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Detection and quantification of chlordecone in contaminated soils from the French West Indies by GC-MS using the $^{13}C_{10}$-chlordecone stable isotope as a tracer

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Abstract Chlordecone is an organochlorine insecticide that has been widely used to control banana weevil in the French West Indies. As a result of this intense use, up to 20,000 ha are contaminated by this insecticide in the French West Indies, and this causes environmental damage and health problems. A scenario of exposure was drawn by French authorities, based on land usage records. Many efforts have been made to monitor the occurrence of chlordecone and its main metabolites using different analytical methods, including GC, GC/MS, LC/MS, and NIRS. Although these different methods allow for the detection and quantification of chlordecone from soils, none of them estimate the bottleneck caused by extraction of this organochlorine from soils with high adsorption ability. In this study, we used $^{13}C_{10}$-chlordecone as a tracer to estimate chlordecone extraction yield and to quantify chlordecone in soil extracts based on the $^{13}C/^{12}C$ isotope dilution. We report the optimization of $^{13}C_{10}$-chlordecone extraction from an Andosol. The method was found to be linear from 0.118 to 43 mg kg^{-1} in the Andosol, with an instrumental detection limit estimated at 8.84 μg kg^{-1}. This method showed that chlordecone ranged from 35.4 down to 0.18 mg kg^{-1} in Andosol, Nitisol, Ferralsol, and Fluvisol soil types. Traces of the metabolite β-monohydrochlordecone were detected in the Andosol, Nitisol, and Ferralsol soil samples. This last result indicates that this method could be useful to monitor the fate of chlordecone in soils of the French West Indies.

Keywords Chlordecone · Organochlorine pesticide · Soil contamination · Banana plantation

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Introduction

Chlordecone (decachlorooctahydro-1,3,2-metheno-2H-cyclobuta[c,d]pentalen-2-one) is an organochlorine insecticide that was once used worldwide. In the French West Indies, it was used in banana plantations to control the development of weevil, *Cosmopolites sordidus*. Over the 1978–1993 period, approximately 300 t of chlordecone was applied to banana plantations in the French West Indies. As a result of this intensive use, the survey conducted in the context of the French National Action Plan for chlordecone revealed its presence in soils, rivers, springs, and drinking water (PNAC 2008–2010). Since May 2009, chlordecone has been classified as a persistent organic pollutant and listed in Annex A of the Stockholm Convention. Its persistence in the agricultural soils of the French West Indies (i.e., 20,000 ha that represents up to 25 % of the agricultural surface of the FWI) was estimated to last for several decades in Nitisol, centuries in Ferralsol, and half a millennium in Andosol soils (Cabidoche et al. 2009). On the basis of land usage, a map estimating the levels of chlordecone contamination was drawn in order to help the French authorities to establish land usage

recommendations (Le Déault and Procaccia 2009). It gives a first approach of local people's exposure risk to chlordecone. However, it is important to monitor chlordecone concentrations in various soil types of the French West Indies to test the validity of the scenario of exposure to chlordecone and to revise it if discrepancies between expected and measured chlordecone concentrations are recorded.

In a methodological review, Faroon and Kueberuwa (1995) report the detection of mirex and photomirex from soil samples using gas chromatography (GC) and capillary gas chromatography with electron capture detection. Soil samples are usually solvent extracted, cleaned up with Florisil columns, and analyzed by GC. More recently, chlordecone residues extracted from soil were detected by GC coupled with mass spectrometry (MS) (Frenich et al. 2000). In addition, Moriwaki and Hasegawa (2004) proposed to detect chlordecone by liquid chromatography with tandem mass spectrometry. More recently, Brunet et al. (2009) explored the potential of near-infrared reflectance spectroscopy (NIRS) for determining chlordecone contents in Andosols, Nitisols, and Ferralsols from Martinique. NIRS was proposed for classifying the chlordecone contamination levels of agricultural soils but did not give a sharp estimation of these levels. Mirex was also quantified from Chinese soil using high-resolution gas chromatography/high-resolution mass spectrometry (Wang et al. 2010). Keeping in mind the very low detection limit (0.01 mg kg^{-1}), GC-MS remains the reference method for quantifying chlordecone residues in soils. The usual method, notably applied by the laboratory analyses commissioned by the French authorities to analyze soil contamination, involves extraction with two solvents followed by a GC-MS analysis using $^{13}C_{10}$-chlordecone as an injection tracer. However, although chlordecone is known to be highly adsorbed onto soil components (i.e., Koc values ranging from 2,000 to 2,500 l kg^{-1}; ATSDR 1995), the yield of chlordecone extraction from different soil matrices is hardly ever considered.

In order to take into account this missing parameter to optimize detection and tune the detection of chlordecone in contaminated soils of the French West Indies, we proposed the use of $^{13}C_{10}$-chlordecone as a tracer to not only estimate the yield of chlordecone extraction, but also to quantify chlordecone in soil extracts based on the $^{13}C/^{12}C$ isotopic ratio. Here, we report the development of an analytical method for the detection and quantification of chlordecone and its known degradation products (β-monohydrochlordecone and dihydrochlordecone) in contaminated soils spiked with $^{13}C_{10}$-chlordecone, using GC-MS. The optimization of the extraction procedure for a typical West Indies clayish soil is described. This technique was evaluated on four different soil types representative of French West Indies soils.

Materials and methods

Chemicals and reagents

[^{12}C U]-Kepone PESTANAL® (kepone; 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one) was purchased from Sigma-Aldrich (Schnelldorf, Germany) (chemical purity 99.7 %), whereas [$^{13}C_{10}$ U]-chloredecone® (kepone; 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one) was from LGC standards (100 μg/ml solution in nonane, chemical purity 99.0 %). Anthracene-d10, used as an internal standard, was obtained from Sigma-Aldrich. It was diluted to a final concentration of 0.1 mg l^{-1} in dichloromethane used to resuspend the soil extracts.

Soil samples

Experiments were performed on four soils collected in the French West Indies (i.e., Andosol, Ferralsol, Fluvisol, and Nitisol) which were chosen for their physicochemical diversity and for their representativeness of the different classes of soils found on the two islands—Guadeloupe and Martinique. The soil physicochemical properties were estimated by the Laboratory of Soil Analysis (INRA, Arras, France) using ISO procedures (Table 1). Concentrations of chlordecone residues determined by La Drôme Laboratoire (LDA26, Valence, France) working under the French accreditation committee "COFRAC" and norm NF17025 are also shown. These measurements were done by GC-MS. Calibration was performed using the standard addition method, with $^{12}C_{10}$-chlordecone and two internal standards, hexabromobenzene and triphenylphosphate. The results are given with a 30 % confidence interval. The extraction procedure was developed on the Andosol that had the worst profile for chlordecone extraction, with Koc values averaging 20 m^3 kg^{-1} (Cabidoche et al. 2009) and then applied to the other soil types.

GC-MS analysis

GC-MS was performed using a QP2010+ (Shimadzu GC-MS 2010) equipped with an RTx-5 column (10 m×0.18 mm i.d., 0.18 μm film) purchased from Restek, using helium as a carrier gas, with a temperature ramp from 80 to 280 °C at 40 °C/min. Anthracene-d10 was used as an internal standard. Soil extracts were injected into the column using a splitless/split injector in splitless mode. Detection of ^{12}C-chlordecone, its two metabolites, and $^{13}C_{10}$-chlordecone was performed using electronic impact at 70 eV, which breaks up the compounds into several fragments that display different mass spectra. The full mass spectra of ^{12}C-chlordecone, its two metabolites, and $^{13}C_{10}$-chlordecone were acquired by full-scan single-quadrupole mass spectra in the 29 to 550

Table 1 Soil physicochemical properties

Soil type	Andosol	Nitisol	Ferralsol	Gleyic Fluvisol	Gleyic Fluvisol
Horizon	0–20 cm	0–20 cm	0–20 cm	0–20 cm	30–50 cm
Latitude	61°37′15″W	61 °34′20.3″W	61°34′16.5″W	61°33′51″W	61°33′51″W
Longitude	16°03′57″N	16°05′29.5″N	16°06′08.4″N	16°07′54″N	16°07′54″N
Clay (<2 μm, g/kg)	128	666	na	288	208
Fine silt (2/20 μm, g/kg)	99	216	na	353	383
Coarse silt (20/50 μm, g/kg)	60	43	na	171	209
Fine sand (50/100 μm, g/kg)	37	28	na	164	181
Coarse sand (200/2,000 μm, g/kg)	676	47	na	24	19
Organic C (g/kg)	79.8	23.3	na	17.7	12
Total N (g/kg)	6.11	2.02	na	1.51	0.99
C/N	13.0	11.6	na	11.7	12.0
Organic matter (g/kg)	138	40.3	na	30.5	20.8
pH water (a.u.)	5.21	5.48	na	7.41	7.97
pH KCl (a.u.)	4.53	4.66	na	6.18	6.56
$CaCO_3$ (g/kg)	<1	<1	na	<1	<1
P_2O_5 (g/kg)	0.042	0.014	na	0.104	0.015
CEC Metson (cmol+/kg)	29.6	15.2	na	20.7	19.0
Chlordecone (mg/kg)[a]	33.6	1.61	1.79	0.06	0.06

na not available

[a] Chlordecone analysis done by LDA26 (France)

m/z range. Mass spectra and *m/z* fragments characteristic of these compounds are given in Fig. S1. Each prepared extract was then injected and detected in SIM mode in order to improve MS analysis sensitivity and accuracy. Target-specific fragments for each compound are listed hereafter: (a) ^{12}C-chlordecone *m/z* 490 (molecular ion), 455 ($C_{10}Cl_9O^{\cdot+}$), 272 ($C_5Cl_6^{\cdot+}$), 237 ($C_5Cl_5^{\cdot+}$), and 218 ($C_5Cl_4O^{\cdot+}$); (b) $^{13}C_{10}$-chlordecone *m/z* 502 (molecular ion), 465 ($C_{10}Cl_9O^{\cdot+}$), 277 ($C_5Cl_6^{\cdot+}$), 242 ($C_5Cl_5^{\cdot+}$), and 223 ($C_5Cl_4O^{\cdot+}$); (c) β-monohydrochlordecone *m/z* 456 (molecular ion), 421 ($C_{10}HCl_8O^{\cdot+}$), 272 ($C_5Cl_6^{\cdot+}$), 237 ($C_5Cl_5^{\cdot+}$), 203 ($C_5HCl_4^{\cdot+}$); and (d) dihydrochlordecone *m/z* 418 (molecular ion), 238 ($C_5HCl_5^{\cdot+}$) and 203 ($C_5HCl_4^{\cdot+}$). The amounts of ^{12}C-chlordecone and of ^{13}C-chlordecone in the samples were estimated by quantifying *m/z* 272 and 277 ions, respectively. ^{12}C-chlordecone (*m/z* 272) was directly quantified relative to $^{13}C_{10}$-chlordecone (*m/z* 277) spiked into the soil samples prior to chemical extraction. The yield of $^{13}C_{10}$-chlordecone extraction from the soil was calculated by measuring how much was recovered from soil extracts as compared to the injection of a standard solution containing $^{13}C_{10}$-chlordecone in GC-MS. In addition, the amounts of β-monohydrochlordecone and dihydrochlordecone were quantified by quantifying *m/z* 272 and 238 ions, respectively. We observed that although chlordecone and β-monohydrochlordecone were both quantified using the *m/z* 272 ion, the metabolite had a shorter migration time than the mother compound, and this allowed for their discrimination. Similarly, the amount of chlordecone metabolites was calculated by taking into account the response factor between *m/z* 272 and 238 ions and the *m/z* 277 ion of $^{13}C_{10}$-chlordecone. Those response factors were determined—thanks to a separate injection that contained the two metabolites and $^{13}C_{10}$-chlordecone. By doing so, we assumed that the extraction yield of the two metabolites was equivalent to that of chlordecone.

Results and discussion

In a first approach, the extraction procedure was optimized on the Andosol which represented the worst case scenario: it was rich in clay and presented a very high adsorption capacity. Three parameters were optimized: (a) soil sample homogenization (aqueous suspension, soil matrix dispersion), (b) type of solvent used for the extractions (dichloromethane versus pentane/acetone), and (c) pH of the extractant (pH 3, 7, or 11). The influence of the homogenization of soil samples on chlordecone quantification was addressed. To do so, 10 g (dry weight equivalent) of air-dried and sieved Andosol was spiked with 10 μl of $^{13}C_{10}$-chlordecone dissolved in nonane at 100 μg ml^{-1}. After evaporation of the solvent by incubating the soil samples at room temperature for 2 h, the samples were then homogenized in 10 ml of 100 mM phosphate buffer, pH 7. The soil mixtures were then placed in an ultrasonic bath for 10 min at room temperature. Half of the samples were mixed with 1:8

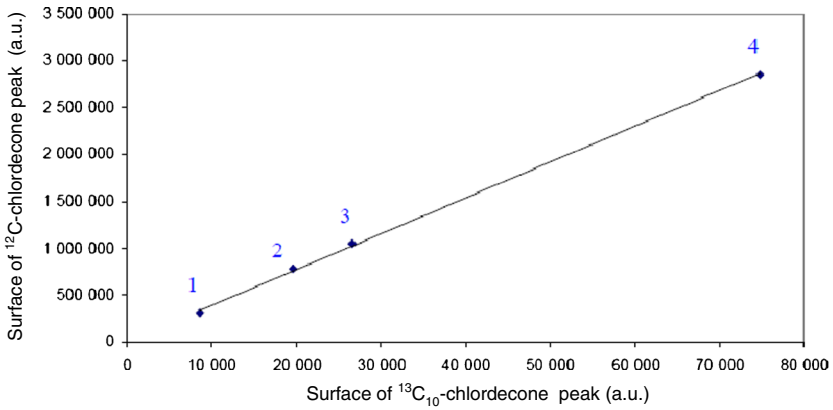

Fig. 1 Linear regression between ^{12}C- and $^{13}C_{10}$-chlordecone in the Andosol. Evolution of the amount of ^{12}C-chlordecone quantified in the soil extract as a function of the ^{13}C-chlordecone spiked into the Andosol prior to extraction

anhydrous sodium sulfate (*w*/*w*). Homogenized samples were then extracted with 80 ml of distilled dichloromethane. The extracts were shaken with a magnetic stirrer and placed in an ultrasonic bath for 10 min at room temperature. After decanting, the organic phase was recovered and purified by solid phase extraction using Florisil adsorbent. The extract was dried, concentrated, and redissolved in 200 μl of dichloromethane containing anthracene-d10 used as an internal standard. The purified extracts were then injected and analyzed in GC-MS. Quantification of the surface of the *m*/*z* 272 fragment revealed that soil homogenization in phosphate buffer with anhydrous sodium sulfate added increased the yield of chlordecone extraction 9.5-fold as compared to soil homogenization in phosphate buffer (Fig. S2). Therefore, this homogenization method was kept for the rest of the study. The influence of the solvent used for the extraction of homogenized soil was also studied, by extracting homogenized soil using either dichloromethane or a mixture made of pentane/acetone (1/1; *v*/*v*). Extraction, purification, and injection conditions were similar to those described above. Quantification of the surface of the *m*/*z* 272 peak revealed that extraction with dichloromethane yielded 2.5-fold more chlordecone as compared to extraction with the pentane/acetone mixture (Fig. S3). Dichloromethane was therefore chosen as the solvent for extracting chlordecone from the soil. Finally, the influence of the pH of the extractant on chlordecone extraction was studied by conducting homogenization in 100 mM phosphate buffer at pH 3, pH 7, or pH 11. Anhydrous sodium sulfate was added, extraction was done with dichloromethane, and purification and injection conditions were similar to those described above. Quantification of the surface of the *m*/*z* 272 peak revealed that soil homogenization with phosphate buffer at pH 7 yielded 1.3-fold more chlordecone as compared to homogenization with phosphate buffer at pH 3, while hardly any difference was recorded between the soil samples homogenized with phosphate buffer at pH 7 or pH 11 (Fig. S4). The extractant at pH 7 was therefore chosen to conduct chlordecone extraction from the soil.

In order to validate the use of $^{13}C_{10}$-chlordecone as a tracer to estimate chlordecone extractability from the soil, $^{13}C_{10}$-chlordecone was spiked into the soil and extracted using the different procedures tested above. The surfaces of the *m*/*z* 272 and *m*/*z* 277 peaks specific for ^{12}C-chlordecone and $^{13}C_{10}$-chlordecone, respectively, were recorded (Fig. S4). It is noteworthy that whatever the procedure used to extract chlordecone from the soil, a linear relationship was found between the amounts of $^{13}C_{10}$- and $^{12}C_{10}$-chlordecone (Fig. 1). This observation suggests that $^{13}C_{10}$-chlordecone was extracted from the Andosol in a similar manner to unlabelled chlordecone from the Andosol. It also suggests that the protocol used for addition of $^{13}C_{10}$-chlordecone to soil yields a distribution within the soil which

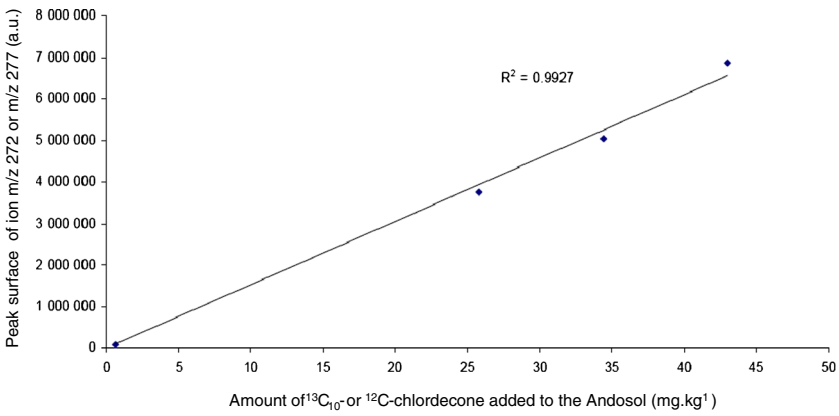

Fig. 2 Linear regression between the amount of chlordecone spiked into the Andosol and the quantification of the corresponding ion *m*/*z* by GC-ECD-MS. Evolution of the peak surface of *m*/*z* 272 or 277 ions quantified by GC-ECD-MS in the soil extract as a function of the $^{13}C_{10}$- or ^{12}C-chlordecone spiked into the Andosol, respectively

Table 2 Estimation of chlordecone and metabolites β-monohydrochlordecone and dihydrochlordecone in different soil types

Soil type	Andosol	Nitisol	Ferralsol	Gleyic Fluvisol (0–20 cm)	Gleyic Fluvisol (30–50 cm)
Chlordecone (mg kg^{-1})	35.4a	1.33b	1.44b	0.10c	0.18c
Coefficient of variation (%)	16.8	2.38	5.40	4.27	4.67
Yield of extraction (%)	21.6	26.9	18.7	16.3	21.5
Coefficient of variation (%)	10.7	15.1	2.68	26.4	11.1
β-monohydrochlordecone (mg kg^{-1})	0.649	0.00945	0.0234	nd	nd
Dihydrochlordecone (mg kg^{-1})	nd	nd	nd	nd	nd
Detection limit (μg kg^{-1})	8.84	5.89	5.50	7.70	4.77

Significant differences in chlordecone concentrations between soils are indicated by different letters

nd not detected

is similar to the unlabelled chlordecone already present, a necessary condition for the use of isotope dilution analysis. We therefore conclude that $^{13}C_{10}$-chlordecone is not only an internal standard, but also constitutes a good tracer that could be used to estimate the yield of chlordecone extraction from the soil.

The linearity of our method was evaluated on the Andosol by spiking different amounts of chlordecone. In order to cover a wide concentration range corresponding to the minimal and maximal concentrations previously reported in French West Indies agricultural soils, the soil was spiked with $^{13}C_{10}$-chlordecone corresponding to 0.69 mg kg^{-1} and with different amounts of ^{12}C-chlordecone (25, 35, and 45 mg kg^{-1}). Analysis of the extracts obtained from spiked Andosol showed that the method had a good linearity, with a calibration curve representing the surface of the *m/z* 272 and *m/z* 277 peaks as a function of $^{13}C_{10}$-chlordecone, with a correlation coefficient (r^2) of 0.99 (Fig. 2). To conclude, our method was found to be linear from 0.118 to 43 mg kg^{-1}. Linearity of the method was not checked beyond this range defined with respect to concentrations previously found in contaminated soils.

In order to test the efficiency and the reliability of the method developed here, its performances were tested on four soil types, i.e., Andosol, Nitisol, Ferralsol, and Fluvisol soils, collected in the French West Indies by Dr. YM Cabidoche (INRA, French West Indies). The soil physicochemical properties are shown in Table 1. All these soils are rich in clay and are characterized by different secondary "clay" materials such as allophane for Andosol, halloysite for Nitisol, and halloysite with Fe and Al oxihydroxides for Ferralsol and Fluvisol (Cabidoche et al. 2009) which account for their high sorption ability. Concentrations of chlordecone residues determined by LDA26 at Valence (France), which works under the French accreditation committee COFRAC and standard NF17025, are also shown. For each soil type tested, five replicates were done. These analyses led to the estimation of chlordecone and two of its metabolites (β-monohydrochlordecone and dihydrochlordecone) in these different soil types (Table 2). In addition, detection limit, yield of extraction, and repeatability were also measured. The detection limit, determined from the signal-to-noise ratio observed for the relevant ion in the GC-MS data, ranged between 4.77 and 8.84 μg kg^{-1} of chlordecone. The yield of chlordecone extraction, estimated using $^{13}C_{10}$-chlordecone, varied from 16 to 26 %, with coefficients of variation between 2.7 and 26 %. This parameter did not seem to be related to soil type. The rather low extractability of chlordecone from these four soil types most likely resulted from the high sorption ability of the four soil types which were rich in clay materials. Nonetheless, chlordecone residues were successfully quantified from the soil extracts. As expected, the highest chlordecone concentration was observed in the Andosol (35.4±5.95 mg kg^{-1}). Nitisol and Ferralsol samples were contaminated with chlordecone to a lesser extent, with 1.33±0.05 and 1.44±0.07 mg kg^{-1}, respectively. The lowest chlordecone concentrations averaged 0.10 mg kg^{-1}. They were quantified in the Gleyic Fluvisol. Traces of β-monohydrochlordecone were detected in the Andosol, Nitisol, and Ferralsol, and the highest concentration was observed in the Andosol (0.649 mg kg^{-1}). Dihydrochlordecone was not detected in any of the four soil types tested here. These results are in agreement with those previously obtained by the LDA 26, given with a 30 % confidence interval (shown in Table 1), except for the Fluvisol samples for which a higher chlordecone concentration was observed with our method (2- and 3-fold for the 0–20- and 30–50-cm horizons, respectively). We noted that the use of the $^{13}C_{10}$-chlordecone tracer allowed us to improve the confidence interval of chlordecone measurements in the soil samples but to a lesser extent for the Andosol, admittedly the worst case scenario. In addition, with our method, traces of β-monohydrochlordecone were quantified from the Andosol, Nitisol, and Ferralsol samples. It is noteworthy that this metabolite is a known contaminant of the Kepone® formulation that was applied to banana plantations in the French West Indies (Cabidoche et al. 2009). Therefore, its detection suggests that chlordecone may not be degraded in the tested soils although the process is thermodynamically possible: chlordecone has redox potentials in the 336–413-mV range

and its Eo′ value is similar to that of other organochlorines (Dolfing et al. 2012).

Conclusions

In light of these results, one can conclude that the GC-MS method using $^{13}C_{10}$-chlordecone as a tracer of extraction is convenient for analyzing chlordecone and its known two metabolites in different soil types representative of the soils found in the French West Indies. This method was shown to have a useful instrumental detection limit and linear ranges for chlordecone. It should be useful for monitoring the fate of chlordecone in soils of the French West Indies in the framework of the National Plan for Chlordecone. It could be applied to conduct a survey of chlordecone soil contamination in order to verify the exposure scenario defined according to land usage considerations.

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