ACN (B), both
150 acidified with 0.1% FAc, were used as eluents for the chromatographic gradient at a flow rate
151 of 0.35 mL/min (60% to 98% B in 6 min, and held for 1 min.). ESI conditions were established
152 from a modified method ³⁹ as follows: negative ESI polarity, 1 kV capillary voltage, 30 V cone
153 voltage, 500 °C desolvation temperature, 150 °C source temperature, 10 L/h cone gas flow rate,
154 1000 L/h desolvation gas flow rate, resolution analyzer mode (up to 18,000 full width at half
155 maximum (FWHM) at 400 Th), and TOF mass range 50-600 Da. Additionally, the mass
156 spectrometer was daily calibrated with a solution of sodium formate (5 mM, HCOONa, Sigma
157 Aldrich) and leucine enkephalin (2 mg/L, C₂₈H₃₇N₅O₇, MW 555.2770, Sigma Aldrich) was
158 constantly infused (lock-mass pump: 7 µL/min) during each run for lock-mass correction and
159 accuracy. Data acquisition and mass spectra treatment were performed using the MassLynx
160 software (v.4.1, Waters).

161 **2.3 Method performance**

162 Chlorine isotope ratios of chlordecone hydrate obtained by LC-QTOF-MS were converted
163 to the international SMOC scale by an external two-point calibration, as suggested elsewhere.
164 ^{29,40} CLD_{SIGMA} and CLD_{CLUZEAU} standards were characterized at the University of Delaware by
165 dual-inlet IRMS after offline conversion using the method of Holt et al. (1997) ⁴¹ after
166 conversion to CO₂ and methyl chloride. CLD_{SIGMA} and CLD_{CLUZEAU} standards had δ³⁷Cl values
167 of -0.11 ± 0.17‰ and +0.52 ± 0.17‰, respectively.

168 Chlordecone samples and standards were injected between 5 to 10 times in our LC-QTOF-
169 MS. These consecutive standard injections are considered as one “sample” measurement. Each
170 sample was bracketed by external chlordecone standards of similar concentrations (±20%), ²⁹
171 and a blank was measured after ten injections. Aeppli and coworkers ¹⁷ showed that when
172 concentrations of samples and standards vary significantly (>20%) chlorine isotopic signatures
173 can be affected and lead to an offset of >2‰. This has also been discussed in Bernstein and co-

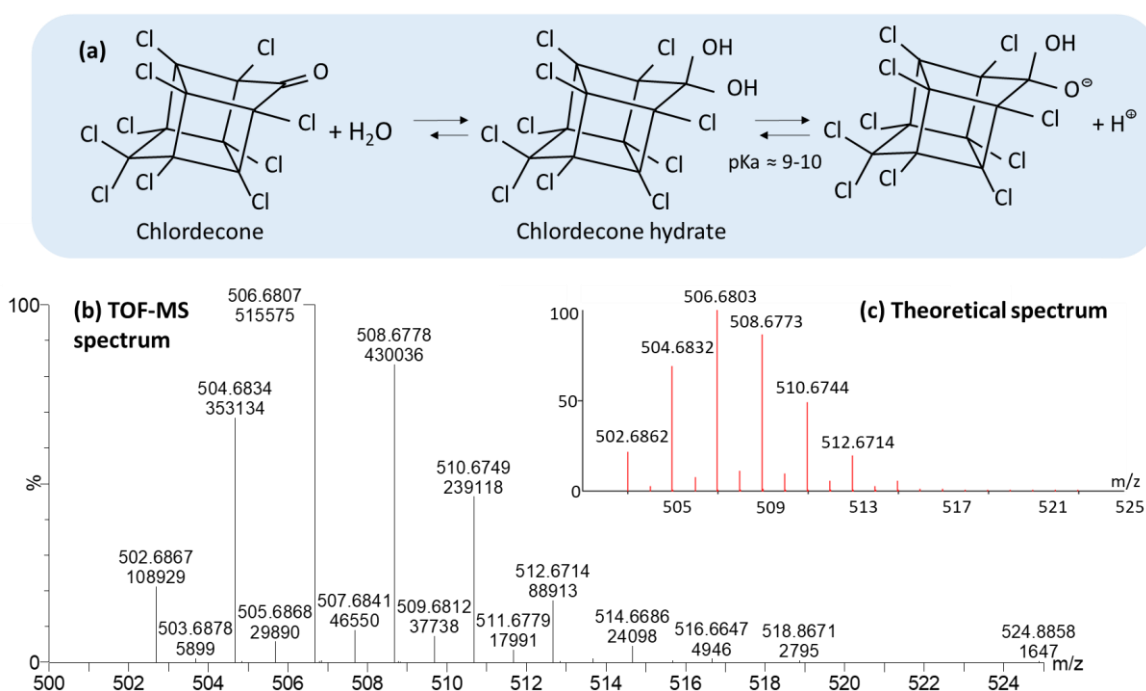
174 workers ²⁹ showing the need for similar amplitudes (i.e., between standards and samples) for
175 accurate chlorine isotope ratios. Each session started and ended with duplicate measurements
176 of both chlordecone standards. The two external standards interspersed along the sequence
177 allowed to evaluate and correct for instrument drift. Measurement uncertainty of chlorine
178 isotope ratios was assessed taking into account the number of injections as $\sigma_m = \sigma/\sqrt{n}$, where
179 σ is the standard deviation of n injections.

180 Precision, trueness, and instrument linearity were assessed to characterize the method
181 performance and the proposed evaluation scheme of chlorine isotope ratios (Section 3).
182 Precision was reported as $\pm \sigma_m$ for n measurements, and trueness was expressed as the offset
183 between the chlorine isotope ratios from LC-QTOF-MS and the referenced SMOC standards.
184 To evaluate the instrument linearity, i.e., the range of amount-dependent induced isotope
185 fractionation, a standard sample (i.e., CLD_{CLUZEAU}) was referenced to average ratios of the
186 second standard (i.e., CLD_{SIGMA}) so that instrument-specific chlorine isotope ratios were
187 obtained. These values were subsequently normalized to the SMOC scale by a two-point linear
188 regression. Reported uncertainties include both accuracy and reproducibility based on long-
189 term measurements and standard errors (σ_m).

190 3. Evaluation scheme of chlorine isotope ratios for polychlorinated pesticides

191 The evaluation scheme presented in this work is based on the mass spectrum of
192 chlordecone hydrate obtained by LC-QTOF-MS. The produced monoprotonated chlordecone
193 hydrate molecule (C₁₀Cl₁₀O₂H⁺) is illustrated in Figure 1a, and its spectrum is depicted in Figure
194 1b. The evaluation scheme of chlorine isotope ratios of chlordecone hydrate focuses on the
195 intensities of the five most abundant ion peaks of the molecular ion, having theoretical masses
196 between 502.6862 and 510.6744 (Figure 1c and Table 1). Ion peaks of higher masses
197 (>510.6878) were not considered due to lower intensities (<5% of relative abundance), resulting

198 in lower repeatability and signal-to-noise ratios (Figure 1b and Supporting information (SI);
 199 Section A).



200

201 **Figure 1.** (a) Chemical structure of chlordecone (also called Kepone, CAS 143-50-0) in its
 202 ketone form (left), and in its gem-diol form (chlordecone hydrate; right). (b) TOF-MS spectrum
 203 (molecular ion) of chlordecone hydrate ($\text{Cl}_{10}\text{C}_{10}\text{O}_2\text{H}_2$) at $500 \mu\text{g/L}$. Ion peak intensities are also
 204 depicted. (c) Theoretical mass spectrum of chlordecone hydrate retrieved from the Isotope
 205 Distribution Calculator at high resolution and with a minimum isotope abundance of 0.0001%.
 206 ⁴²

207

208 The abundance of each isotopologue of chlordecone hydrate can be calculated as
 209 follows: ⁴³

$$210 \quad A = \binom{p}{q} \pi_{37}^q (1 - \pi_{37})^{p-q} \binom{r}{s} \pi_{13}^s (1 - \pi_{13})^{r-s} \binom{t}{u} \pi_{18}^u (1 - \pi_{18})^{t-u} \binom{t}{v} \pi_{17}^v (1 - \pi_{17})^{t-v} \binom{h}{w} \pi_2^w (1 - \pi_2)^{h-w} \quad (1)$$

$$212 \quad \pi_{37} = \frac{\left(\frac{\delta^{37}\text{Cl}}{1000} + 1\right) R_{SMOC}}{\left(1 + \left(\frac{\delta^{37}\text{Cl}}{1000} + 1\right) R_{SMOC}\right)} \quad (2)$$

$$213 \quad \pi_{13} = \frac{\left(\frac{\delta^{13}\text{C}}{1000} + 1\right) R_{VPDB}}{\left(1 + \left(\frac{\delta^{13}\text{C}}{1000} + 1\right) R_{VPDB}\right)} \quad (3)$$

$$214 \quad \pi_{18} = \frac{\left(\frac{\delta^{18}O}{1000} + 1\right) R_{SMOW}}{\left(1 + \left(\frac{\delta^{18}O}{1000} + 1\right) R_{SMOW}\right)} \quad (4)$$

$$215 \quad \pi_{17} = \frac{\left(\frac{\delta^{17}O}{1000} + 1\right) R_{SMOW}}{\left(1 + \left(\frac{\delta^{17}O}{1000} + 1\right) R_{SMOW}\right)} \quad (5)$$

$$216 \quad \pi_2 = \frac{\left(\frac{\delta^2H}{1000} + 1\right) R_{SMOW}}{\left(1 + \left(\frac{\delta^2H}{1000} + 1\right) R_{SMOW}\right)} \quad (6)$$

217 where p , r , t and h are the total numbers of chlorine, carbon, oxygen and hydrogen atoms in the
 218 compound, respectively, and q , s , u , v and w are the numbers of heavy chlorine-37, carbon-13,
 219 oxygen-18, oxygen-17 and deuterium isotopes in a chlordecone isotopologue, respectively. The
 220 π values are the relative abundances of the heavy isotopes of each element. R_{SMOC} is the
 221 abundance ratio of ³⁷Cl in standard mean ocean chloride (0.319766).⁴⁴ R_{VPDB} is the abundance
 222 ratio of ¹³C in standard Vienna Pee Dee Belemnite (0.011237)⁴⁵ and R_{SMOW} are the abundance
 223 ratios of ¹⁸O (0.0020052), ¹⁷O (0.00038) and ²H (0.0001575) in standard mean ocean water.¹⁴
 224 To calculate the relative abundance of the isotopologues, the initial chlorine, oxygen, and
 225 hydrogen isotope signatures of chlordecone hydrate were assumed as 0‰ ($\delta^{18}O = \delta^{17}O = \delta$
 226 ³⁷Cl = $\delta^2H = 0$ ‰). Initial carbon isotope signatures were considered for a $\delta^{13}C$ value of -30
 227 ‰, approaching typical isotope ratios of chlordecone formulations ranging between -34 ‰ and
 228 -31 ‰.¹⁸

229 The abundance of the 30 isotopologues of chlordecone hydrate were computed with eqs.
 230 1-7. This list contains isotopologues of the five most abundant molecular ion peaks of
 231 chlordecone hydrate as a function of number of chlorine, carbon, oxygen and hydrogen
 232 isotopes, and is presented in Table 1. These selected isotopologues have incremental masses of
 233 nearly +2 from mass 502.6862 onward. The sum of the relative abundance of these
 234 isotopologues is 0.8376. All other ion peaks represent the remaining 0.1624 of the total

235 isotopologues and are not used for chlorine isotope ratio calculations. Hence, the relative
 236 abundance of chlordecone hydrate isotopologues was normalized in order that their sum equals
 237 1 (last column; Table 1). For clarity, chlordecone hydrate isotopologues without ^{13}C , ^{17}O , ^{18}O
 238 or ^2H are printed in bold.

239 **Table 1.** Abundance of 30 isotopologues of chlordecone hydrate calculated with eq. 1 for $\delta^{13}\text{C}$
 240 $= -30$ ‰ and $\delta^{18}\text{O} = \delta^{17}\text{O} = \delta^{37}\text{Cl} = \delta^2\text{H} = 0$ ‰. In bold are isotopologues without ^{13}C , ^{17}O ,
 241 ^{18}O or ^2H . Isotopologues are based on the five most abundant molecular ion peaks.

| Isotopologue | Exact Mass ^a | ^{37}Cl | ^{13}C | ^{17}O | ^{18}O | ^2H | Absolute Abundance | Normalized Relative abundance ^b |
|--|-------------------------|------------------|-----------------|-----------------|-----------------|--------------|--------------------|--|
| $^{12}\text{C}_{10}^{35}\text{Cl}_{10}^{16}\text{O}_2\text{H}$ | 502.6862 | 0 | 0 | 0 | 0 | 0 | 0.055694 | 0.066496 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_9^{37}\text{Cl}^{16}\text{O}_2\text{H}$ | 504.6832 | 1 | 0 | 0 | 0 | 0 | 0.178091 | 0.212630 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_{10}^{16}\text{O}^{18}\text{O}^1\text{H}$ | 504.6904 | 0 | 0 | 0 | 1 | 0 | 0.000223 | 0.000267 |
| $^{12}\text{C}_8^{13}\text{C}_2^{35}\text{Cl}_{10}^{16}\text{O}_2\text{H}$ | 504.6929 | 0 | 2 | 0 | 0 | 0 | 0.000298 | 0.000356 |
| $^{12}\text{C}_9^{13}\text{C}^{35}\text{Cl}_{10}^{16}\text{O}^{17}\text{O}^1\text{H}$ | 504.6938 | 0 | 1 | 1 | 0 | 0 | 0.000005 | 0.000006 |
| $^{12}\text{C}_9^{13}\text{C}^{35}\text{Cl}_{10}^{37}\text{Cl}^{16}\text{O}_2\text{H}$ | 504.6958 | 0 | 1 | 0 | 0 | 1 | 0.000001 | 0.000001 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_8^{37}\text{Cl}_2^{16}\text{O}_2\text{H}$ | 506.6803 | 2 | 0 | 0 | 0 | 0 | 0.256264 | 0.305963 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_9^{37}\text{Cl}^{16}\text{O}^{18}\text{O}^1\text{H}$ | 506.6875 | 1 | 0 | 0 | 1 | 0 | 0.000714 | 0.000853 |
| $^{12}\text{C}_8^{13}\text{C}_2^{35}\text{Cl}_9^{37}\text{Cl}^{16}\text{O}_2\text{H}$ | 506.6900 | 1 | 2 | 0 | 0 | 0 | 0.000952 | 0.001137 |
| $^{12}\text{C}_9^{13}\text{C}^{35}\text{Cl}_9^{37}\text{Cl}^{16}\text{O}^{17}\text{O}^1\text{H}$ | 506.6908 | 1 | 1 | 1 | 0 | 0 | 0.000015 | 0.000018 |
| $^{12}\text{C}_9^{13}\text{C}^{35}\text{Cl}_9^{37}\text{Cl}^{16}\text{O}_2^2\text{H}$ | 506.6929 | 1 | 1 | 0 | 0 | 1 | 0.000003 | 0.000004 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_{10}^{18}\text{O}_2\text{H}$ | 506.6947 | 0 | 0 | 0 | 2 | 0 | 0.000000 | 0.000000 |
| $^{12}\text{C}_8^{13}\text{C}_2^{35}\text{Cl}_{10}^{16}\text{O}^{18}\text{O}^1\text{H}$ | 506.6971 | 0 | 2 | 0 | 1 | 0 | 0.000001 | 0.000001 |
| $^{12}\text{C}_6^{13}\text{C}_4^{35}\text{Cl}_{10}^{16}\text{O}_2\text{H}$ | 506.6996 | 0 | 4 | 0 | 0 | 0 | 0.000000 | 0.000000 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_7^{37}\text{Cl}_3^{16}\text{O}_2\text{H}$ | 508.6773 | 3 | 0 | 0 | 0 | 0 | 0.218518 | 0.260898 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_8^{37}\text{Cl}_2^{16}\text{O}^{18}\text{O}^1\text{H}$ | 508.6845 | 2 | 0 | 0 | 1 | 0 | 0.001028 | 0.001227 |
| $^{12}\text{C}_8^{13}\text{C}_2^{35}\text{Cl}_8^{37}\text{Cl}_2^{16}\text{O}_2\text{H}$ | 508.6870 | 2 | 2 | 0 | 0 | 0 | 0.001370 | 0.001636 |
| $^{12}\text{C}_9^{13}\text{C}^{35}\text{Cl}_8^{37}\text{Cl}_2^{16}\text{O}^{17}\text{O}^1\text{H}$ | 508.6879 | 2 | 1 | 1 | 0 | 0 | 0.000021 | 0.000025 |
| $^{12}\text{C}_9^{13}\text{C}^{35}\text{Cl}_8^{37}\text{Cl}_2^{16}\text{O}_2^2\text{H}$ | 508.6899 | 2 | 1 | 0 | 0 | 1 | 0.000004 | 0.000005 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_9^{37}\text{Cl}^{18}\text{O}_2\text{H}$ | 508.6917 | 1 | 0 | 0 | 2 | 0 | 0.000001 | 0.000001 |
| $^{12}\text{C}_8^{13}\text{C}_2^{35}\text{Cl}_9^{37}\text{Cl}^{16}\text{O}^{18}\text{O}^1\text{H}$ | 508.6942 | 1 | 2 | 0 | 1 | 0 | 0.000004 | 0.000005 |
| $^{12}\text{C}_9^{13}\text{C}^{35}\text{Cl}_6^{37}\text{Cl}_4^{16}\text{O}_2\text{H}$ | 508.6967 | 1 | 4 | 0 | 0 | 0 | 0.000001 | 0.000001 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_6^{37}\text{Cl}_4^{16}\text{O}_2\text{H}$ | 510.6744 | 4 | 0 | 0 | 0 | 0 | 0.122281 | 0.145996 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_7^{37}\text{Cl}_3^{18}\text{O}_2\text{H}$ | 510.6816 | 3 | 0 | 0 | 1 | 0 | 0.000876 | 0.001046 |
| $^{12}\text{C}_8^{13}\text{C}_2^{35}\text{Cl}_7^{37}\text{Cl}_3^{16}\text{O}_2\text{H}$ | 510.6841 | 3 | 2 | 0 | 0 | 0 | 0.001168 | 0.001395 |
| $^{12}\text{C}_9^{13}\text{C}^{35}\text{Cl}_7^{37}\text{Cl}_3^{16}\text{O}^{17}\text{O}^1\text{H}$ | 510.6849 | 3 | 1 | 1 | 0 | 0 | 0.000018 | 0.000022 |
| $^{12}\text{C}_9^{13}\text{C}^{35}\text{Cl}_7^{37}\text{Cl}_3^{16}\text{O}_2^2\text{H}$ | 510.6870 | 3 | 1 | 0 | 0 | 1 | 0.000004 | 0.000004 |
| $^{12}\text{C}_{10}^{35}\text{Cl}_8^{37}\text{Cl}_2^{18}\text{O}_2\text{H}$ | 510.6888 | 2 | 0 | 0 | 2 | 0 | 0.000001 | 0.000001 |
| $^{12}\text{C}_8^{13}\text{C}_2^{35}\text{Cl}_8^{37}\text{Cl}_2^{16}\text{O}^{18}\text{O}^1\text{H}$ | 510.6912 | 2 | 2 | 0 | 1 | 0 | 0.000005 | 0.000007 |
| $^{12}\text{C}_6^{13}\text{C}_4^{35}\text{Cl}_8^{37}\text{Cl}_2^{16}\text{O}_2\text{H}$ | 510.6937 | 2 | 4 | 0 | 0 | 0 | 0.000001 | 0.000001 |
| Total | | | | | | | 0.8376 | 1.0000 |

242 ^a From Isotope Distribution Calculator ($\text{C}_{10}\text{Cl}_{10}\text{O}_2\text{H}^+$): <https://www.sisweb.com/mstools/isotope.htm>

243 ^b Relative to the total abundance of masses from the molecular ion in column "absolute abundance", the latter calculated with
 244 Eq. 1.

245

246 Low resolution mass spectrometers such as quadrupole MS will not give the abundance
247 of all molecular ion peaks presented in Table 1. Rather, they will be added into the same peak
248 of rounded mass (502.7, 504.7, and so on). In case of TOF-MS, all peaks may be discriminated
249 in mass ratios. Nonetheless, the rare chlordecone hydrate isotopologues (non-bold in Table 1)
250 could not be isolated from the noise in our LC-QTOF-MS, because of their very low relative
251 abundance (<0.1%). Cutting-edge ultra-high resolution mass spectrometers such as
252 Electrospray-Orbitrap may offer sufficient resolution and enough sensitivity to enable the
253 detection of low abundant isotopologues of organic molecules.⁴⁶

254 Correction factors for the five most abundant ion peaks of chlordecone hydrate (in bold
255 in Table 1) were included in this evaluation scheme. In the second column of Table 2, only the
256 relative abundances of the bold isotopologues are listed (i.e., without the presence of other
257 heavy isotopes and calculated with Eq. 1). In the third column, the relative abundance of each
258 mass summing up all 30 isotopologues of the five most abundant ion peaks are given,
259 considering the presence of heavy isotopes of C, O and H (i.e., the numbers in column 3 are
260 additions of the numbers of the last column in Table 1 for each rounded mass). In the fourth
261 column, correction factors are presented which represent the offset between calculating chlorine
262 isotope ratios with and without the presence of other heavy elements. They can be called a
263 “theoretical” reference spectrum of rounded masses 502.7 to 510.7. Theoretical correction
264 factors for chlorine isotopes of chlordecone hydrate ranged from 1.066 to 1.084 (Table 2).

265 **Table 2.** Calculation of theoretical correction factors for the abundance of impair masses of
 266 chlordecone hydrate (rounded masses 502.7 to 510.7) without or with the presence of ¹³C, ¹⁸O,
 267 ¹⁷O, ²H isotopes.

| Mass (rounded) | Relative Abundance if no isotopes of C, O or H are present ^a | Relative Abundance with isotopes of C, O and H ^b | Theoretical correction factors ^c | Number of ³⁷ Cl atoms ^d |
|----------------|---|---|---|---|
| 502.7 | 0.062378 | 0.066496 | 1.06601 | 0 |
| 504.7 | 0.199463 | 0.213259 | 1.06916 | 1 |
| 506.7 | 0.287017 | 0.307976 | 1.07302 | 2 |
| 508.7 | 0.244742 | 0.263797 | 1.07786 | 3 |
| 510.7 | 0.136955 | 0.148472 | 1.08409 | 4 |
| Total | 0.930556 | 1.000000 | | |

268 ^a calculated with the chlorine term of eq. 2 uniquely

269 ^b sum of the normalized relative abundances of a given rounded mass with the presence of other heavy isotopes
 270 (see Table 1)

271 ^c theoretical correction factors = value of column 3/value of column 2

272 ^d number to be used in eq. 7

273

274 Aeppli and co-workers (2010) ¹⁷ used only the first two ion pair intensities of the mass
 275 spectrum of pentachlorophenol and DDT to calculate ³⁷Cl/³⁵Cl isotope ratios. However, Jin and
 276 co-workers ³⁰ recommended that more than one couple of ion intensities are exploited and
 277 proposed the so-called multiple ion method. This includes information taken from several or all
 278 ion mass intensities of the molecular ion mass spectrum, weighted as a function of their relative
 279 intensities. We will adopt such a scheme in the following and call it multiple ion method with
 280 correction factors, accounting for the five most abundant ion peaks of chlordecone hydrate
 281 (rounded masses 502.7; 504.7; 506.7; 508.7; 510.7).

282 The relative abundances of the heavy chlorine isotopes accounting for the couple of
 283 intensities of mass peaks differing by two mass units ($\pi_{37,j}$) can be calculated with eq. 7, which
 284 is to our best knowledge new.

$$285 \pi_{37,j} = \frac{\binom{p}{j}}{\left(\frac{I_j c_j}{I_{j+1} c_{j+1}} \binom{p}{j+1} + \binom{p}{j} \right)} \quad (7)$$

286 Where I_j is the measured intensity of an ion peak j , C_j is the correction factor for each
 287 ion peak accounting for ³⁷Cl isotopologues (Table 2), j is a counter of chlorine atoms in peaks
 288 from 0 to n , and p is the total number of chlorine atoms (10 for chlordecone) as in eq. 1. $\binom{p}{j}$
 289 are the binomial factors calculated as in equation 8³⁰ and are numbers between 1 and 210 for
 290 the five peaks chosen for chlordecone hydrate (SI; MS Excel worksheet).

$$291 \binom{p}{j} = \frac{p!}{j!(p-j)!} \quad (8)$$

292 Thus, equation 7 is evaluated for $j = 0$ to 4. Chlorine isotope composition of each couple
 293 of ion intensities can be obtained using eq. 9.⁴⁷

$$294 \delta^{37}Cl_j = \left(\frac{\frac{\pi_{37,j}}{(1-\pi_{37,j})}}{R_{SMOC}} - 1 \right) \times 1000 \quad (9)$$

295 For any number j , a ³⁷Cl/³⁵Cl isotope ratio is therefore calculated. These may slightly
 296 differ in practice due to instrumental non-linearity or uneven isotopic enrichment of the
 297 molecular ions. To compute a weighted average of all $\delta^{37}Cl$ values into one mean value,
 298 weighting factors w_j are computed from the relative intensities of dual peak groups (eq. 10)³⁰

$$299 w_j = \frac{I_j + I_{j+1}}{2 \sum_{j=1}^{p-1} I_j + I_0 + I_p} \quad (10)$$

300 The mean chlorine isotope composition is finally calculated with eq. 11³⁰:

$$301 \delta^{37}Cl = \sum w_j \delta^{37}Cl_j \quad (11)$$

302 Derivation of equation 7 is given in SI, Section B. All equations were programmed in
 303 an MS Excel worksheet, which is given as supplementary information for calculation of
 304 chlorine isotope ratios based on multiple ions and molecular ion.

305 4. Results and Discussion

306 4.1 Validation of the evaluation scheme based on a theoretical mass spectrum

307 4.1.1 Chlorine isotope ratios of chlordecone hydrate

308 The evaluation scheme of ³⁷Cl isotope analysis of chlordecone hydrate was first tested
309 on its theoretical mass spectrum obtained from the *Isotope Distribution Calculator and Mass*
310 *Spec Plotter* software available online,⁴² and depicted in Figure 1c. For this, ion peak intensities
311 were retrieved from a low mass resolution spectrum with 0.0001% minimum abundance and
312 are given in Table 3. This theoretical mass spectrum was in accordance with the measured TOF
313 mass spectrum of chlordecone hydrate (SI, Section A).

314 Evaluation of chlorine isotope ratios of chlordecone hydrate from the theoretical mass
315 spectrum, including correction factors shown in Table 2, resulted in a $\delta^{37}\text{Cl}$ value of +13.1 ‰
316 (Table 3). When chlorine isotopes were evaluated without including correction factors, a drift
317 of $\delta^{37}\text{Cl}$ values of +17.4 ‰ was calculated (Table 3). Neither of these values are close to the
318 anticipated $\delta^{37}\text{Cl}$ value of 0‰ referenced to the SMOC scale. It must be noted that the
319 theoretical mass spectrum of chlordecone hydrate (Figure 1c) is calculated based on the
320 international standard ratio R_{SMOC^*} of 0.324. This value is often used in some literature
321 dedicated to structural identification by mass spectrometry.⁴⁸ This R_{SMOC^*} value is
322 approximately 13.1 ‰ higher than the international standard ratio typically used for chlorine
323 isotope analysis in environmental studies ($R_{\text{SMOC}} = 0.319766$)⁴⁴. If we subtract 13.1 ‰ from
324 results in the last two columns of Table 3 we get a $\delta^{37}\text{Cl}$ value of 0 ‰ for the evaluation method
325 with correction factors and +4.3 ‰ for the method without correction factors. The first value
326 results in the anticipated value of 0‰ referenced to the SMOC scale, thus satisfactory for mass
327 spectrometry and probably better than any measurement error. The significant drift between
328 $\delta^{37}\text{Cl}$ values calculated with and without correction factors (>4 ‰) suggests that the integration
329 of correction factors is needed for calculating chlorine isotope ratios of polychlorinated

330 compounds, such as chlordecone hydrate. This is also necessary as changes in $\delta^{37}\text{Cl}$ values in
 331 biochemical reactions of polychlorinated compounds are usually not much larger than 5%.⁴⁹

332

333 **Table 3.** Evaluation of $\delta^{37}\text{Cl}$ values from the theoretical spectrum of chlordecone hydrate.^a

| No. of Cl atoms j | Mass (rounded) | Relative Intensity ^a | Calculated $\delta^{37}\text{Cl}_j$ (‰) ^b | Weighted Factor w_j | Calculated with correction $\delta^{37}\text{Cl}_j$ (‰) ^c | Calculated without correction $\delta^{37}\text{Cl}_j$ (‰) ^{b, c, d} |
|---------------------|----------------|---------------------------------|--|-----------------------|--|---|
| 0 | 502.7 | 0.0646 | 13.1 | 0.15475 | 2.03 | 2.48 |
| 1 | 504.7 | 0.2099 | 13.1 | 0.29063 | 3.81 | 4.86 |
| 2 | 506.7 | 0.3071 | 13.1 | 0.32129 | 4.20 | 5.67 |
| 3 | 508.7 | 0.2665 | 13.1 | 0.23333 | 3.04 | 4.44 |
| 4 | 510.7 | 0.1519 | n.a. ^e | n.a. | n.a. | n.a. |
| | | | | | $\delta^{37}\text{Cl}$ | $\delta^{37}\text{Cl}$ |
| | Sum | 1 | n.a. | 1 | +13.1^c | +17.4 |

334 ^a theoretical mass spectrum of chlordecone hydrate (SI, Section C and MS Excel supplementary information)

335 ^b calculated with equations 7-9 with R_{SMOC} of 0.319766

336 ^c calculated with equation 10, including weighted average calculated with equation 11

337 ^d correction factors were set to 1

338 ^e n.a. not applicable

339

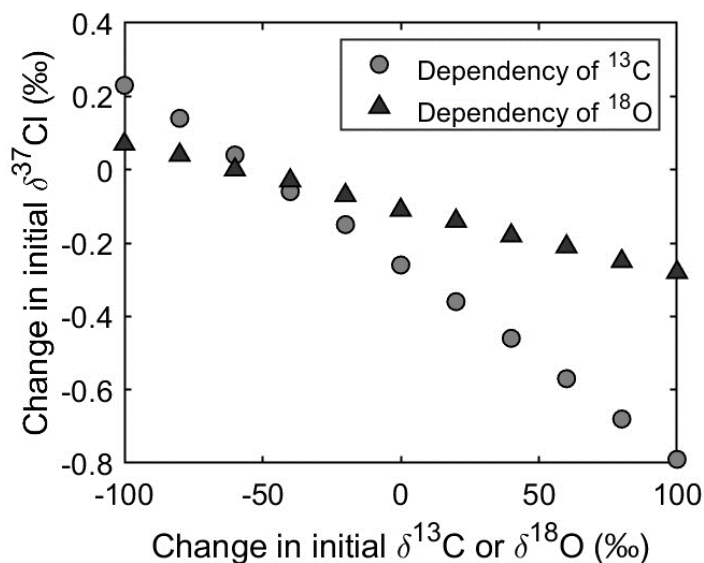
340 Column 4 in Table 3 also shows that calculated $\delta^{37}\text{Cl}$ values from peaks of high
 341 intensities are constant and close to the mean $\delta^{37}\text{Cl}$ value of +13.1 ‰. When the evaluation of
 342 chlorine isotope ratios of chlordecone hydrate includes all ion peaks, $\delta^{37}\text{Cl}$ values from peaks
 343 of low intensities (rounded mass of 512.7 and higher) significantly vary of ± 41 ‰ (SI, Section
 344 D). The sum of weighted $\delta^{37}\text{Cl}$ values is, however, well calculated since weighted values of
 345 these low abundant ions are very small (<3.5%; SI, Section D). Moreover, TOF-MS
 346 measurements did not show good reproducibility of the low abundant ion peaks (SI, Section
 347 A). This supports the recommendation of only using the 5 most abundant ion peaks of
 348 chlordecone hydrate for accurate chlorine isotope analysis by LC-QTOF-MS.

349 4.1.2 Correction for ^{13}C , ^{17}O , ^{18}O and ^2H on the isotope ratio of ^{37}Cl of chlordecone hydrate

350 Correction factors accounting for variations of different elements in an isotopologue are
 351 often considered. For chlordecone hydrate, the presence of ^{13}C , ^{17}O , ^{18}O and ^2H can interfere in

352 the measurement of chlorine isotope ratios, as shown in Table 3 for $\delta^{37}\text{Cl}$ values calculated with
353 and without correction factors. In the TOF mass spectrum of chlordecone hydrate (Figure 1b),
354 a substitution of ¹⁸O for ¹⁶O will mimic the presence of one ³⁷Cl instead of a ³⁵Cl. The same can
355 be observed when a ¹³C is present together with either a second ¹³C, a ¹⁷O or a ²H isotope.

356 Variations in the initial isotope signature of heavy C, O and H also can result in different
357 $\delta^{37}\text{Cl}$ values of chlordecone hydrate. The correction factors for this study were initially
358 calculated for typical stable carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotope signatures of -30% and
359 of $+0\%$, respectively, and are presented in Table 2. We further investigated how the calculated
360 isotope ratio of ³⁷Cl would deviate from a true value if ¹³C and ¹⁸O isotope ratios were different
361 than -30% and $+0\%$, respectively. To this end, both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values were varied from
362 -100 to $+100\%$. Figure 2 shows that the variation in $\delta^{18}\text{O}$ values affects $\delta^{37}\text{Cl}$ values by an
363 error smaller than 0.2% . Thus, calculations of $\delta^{37}\text{Cl}$ values in chlordecone hydrate will be
364 accurate even if the oxygen isotope ratio in the compound is unknown and largely differs from
365 zero. This is mainly because chlordecone hydrate has only two oxygen atoms (Figure 1a). For
366 polychlorinated compounds having more than two oxygen atoms, a more extended table of
367 isotopologues should be made analogously to Table 1 to quantify the effect of oxygen on
368 chlorine isotope ratios.



369

370 **Figure 2.** Influence of heavy carbon (^{13}C) and oxygen (^{18}O) isotope ratios on the computed
371 $\delta^{37}\text{Cl}$ value of chlordecone hydrate.

372 For variations in carbon isotope ratios in chlordecone hydrate, $\delta^{37}\text{Cl}$ values showed
373 variations leading to an error of up to 1‰ (Figure 2). It is thus recommended that the initial
374 $\delta^{13}\text{C}$ value of the target compound is known and that this value should not be too far away from
375 the $\delta^{13}\text{C}$ value used for calculating the correction factors.¹⁷ For chlordecone hydrate, four
376 commercial formulations from different manufacturers were analyzed previously with $\delta^{13}\text{C}$
377 values ranging between -34 ‰ and -31 ‰.¹⁸ This is very close to the $\delta^{13}\text{C}$ value of -30 ‰
378 used for the theoretical correction factors (Table 2). Figure 2 shows that the deviation in $\delta^{37}\text{Cl}$
379 for values close to -30 ‰ will be negligible (<0.02 ‰). Hence, the evaluation scheme presented
380 in this work for chlorine isotope analysis of highly chlorinated compounds can be validated for
381 chlordecone hydrate following the multiple ion method with correction factors, as long as the
382 $\delta^{13}\text{C}$ values remain close to -30 ‰.

383 4.2 Validation of the evaluation scheme based on experimental LC-QTOF-MS data

384 4.2.1 Optimization of instrument parameters

385 To further assess the applicability of the proposed evaluation scheme with an
386 experimental mass spectrum, a series of chlordecone hydrate standards were analyzed using

387 LC-QTOF-MS. The full spectral data (Figure 1b) was used for the 5 most abundant ion peaks
388 with rounded masses of 502.7 to 510.7. The precision and reproducibility of the method was
389 first evaluated for different TOF-MS conditions. First, formic acid (FAc) and FAc with
390 ammonium formate (AF) were tested to evaluate chlordecone signal and optimize the mobile
391 phase. FAc is one of the most common mobile-phase modifiers used for LC-based methods.
392 However, the addition of AF to FAc mobile phases has been suggested to improve signal
393 intensity, peak capacity and ionization efficiency.⁵⁰

394 Evaluation of the chlordecone hydrate TOF-MS spectrum from standards infused at
395 1000 and 5000 µg/L, with and without AF, showed variations in chlordecone signal intensity
396 and distortion of the isotope distribution (Table 4 and SI, Section F). From each chlordecone
397 infusion, 25 scan points were retrieved. Chlordecone hydrate solutions amended with AF
398 resulted in higher peak intensity signals for rounded mass 506.7 compared to solutions acidified
399 with FA only, at a concentration of 1000 µg/L. While higher chlordecone peak intensity was
400 observed in solutions amended with AF, the standard deviation of the ten consecutive infusions
401 was significantly higher ($\sigma = 40\%$) compared to solutions acidified with FAc only ($\sigma = 2.5\%$).
402 A distortion of the isotope distribution of chlordecone hydrate also was observed with AF
403 amendments, in which the normalized abundance of the measured peak intensity of rounded
404 mass 506.7 was 0.3262 compared to the “theoretical” value of 0.3070 (Table 2). This effect was
405 also observed for chlordecone standard solutions at 5000 µg/L (Table 4). This suggests that AF
406 is not suitable as a mobile-phase modifier for chlordecone hydrate analysis, producing
407 deformation of the chlordecone hydrate isotope distribution by LC-QTOF-MS. Moreover,
408 chlordecone solutions above 1000 µg/L likely exceed the detector capacity, resulting in
409 saturated peak signals which are often observed in TOF-MS.^{51,52} This is also indicated by the
410 comparison of the measured saturated peak signals with the theoretical mass spectra of the
411 target compound⁵³ (SI, Section F).

412 **Table 4.** Effect of mobile-phase modifiers on ion intensity signals and mass spectra from
 413 which chlorine isotope ratios of chlordecone hydrate are calculated.

| Infused chlordecone solutions | Mobile-phase modifiers | Measured peak intensity of rounded mass 506.7 ^a | STDEV (%) (n=10) | Normalized abundance of rounded mass 506.7 ^b |
|-------------------------------|------------------------|--|------------------|---|
| 1000 µg/L | 0.1% FAc + 5 mM AF | $5.75 \times 10^5 \pm 2.33 \times 10^5$ | 40.6 | 0.3262 |
| 1000 µg/L | 0.1% FAc | $2.0 \times 10^5 \pm 5.65 \times 10^3$ | 2.5 | 0.3115 |
| 5000 µg/L | 0.1% FAc + 5 mM AF | $1.20 \times 10^6 \pm 3.64 \times 10^5$ | 30.5 | 0.3282 |
| 5000 µg/L | 0.1% FAc | $2.4 \times 10^5 \pm 7.38 \times 10^4$ | 30.0 | 0.3163 |

414 ^a measured by LC-TOF-MS (SI, Section F)

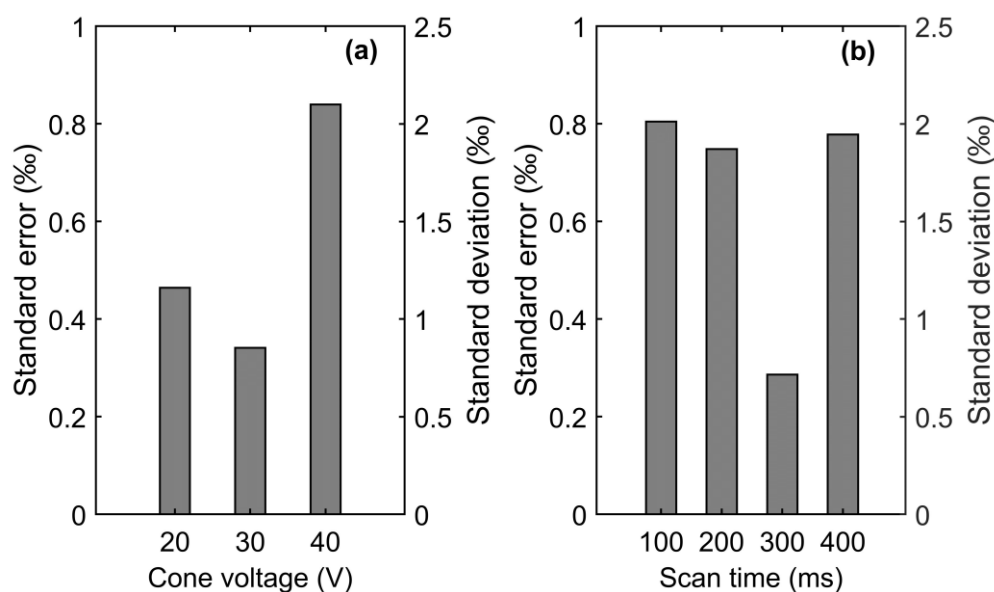
415 ^b theoretical values are presented in Table 3

416

417 Chromatographic separation was then performed with the optimized mobile phase and
 418 following classical column gradients for chlordecone analysis. ^{39,54} To test the effect of TOF-
 419 MS instrument parameters on chlorine isotope ratios, chlordecone standard “samples”
 420 (CLD_{CLUZEAU}) were first analyzed at different sampling cone tensions (20-40 V) and scan times
 421 (100-400 ms). Each chlordecone standard sample represents ten consecutive injections
 422 performed at 500 µg/L. The precision of the method was evaluated based on standard errors
 423 (σ_m). Overall, σ_m ranged from 0.34 to 0.83‰, equivalent to a standard deviation (σ) of 0.76 to
 424 2.22‰, respectively (Figure 3a). The method was thus optimized according to the highest
 425 precision obtained at an ionization energy of 30 V.

426 Different scan times can result in significant effects on method performance for chlorine
 427 isotope ratios. ³⁰ For TOF-MS performance, the highest precision of the method was achieved
 428 at a scan time of 300 ms, corresponding to approximately 25 scan points for background peak
 429 definition. This resulted in a standard error (σ_m) of 0.28‰, which corresponds to a standard
 430 deviation (1σ , $n = 8$) of 0.64‰ (Figure 3b). Particularly, the low and large scan times of 200
 431 ms and 400 ms, respectively, resulted in $\sigma_m > 1\%$. Generally, a large scan time reduces the scan
 432 rate at which data is collected resulting in fewer data points from which the chromatogram peak
 433 shape is obtained. At 400 ms, the peak shape of chlordecone hydrate consisted of approximately
 434 14 scan points. Low numbers of scan points have been suggested to introduce errors in
 435 calculating chlorine isotope ratios from conventional MS instruments. ¹⁷ While the peak

436 capacity can be affected at large scan times, short scan times (e.g., 100 ms) can introduce more
437 variability and compromise sensitivity of ESI-MS due to lower signal-to-noise ratios.⁵⁵ Here,
438 chromatographic peak shape of chlordecone hydrate consisted of more than 70 scan points,
439 which likely introduced instrument variability affecting chlorine isotope ratios. Hence,
440 optimization of scan time is necessary to ensure the fidelity of chlorine isotopic data.



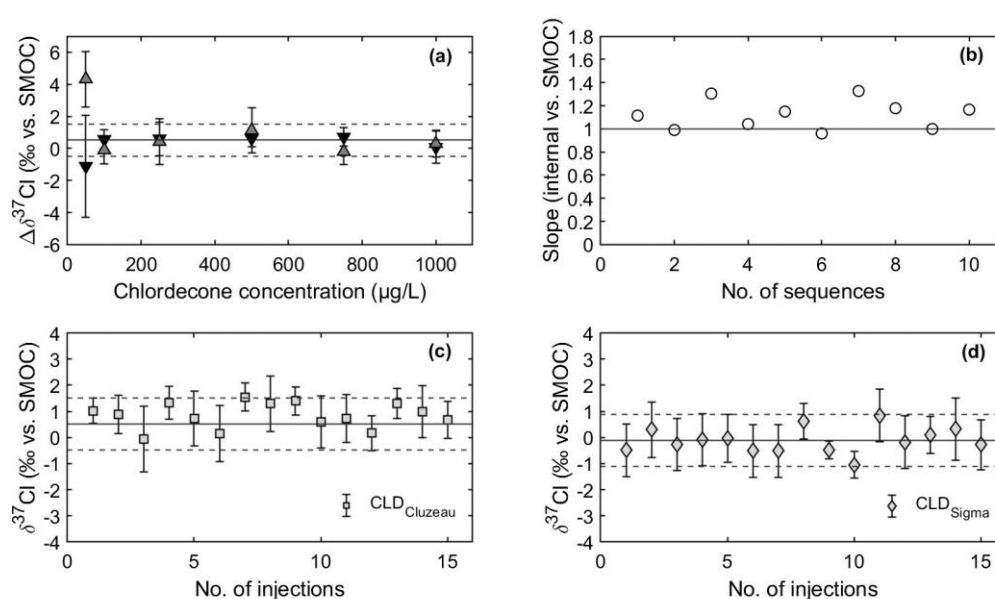
441 **Figure 3.** Effect of TOF-MS instrument parameters on the precision of chlorine isotope ratios
442 of chlordecone hydrate. (a) Influence of sampling cone voltage which varied from 20 to 40 V.
443 (b) Influence of scan time which varied from 100 to 400 ms. Standard errors (σ_m) are for $n = 5$ -
444 10. For comparison, standard deviation (σ) is also included.
445

446

447 4.2.2 Method performance, linearity and two-point calibration

448 The amount of analyte entering the column and reaching the MS can affect both the
449 precision and accuracy of stable chlorine isotope analysis. The precision and linearity range of
450 our TOF-MS instrument was thus tested after optimization of the MS configuration.
451 CLD_{CLUZEAU} standard samples were injected at varying concentrations of analyte (50-1000
452 $\mu\text{g/L}$; $n = 5$ -10) and bracketed with CLD_{SIGMA} which served as an external standard. This
453 bracketed standard was prepared at concentrations varying $\pm 20\%$ from the CLD_{CLUZEAU}
454 standard sample concentrations. The linearity range of the instrument was tested in two different

455 sessions. Figure 4a shows the $\delta^{37}\text{Cl}$ values of $\text{CLD}_{\text{CLUZEAU}}$ obtained with TOF-MS, referenced
 456 to the external standard and subsequent normalization relative to the SMOC scale with the two-
 457 point linear regression.²⁹ Following the multiple ion method with corrected values (Section 3),
 458 the lower and upper detection limits of chlordecone hydrate for valid chlorine isotope ratios
 459 were 100 $\mu\text{g/L}$ (i.e., 0.2 mM) and 1000 $\mu\text{g/L}$ (i.e., 2 mM), respectively. Overall, a good
 460 precision of chlorine isotope ratios was observed within the total accepted uncertainty of (σ_m)
 461 $\pm 1\text{‰}$ (Figure 4a).



462

463 **Figure 4.** Precision, trueness and instrument linearity for chlorine isotope analysis of
 464 chlordecone hydrate. (a) Instrument linearity of amount of analyte injected for the chlordecone
 465 standard $\text{CLD}_{\text{CLUZEAU}}$. Symbols represent two independent sequences, and each symbol
 466 represents $n = 5-10$. Solid horizontal lines represent the SMOC value of $\text{CLD}_{\text{CLUZEAU}}$ obtained
 467 by dual-inlet IRMS. Dashed horizontal lines represent the accepted total uncertainty of TOF-
 468 MS of $\pm 1\text{‰}$ (σ_m). Error bars represent standard error (σ_m) for $n = 5-10$. (b) Evolution of the
 469 calibration slope (two-point calibration against the international SMOC scale) against number
 470 of sequences. Each symbol represents $n = 5-10$, and sequences expanded for more than 3
 471 months. Solid horizontal line represents the expected slope of 1 (QTOF-MS vs. SMOC values).
 472 (c, d) Temporal monitoring of measured chlorine isotope ratios of chlordecone standards
 473 $\text{CLD}_{\text{CLUZEAU}}$ (500 $\mu\text{g/L}$) and $\text{CLD}_{\text{SIGMA}}$ (500 $\mu\text{g/L}$), respectively, after conversion to the SMOC
 474 scale and expressed as $\delta^{37}\text{Cl}$ values (‰). Solid horizontal lines are the average $\delta^{37}\text{Cl}$ values
 475 obtained by dual-inlet IRMS of $+0.52 \pm 0.17\text{‰}$ and $-0.11 \pm 0.17\text{‰}$, respectively. Dotted
 476 horizontal lines represent the accepted total uncertainty of QTOF-MS of 1‰ (σ_m). Standard
 477 errors of measured $\text{CLD}_{\text{CLUZEAU}}$ and $\text{CLD}_{\text{SIGMA}}$ standards by LC-QTOF-MS were 0.82‰ and
 478 0.89‰ , respectively. Error bars represent standard error (σ_m) for $n = 5-10$.

479

480 The trueness of the calculated chlorine isotope ratios of chlordecone standards, i.e., the
481 offset between the TOF-MS values and the two SMOC referenced standards obtained by dual-
482 inlet IRMS, was also evaluated. For each TOF-MS sequence, consisting approximately of 15
483 measurements of $n = 5-10$, both CLD_{SIGMA} and CLD_{CLUZEAU} standards were tested, allowing
484 evaluation of the instrument drift. Bernstein and co-workers (2011)²⁹ suggested that instrument
485 drift can produce nonsystematic changes in the analysis of chlorine isotope ratios, which can
486 affect the evolution of the slope of the external two-point regression.^{29,35} Figure 4b shows the
487 normalized TOF-MS values relative to the SMOC scale. An average slope of 1.19 ± 0.04 was
488 obtained for sequences performed within a span of three months, indicating a good long-term
489 accuracy of chlorine isotope ratios by TOF-MS. This also indicated that chlorine isotope ratios
490 obtained from TOF-MS and the two SMOC referenced standards were in good agreement
491 (Figure 4c-d).

492 Method performance between two independent sequences of CLD_{CLUZEAU} and
493 CLD_{SIGMA} resulted in accurate chlorine isotope ratios with no systematic drift (Figure 4c-d).
494 Standard errors of measured CLD_{CLUZEAU} and CLD_{SIGMA} standards by LC-QTOF-MS were
495 0.82‰ and 0.89‰, respectively, which are satisfactory for environmental studies.³⁵ This also
496 supports the reproducibility and accuracy of bracketed external standards within a sequence
497 consisting of 15 measurements for our LC-QTOF-MS. It is important, however, that the quality
498 of the raw data for each injection should be carefully evaluated, as aberrant values can be
499 introduced from unexpected instrument instability. For instance, TOF-MS instruments are
500 highly sensitive and prone to mass drift due to ambient temperature fluctuations,⁵⁶ thus
501 requiring the use of a lock-mass calibration solution. For this study, a series of daily TOF-MS
502 instrument calibrations were performed prior to each sequence, and mass accuracy was
503 calibrated during each run. Moreover, cone cleaning was performed after 3 sequences to avoid

504 overload MS signals. It is thus suggested that in-house TOF-MS instrument calibrations are
505 carried out prior to a sequence and during each run to ensure accurate chlorine isotope analysis
506 of polychlorinated compounds by LC-QTOF-MS.

507 **5. Conclusions and outlook**

508 The present work proposes a new evaluation scheme for obtaining chlorine isotope
509 ratios of polychlorinated compounds using conventional/common mass spectrometers, such as
510 LC-QTOF-MS. The proposed scheme goes further than Aeppli and co-workers (2010)¹⁷ who
511 corrected only for double presence of ¹³C and made use only of the intensities of the two peaks
512 of lowest mass with 0 or 1 heavy chlorine atoms. Integration of corrections factors for heavy
513 C, O and H isotopes was necessary to accurately calculate ³⁷Cl isotopes of chlordecone hydrate,
514 following our multiple ion method of the five most abundant ion peaks. Calculations of chlorine
515 isotope ratios for chlordecone hydrate are provided in an MS Excel worksheet. This Excel
516 worksheet can be adapted for other polychlorinated compounds with different numbers of
517 carbon, chlorine, oxygen or even hydrogen atoms.

518 Despite the improved evaluation scheme, great care must be given to account for
519 instrumental non-linearities. This issue has been discussed in detail by Ebert and co-workers⁴⁰
520 and can only be resolved when two isotope standards of different $\delta^{37}\text{Cl}$ values are measured
521 along with the unknown samples. The problem today is that such standards are not available
522 for every compound and must be synthesized or enriched from case to case. Chlordecone
523 exemplifies this by having only two available commercial standards, which can be
524 characterized and used for external calibrations. Overall, the good accuracy of chlorine isotope
525 ratios of chlordecone hydrate by our evaluation scheme with LC-QTOF-MS data demonstrates
526 the promising use of C-Cl CSIA to investigate degradation pathways and mechanisms of
527 chlordecone hydrate.

528 Acknowledgments

529 This work is funded by the French National research Agency ANR through grant ANR-18-
530 CE04-0004-01 project DECISIVE. We thank Neil Sturchio and Linnea Heraty from University
531 of Delaware for the calibration of chlordecone standards by dual-element IRMS. We thank
532 Amandine Durand, Stéphanie Lebarillier and Asma Ben Salem for laboratory assistance.

533 Author contributions

534 Maria Prieto-Espinoza: Methodology, Data acquisition, Writing, Reviewing. Laure Malleret:
535 Conceptualization, Methodology, Writing, Reviewing. Sylvain Ravier: Data acquisition,
536 Reviewing. Patrick Höhener: Conceptualization, Methodology, Writing, Reviewing,
537 Supervision, Fund acquisition.

538 Supporting information

539 All equations were programmed in an MS Excel worksheet. TOF-MS data from 10 consecutive
540 injections of chlordecone hydrate, derivation of equation 7 and information of measured
541 chlordecone mass spectrum, number of injections and stable chlorine isotope analysis of
542 chlordecone hydrate is provided (PDF).

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