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Compound specific isotope analysis of organophosphorus pesticides

Langping Wu [a], Jun Yao [a,1], Polonca Trebse [b,*], Ning Zhang [c], Hans H. Richnow [c]

[a] School of Civil and Environmental Engineering, and National International Cooperation Based on Environment and Energy, University of Science and Technology Beijing, Xueyuan Road No. 30, Haidian District, Beijing 100083, PR China
[b] Laboratory for Environmental Research, University of Nova Gorica, Vipavska 13, Nova Gorica SI-5000, Slovenia
[c] Department of Isotope Biogeochemistry, Helmholtz Centre for Environmental Research-UFZ, Permoserstraße 9 15, Leipzig 04318, Germany

HIGHLIGHTS

- First report on stable carbon isotope fractionation of organophosphorus (OP) pesticides.
- Method development for analyzing carbon isotope composition of OP pesticides.
- Isotope fractionation characterising decomposition pathways of OP pesticides.

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ABSTRACT

Compound-specific isotope analysis (CSIA) has been established as a tool to study the environmental fate of a wide range of contaminants. In this study, CSIA was developed to analyse the stable carbon isotope signatures of the widely used organophosphorus pesticides: dichlorvos, omethoate and dimethoate. The linearity of the GC–C–IRMS system was tested for target pesticides and led to an acceptable isotope composition within the uncertainty of the instrument. In order to assess the accuracy of the developed method, the effect of the evaporation procedure on measured carbon isotope composition ($\delta^{13}C$) values was studied and showed that concentration by evaporation of solvents had no significant isotope effect. The CSIA was then applied to investigate isotope fractionation of the hydrolysis and photolysis of selected pesticides. The carbon isotope fractionation of tested pesticides was quantified by the Rayleigh model, which revealed a bulk enrichment factor (ε) of -0.2 ± 0.1‰ for hydrolysis of dichlorvos, -1.0 ± 0.1‰ and -3.7 ± 1.1‰ for hydrolysis and photolysis of dimethoate respectively. This study is a first step towards the application of CSIA to trace the transport and degradation of organophosphorus pesticides in the environment.

1. Introduction

A growing food demand in the world forces intensive agriculture accompanied by releasing of a variety of agrochemicals into the environment. There are public concerns regarding the use of pesticides and adequate monitoring of the fate of pestidies is an urgent objective. Today, over 500 compounds are registered worldwide as pesticides, or metabolites of pesticides, of which organophosphorus compounds are a highly diverse family of organic chemicals used in high amounts. For example, the annual production of organophosphorus pesticides (OP pesticides) is more than 100.000 t in China, which accounts for more than 80% of total pesticide production (Liu, 2010). In our study we focused on three representatives from the group of OP pesticides: dichlorvos, omethoate and dimethoate. These three OP pesticides are widely used in China, are of public concern, and are on the list of Priority Monitoring Pesticides published by the Ministry of Environmental Protection of the People's Republic of China due to their high toxicity, frequent use and appearance (Jiang, 1993).

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is a volatile organophosphorus insecticide with fumigant and penetrant action. It is predominantly used as a fumigant or spray for stored grain and for grain handling equipment (Oncescu et al., 2010). Dimethoate (O,O-dimethyl S-[2-(methylamino)-2-oxoethyl] dithiophosphate) is an OP pesticide which has both direct and systemic action against a broad range of insect pests. It is considered as 'moderately hazardous, class II' compound by WHO with a permissible limit of 0.006 mg L^{-1} in drinking water. Omethoate (2-[(dimethoxyphosphoryl)sulfanyl]-N-methyl-acetamide) is an structural analog of dimethoate, and appears to play a dominant role in the

* Corresponding author. Tel.: +386 5 331 5238; fax: +386 5 331 5296.
E-mail addresses: yaojun@ustb.edu.cn (J. Yao), polonca.trebse@ung.si (P. Trebse).
[1] Tel./fax: +86 10 62333305.

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toxicity of dimethoate for insects and mammals. These pesticides may be characterised by classical analytical methods, like GC and LC in combination with different detection techniques, like FID, ECD, NPD and MS (Pappas and Kyriakidis, 2003; Evgenidou et al., 2006; Priya et al., 2011). The complexity of aquatic systems always makes it difficult to assess degradation based on concentration data alone, especially, to distinguish degradation from dilution processes in the environment.

Recently, CSIA has opened a promising avenue to study the contaminants behaviours in the environment employing isotope fractionation to trace reactivity. The stability of chemical bonds is dependent on the mass of substituent thus higher activation energy is needed to cleave a bond formed by heavy isotopomers leading to kinetic isotope fractionation in chemical processes (Bigeleisen and Wolfsberg, 1958). This principle controls the reactivity of the individual stable isotopes in the environment and determines isotope fingerprints during synthesis of organic compounds. The isotope composition provides clues that can be used to identify sources, transformation reactions, and sinks of organic compounds in the environment (Meckenstock et al., 2004). The coupling of gas chromatographs with IRMS makes it possible to analyse isotope ratios of individual compounds in complex mixtures and is known as CSIA. CSIA has offered novel avenues to trace transformation processes of contaminants in complex environments because it can be used to identify (Hirschorn et al., 2004; Elsner et al., 2005; Fletcher et al., 2009; Hofstetter and Berg, 2011) and quantify (Abe and Hunkeler, 2006; Aeppli et al., 2010) transformation reactions by determining the isotope composition of organic compound. Over recent years, CSIA has become an increasingly valuable tool and has been applied to study several groups of contaminants, mostly including benzene homologues (Mancini et al., 2003; Fischer et al., 2008), chlorinated ethenes (Vieth et al., 2003; Van Breukelen et al., 2005; Nijenhuis et al., 2007), petroleum hydrocarbons (Richnow et al., 2003a, 2003b), and fuel oxygenates (Mckelvie et al., 2007; Rosell et al., 2007, 2010, 2012). CSIA has been developed for several pesticides, such as Lindane (Badea et al., 2009), isoproturon (Penning et al., 2010) and 2,6-dichlorobenzamide (BAM) (Reinnicke et al., 2012). However, to our best knowledge, the evaluation of OP pesticides by CSIA has, to date, not been reported.

The aim of this study was to develop a method for the analysis of carbon isotope signatures of three OP pesticides (dichlorvos, omethoate and dimethoate) extracted from aqueous samples and to explore the applicability of CSIA to characterise the transformation of OP pesticides. For each compound, the precision of the method was tested as well as the detection limits of precise isotope analysis. All measurements were initially analysed by GC–FID in order to find appropriate chromatographic conditions. Organophosphorus esters are susceptible to hydrolysis, therefore this is the most common environmental degradation pathway, so the method was then applied to assess their isotope fractionation changes during hydrolysis. Photolytic degradation of dimethoate was conducted to demonstrate that CSIA could be used to explore different degradation mechanisms by isotope fractionation.

2. Materials and methods

2.1. Chemicals

High purity standards of three pesticides were selected: dichlorvos (PESTANAL®, analytical standard, 98.8% pure), omethoate (PESTANAL®, analytical standard, 97.0% pure), dimethoate (PESTANAL®, analytical standard, 99.6% pure) were purchased from Fluka (Sigma–Aldrich, Germany). Methanol of HPLC gradient grand (purity ⩾ 99.8%) was supplied by J.T. Baker (Netherlands), while dichloromethane (Assay (GC), purity > 99.9%) were supplied by Fluka (Sigma–Aldrich, Germany). Stock and standard solutions of pesticides were stored at −4 °C. All other chemicals were analytical grade and used without further purification. 2 × DI water was obtained by a NANOpure® ultrapure water system (Barnstead, USA).

2.2. Pesticides extraction

Solid-phase extraction (SPE) using 3 mL DSC-18 cartridges (Discovery®, Bellefonte, USA) were used for extraction of pesticides from aqueous solution. Before extraction, the SPE cartridges were activated by passing consecutively 5 mL of dichloromethane, 5 mL of 2 × DI water, and 5 mL of purified water alkalized to pH 10 with 0.1 M NaOH (or acidified to pH 3 with 0.1 M HCl for control experiments) (Demoliner et al., 2010). Cartridges were then loaded with 6 mL samples and eluted with 1 mL of dichloromethane to 2 mL vials. The extracted phase was stored for subsequent analysis. All extractions were performed in two parallels for each time point of all experiments, one of them was immediately analysed by GC–FID as described below, and the other one was stored at −4 °C for isotope analysis.

2.3. Evaporation experiment

The evaporation test was conducted to quantify the effect of evaporation procedures on isotope fractionation. Standard dichloromethane solutions of dichlorvos, omethoate and dimethoate mixture (100 mg L^{-1} 1:1:1) were evaporated under a gentle stream of N_2 to volume of 15%, 25%, 40%, 60%, 80% and 100%, respectively, and changes in their carbon isotope compositions were determined.

2.4. Analysis methods

2.4.1. GC–FID analysis

An Agilent 6890 series gas chromatograph (GC, Agilent Technologies, Germany) equipped with a flame ionization detector (FID) was used. OP pesticides were separated in a HP-608 column (30 m × 0.53 mm × 0.5 μm, USA) with helium as the carrier gas (flow of 6.0 mL min^{-1}). The column was initially held at 60 °C for 1 min, ramped at 30 °C min^{-1} to 300 °C, and held for 2 min. Injector and detector temperatures were set to 180 °C and 250 °C, respectively. The samples were injected in splitless mode with injection volumes of 1 μL. Each sample was measured in triplicate. Calibration of three tested pesticides was measured by diluting it with dichloromethane.

2.4.2. EA–IRMS analysis

To validate the results of the GC–C–IRMS method, the carbon isotope compositions of the reference compounds were determined with an elemental analyser (EuroVector, Milan, Italy) directly coupled via a ConFlo III (open split, Thermo Fisher Scientific, Bremen, Germany) to a MAT 253 isotope ratio mass spectrometer (Thermo Fisher Scientific), as described elsewhere (Badea et al., 2009).

2.4.3. CSIA analysis

The carbon isotope composition of dichlorvos, omethoate and dimethoate was analysed by a GC–C–IRMS system consisting of a gas chromatograph (Agilent 6890) coupled via a GC/C III interface to isotope ratio mass spectrometer (Finnigan MAT 252, Thermo Fischer Scientific). The oxidation furnace of the GC/C III interface containing (Pt, Ni, CuO) was set to 980 °C. A DB-608 column (30 m × 0.32 mm × 0.5 μm, USA) was used for pesticides separation, with helium as the carrier gas at a flow rate of 1.3 mL min^{-1}.

The column was initially held at 60 °C for 2 min, ramped at 12 °C min^{-1} to 225 °C, then up to 280 °C at 7 °C min^{-1} and finally held for 2 min. The injector was set to 180 °C. Samples were injected in the split injection mode (the split ratio was from 1:1 to 1:5, which was adjusted to concentrations resulting in suitable peak areas). At least three replicates were measured per sample in order to check the reproducibility. If necessary, the samples were reduced under a gentle stream of N_2 to increase the concentration for isotope analysis.

2.5. Hydrolysis experiment

OP pesticides can be hydrolyzed rapidly in alkaline solution, but are more stable in acidic solution. Thus, the hydrolysis experiments of selected pesticides were carried out in buffer solution of pH 10, while hydrolysis in solutions of pH 3 was used as control experiments. Hydrolysis experiments were carried out at 22 °C in 200 mL buffer solution (pH 10) which was prepared with 0.1 M NaOH (purity ⩾ 99%) and 0.1 M KCl–boric acid (purity > 99.5%). Control experiments were performed in 50 mL buffer solution (pH 3) which contains 0.1 M HCl (32%, Baker analytical grade) and 0.1 M $C_8H_5KO_4$ (potassium hydrogen phthalate, 99% purity, Alfa Aesar, Germany). All hydrolysis experiments were conducted in grinding mouth Erlenmeyer flasks with initial concentration of 100 mg L^{-1} of respective pesticides. Samples were collected at regular time intervals for further analysis. Remaining concentrations of compounds during degradation were determined by GC–FID, and then calculated according to the calibration curve of OP pesticides (see Supporting Information, Fig. S3).

2.6. Photolysis experiment

Photolysis experiment was conducted in a tailor-made chamber photoreactor using six mercury fluorescent lamps as a UVA radiation source (CLEO 20 W, 438 mm × 26 mm, Philips; broad maximum at 355 nm) which was described in details elsewhere (Černigoj et al., 2007). Before starting the degradation experiment, the photoreactor was preheated for 15 min by turning on lamps to keep stable temperature at 35 °C. 200 mL aqueous solution of dimethoate (100 mg L^{-1}) was taken in the reaction tube, and 50 mL of dimethoate solution was performed as dark control experiment. Samples were collected at regular irradiation time intervals for further analysis.

2.7. Quantification of carbon isotope fractionation

The quantification of carbon isotope fractionation has been described in (Meckenstock et al., 2004). Briefly, the isotope ratios measured by CSIA are reported in δ notation in parts per thousand (‰) relative to the international carbon isotope standard (VPDB) (Coplen, 2011). Then bulk isotope enrichment factors (ε) can be obtaining from the slope of the Rayleigh equation. Eq. (1):

$$\varepsilon_C = ln\left[\frac{(\delta_t + 1)}{(\delta_0 + 1)}\right] \Big/ \ln\left(\frac{C_t}{C_0}\right) \quad (1)$$

Isotope fractionation occurs during the chemical reaction step. The apparent kinetic isotope effect (AKIE) was calculated to quantify the intrinsic isotope effect of the bond cleavage. AKIE values were calculated by Eq. (2) (Elsner et al., 2005)

$$\mathrm{AKIE}_C = \left(\frac{1}{z \times \frac{n}{x} \times \varepsilon_C + 1}\right) \quad (2)$$

where n is the number of carbon atoms in the molecule, x is the number of carbon atoms in the reactive position, and z is the number of indistinguishable reactive sites.

3. Results and discussion

3.1. CSIA development

The mixtures of OP pesticides were successfully separated by GC–FID and GC–C–IRMS system under selected temperature programs and columns (see Supporting Information, Fig. S1).

The linearity of the method was analysed using a stock solution of OP pesticides, dissolved in DCM to different final concentrations (concentrations of 40, 50, 100, 200, 300, 400, 500, 600, 800, 1000 mg L^{-1}). A new glass liner, 4 mm ID filled with single taper and quartz wool (SGE), was used for split/splitless injection into GC system caused significant shifts in $\delta^{13}C$ values and gave a poor linearity range, especially for omethoate (see Supporting Information, Fig. S2). Glass liner deactivation using BSTFA gave a better linearity and the isotope composition was almost identical to the elementary analysis coupled to isotope ratio determination. Therefore, we suggest that OP pesticides were decomposed in the liner. In order to circumvent this problem, 1 μL of BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide, SUPELCO) was injected manually three times into the GC inlet (The Agilent Multimode Inlet (MMI)), adjusted to 100 °C with split ratio of 200:1. Then the injector was heated to 180 °C. After deactivation of the liner, the linearity ranges of OP pesticides was improved (Fig. 1). Linearity ranges were shown for a range of signal areas: 12–35 vs (200–600 mg L^{-1}), 1–68 vs (40–1000 mg L^{-1}) and 5.5–60 vs (100–1000 mg L^{-1}) for dichlorvos, omethoate and dimethoate respectively, showing that the linearity of our method led to an acceptable isotope composition of pesticides within the uncertainty of the instrument. Only signals with this range of areas were used for evaluation of isotope values.

To assess the trueness of the GC–C–IRMS method, the isotope composition of three pure compounds was analysed by EA–IRMS system. The values obtained by two methods were compared in Table 1. The systematic shifts in averaged $\delta^{13}C$ values determined by the EA–IRMS and GC–C–IRMS systems were 0.8‰, 0.3‰ and 0.7‰ for dichlorvos, omethoate and dimethoate respectively, thus showing relatively good agreement between the two methods.

The isotope effect of the evaporation procedure was evaluated. The evaporation of mixture solution from the original concentration of 100% to 80%, 60%, 40%, 25% and 15% of the original volume shows almost no difference compared with original $\delta^{13}C$ value of the initial compound (Fig. 2). The standard deviations (2σ) of 6 $\delta^{13}C$ values of dichlorvos, omethoate and dimethoate were 0.15‰, 0.14‰ and 0.11‰, which fit to the reproducibility by CSIA for carbon isotope ($2\sigma \leqslant \pm 0.5$‰). Therefore, the precision of the measurement was demonstrated as well. The evaporation procedure is unlikely to induce significant isotope effects, thus, concentration of components by evaporation can be used for sample preparation. As the sensitivity of the GC–C–IRMS is relatively low compared to GC–MS techniques, further efforts are needed for isolation of OP pesticides from environmental samples. For example, the limits of the source of drinking water in China are 0.08 and 0.05 mg L^{-1} for dimethoate and dichlorvos, respectively. For monitoring of environmentally relevant concentration, isolation and enrichment strategies need to be developed. However, as evaporation does not affect the isotope composition, solvent extraction of large sample volume and subsequent enrichment by careful evaporation might be employed.

3.2. Carbon isotope fractionation during degradation

Hydrolysis occurs at several reactive positions in OP pesticide molecules. It can occur by a homogeneous mechanism, where H_2O and OH^- act as nucleophiles (Bavcon et al., 2003). OP pesticides can be hydrolyzed rapidly in alkaline solution, but are more

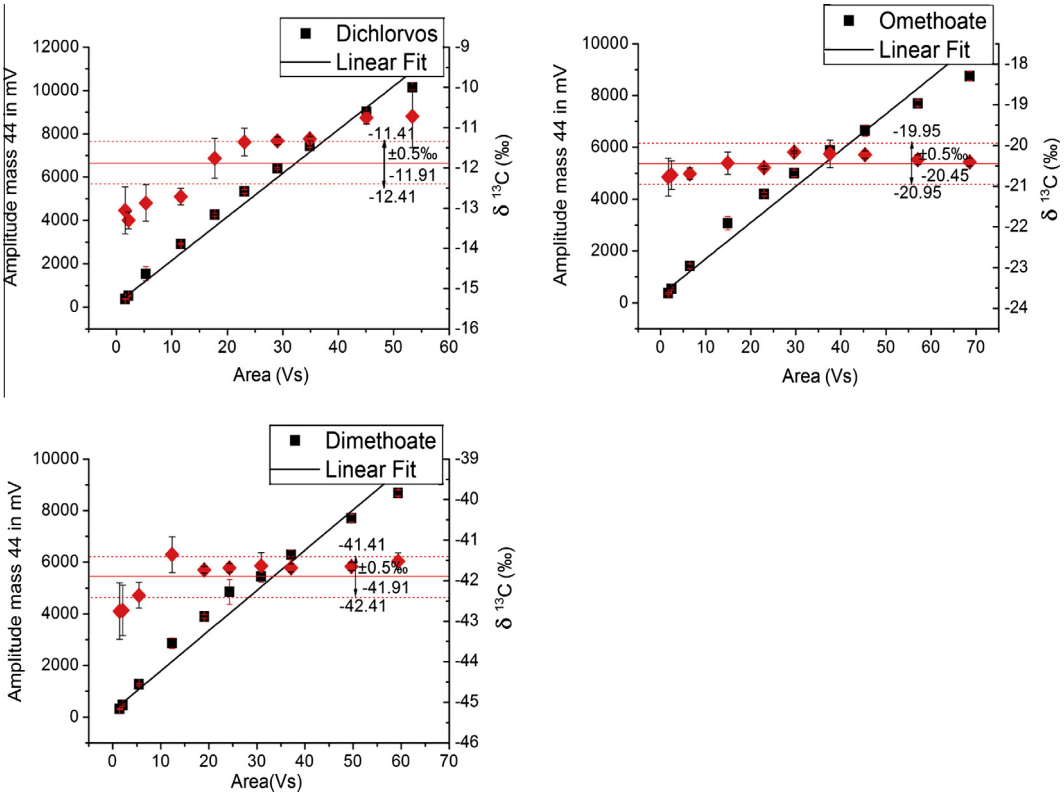

Fig. 1. Linearity test for dichlorvos, omethoate and dimethoate after deactivation of the liner. Signal area represent here correspond to concentrations of 40, 50, 100, 200, 300, 400, 500, 600, 800, 1000 mg L^{-1}. Red diamonds (♦) indicate $\delta^{13}C$ values and black squares (■) indicate amplitude values. Solid lines represent the means of all measurements; dotted lines represent one standard deviation (2σ) of all measurements. Error bars represent one 2σ of triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparison of mean $\delta^{13}C$ (‰) values between EA–IRMS and GC–C–IRMS.

Compound	$\delta^{13}C$ (‰)		$\Delta\delta^{13}C$ (‰)
	EA–IRMS	GC–C–IRMS	
Dichlorvos	−10.4 ± 0.1	−11.2 ± 0.4	+0.8
Omethoate	−20.8 ± 0.0	−20.5 ± 0.2	−0.3
Dimethoate	−42.6 ± 0.1	−41.7 ± 0.3	−0.7

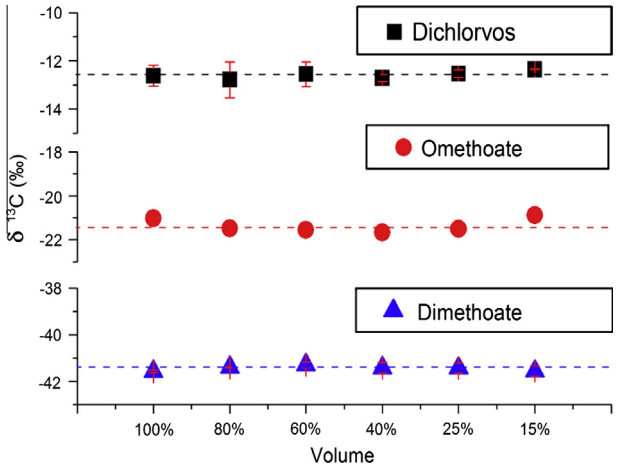

Fig. 2. Evaporation experiment for dichlorvos, omethoate and dimethoate. Isotope composition was determined after reduction of the solvent volume from 100% to 80%, 60%, 40%, 25% and 15%. Dotted lines represent the average value of all measurements. Error bars represent one 2σ of two measurements.

stable in acidic solution. In all of our experiments, the heavier carbon isotope was enriched for tested compounds during the degradation. Data from single experiments are shown in Fig. 3 (data of control experiments are reported in Table S1). After 98.3% was hydrolyzed, dichlorvos was detected with a carbon isotopic composition of −13.7 ± 0.2‰ (Fig. 3a), which is slightly heavier than the isotopic signature of the parent compound at the beginning of the experiment ($\delta^{13}C$ = −14.5 ± 0.2‰). 97.7% of omethoate was degraded within 8 h, and the corresponding carbon isotope signatures were enriched from −22.2 ± 0.1‰ to −20.5 ± 0.2‰ (Fig. 3b). Similarly, during the hydrolysis of dimethoate, significant carbon isotope fractionation was observed. The carbon isotope ratio of dimethoate enriched from −42.8 ± 0.0‰ to −40.9 ± 0.2‰ (Fig. 3c) within a 96 h experiment. The carbon isotope composition enriched from −42.5 ± 0.3‰ to −40.4 ± 0.2‰ upon photolysis of dimethoate over experimental period of 56 h (Fig. 3d).

3.3. AKIE and degradation mechanisms

The isotope fractionation process was quantified by the isotope enrichment factor (ε) using the Rayleigh equation (Eq. (1)), obtaining enrichment factors of −0.2 ± 0.1‰ for hydrolysis of dichlorvos and −1.0 ± 0.1‰ and −3.7 ± 1.1‰ for hydrolysis and photolysis dimethoate respectively (Fig. 4). For further elucidation of the reaction mechanism, AKIE values were calculated to characterise the isotope effect of the cleavage of the chemical bond at the reactive positions. According to Oncescu and Oancea (2007), the hydrolysis of dichlorvos may occur by a homogeneous mechanism where $^{+}H_3O/OH^{-}$ act as nucleophiles in a S_{N2} mechanism. Its a general base-catalyzed reaction and has two parallel routes (Fig. S4). Both routes show no carbon atoms are involved in the reactive

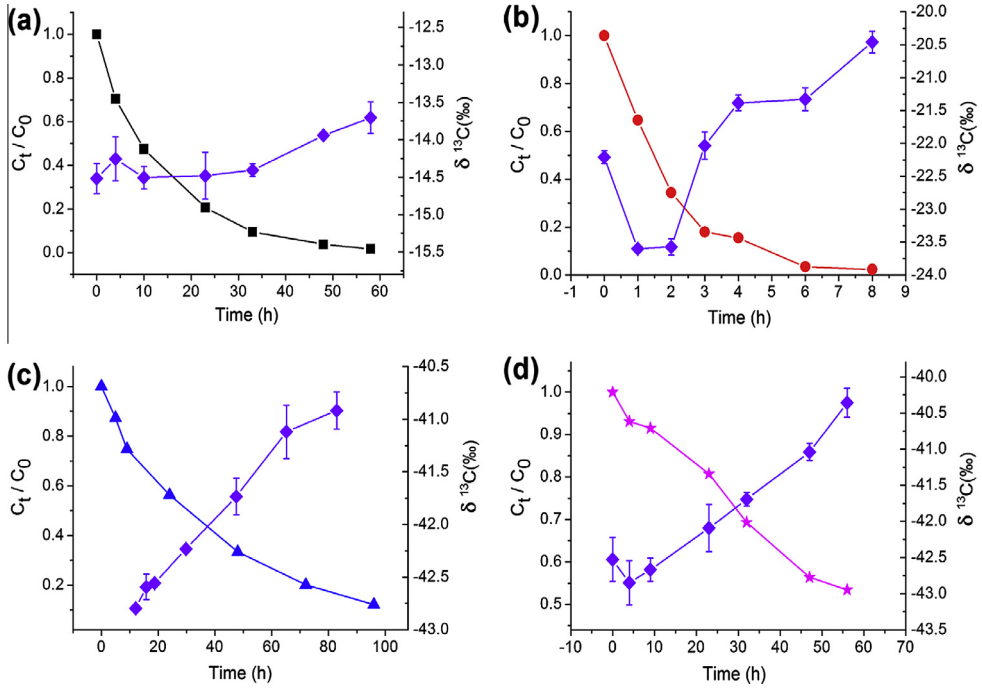

Fig. 3. Changes in concentrations (C_t/C_0) and carbon isotope ratios ($\delta^{13}C$) of OP pesticides during degradation. (a–c) Demonstrate the hydrolysis degradation of dichlorvos (■), omethoate (●) and dimethoate (▲); (d) demonstrates the photolysis of dimethoate (★). (♦) Indicate $\delta^{13}C$ values.

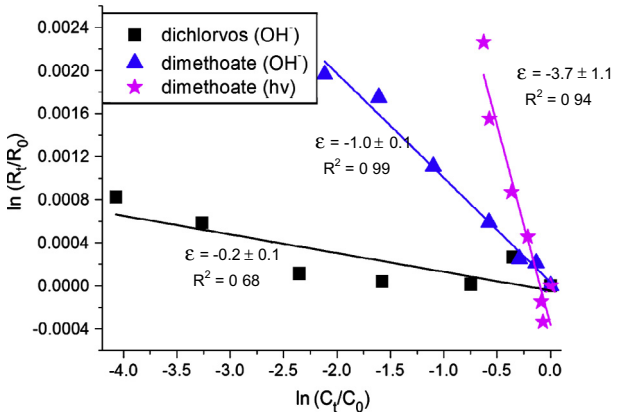

Fig. 4. Double logarithmic plot according to the Rayleigh equation of the isotopic composition vs the residual concentration of dichlorvos (■) form hydrolysis and dimethoate form hydrolysis (▲) and photodegradation (★). ε values were calculated according to equation (Eq. (1)) using the slope of the linear regression.

positions. Oncescu et al. found that the lowest value (0.61) of bond energy in dichlorvos molecule belongs to phosphate–dichlorviniyl bond, which indicates that this bond has to cleave first by hydrolysis. Thus, we suggest that there is no primary carbon isotope fractionation occurs during the hydrolysis of dichlorvos. The obtained relatively lower enrichment factors of -0.2 ± 0.1‰ may be caused by the secondary isotope effects. Thus, changes in carbon isotope ratios of dichlorvos induced by hydrolysis are expected to be low and the enrichment in isotope signature of dichlorvos may offer opportunities for detection of other processes or source allocation.

The fractionation upon hydrolysis of dimethoate (-1.0 ± 0.1‰) was significantly higher than dichlorvos suggesting a primary isotope effect associated with a carbon bond cleavage in the first unidirectional and irreversible reaction step. In analogy to the proposed mechanism for the hydrolysis of omethoate (Farooq et al., 2004), we propose a similar mechanism proceeding by two main pathways. A nucleophilic OH^- attacking the phosphorus atom will lead to P–S bond cleavage without any primary carbon isotope effect. A parallel nucleophilic OH^- attack on the C–O bond may lead to a C–O bond fission associated with a primary stable carbon isotope effect and lead to the formation of O-desmethyl-dimethoate. *O*-demethyl-dimethoate and *O,O*-dimethylhydrogen phosphorothioate acid as products of dimethoate hydrolysis at alkaline conditions (WHO, 2012), suggesting that two parallel reactions are at work. The calculation of AKIE value of 1.0050 ± 0.0005, which is smaller than the theoretical value of KIE (1.061) for C–O bond may reflect that two parallel mechanisms at work and leading to a lower isotope fractionation as expected for an typical S_{N2} reaction such as alkaline hydrolysis of a methoxy bond alone (see Supporting Information).

During photolytic degradation, C–O bonds can be broken under direct UV irradiation (Wang et al., 2006). There are two C–O bonds in dimethoate molecule. Assuming that the photolysis of dimethoate was a concerted reaction, where $n = 5$, $x = 2$, $z = 1$, the AKIE calculation gives a value of 1.0094 ± 0.0027. If the reaction proceeds in stepwise mode, where $n = 5$, $x = 1$, $z = 1$, the AKIE calculation gives a value of 1.0188 ± 0.0054. Both AKIE values are typically below the theoretical KIE O–C bond cleavages, but still in the similar order. However, as documented so far, this is the first study on CSIA of OP pesticides, therefore, no other values are available to date for comparison. Further mechanistic studies employing CSIA need to be conducted and compared with metabolite studies to identify bond cleavage mechanisms in more details.

4. Conclusion

The GC–C–IRMS method for CSIA for dichlorvos, omethoate and dimethoate was developed in this study. Linearity test shows that carbon isotope ratios can be obtained for a signal size of area above 12, 1 and 5.5 vs for dichlorvos, omethoate and dimethoate, respectively. The measurements obtained by GC–C–IRMS system exhibited standard deviations (2σ) that were mostly < ±0.5‰. To explore the application of the developed CSIA, the degradation

experiments revealed a bulk enrichment factor (ε) for hydrolysis of $-0.2 \pm 0.1‰$ and $-1.0 \pm 0.1‰$ for dichlorvos and dimethoate, respectively. The photolysis of dimethoate gave a larger enrichment factor of $3.7 \pm 1.1‰$ and allow to distinct hydrolysis and photolysis. Our study clearly demonstrated that carbon isotope fractionation occurs during degradation of tested pesticides. The isotope enrichment factor may be used to characterise hydrolysis reaction and direct photolysis in field studies.

Monitoring the carbon isotope signatures may be a promising tool for the qualitative and quantitative assessment of the fate of OP pesticides. In order to assess the transport of OP pesticides in the environment and decipher their degradation in more details by CSIA, further laboratory investigations providing reference enrichment factors for degradation mechanisms occurring under different hydrogeochemical conditions are needed. In addition, ^{2}H, ^{18}O and ^{15}N could also be considered for isotope analysis in order to obtain enhanced insight of OP pesticides from multi-element isotope analysis in future studies.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.04.037.

References

Abe, Y., Hunkeler, D., 2006. Does the Rayleigh equation apply to evaluate field isotope data in contaminant hydrogeology. Environ. Sci. Technol. 40, 1588–1596.

Aeppli, C., Hofstetter, T.B., Amaral, H.I.F., Kipfer, R., Schwarzenbach, R.P., Berg, M., 2010. Quantifying in situ transformation rates of chlorinated ethenes by combining compound-specific stable isotope analysis, groundwater dating, and carbon isotope mass balances. Environ. Sci. Technol. 44, 3705–3711.

Badea, S.L., Carsten, V., Stefanie, W., Danet, A.F., Richnow, H.H., 2009. Stable isotope fractionation of α-hexachlorocyclohexane (Lindane) during reductive dechlorination by two strains of sulfate-reducing bacteria. Environ. Sci. Technol. 43, 3155–3161.

Bavcon, M., Trebše, P., Zupančič Kralj, L., 2003. Investigations of the determination and transformations of diazinon and malathion under environmental conditions using gas chromatography coupled with a flame ionisation detector. Chemosphere 50, 595–601.

Bigeleisen, J., Wolfsberg, M., 1958. Theoretical and experimental aspects of isotope effects in chemical kinetics. Adv. Chem. Phys., 15–76.

Černigoj, U., Štangar, U.L., Trebše, P., 2007. Evaluation of a novel carberry type photoreactor for the degradation of organic pollutants in water. J. Photoch. Photobio. A 188, 169–176.

Coplen, T.B., 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Commun. Mass Spectrom. 25, 2538–2560.

Demoliner, A., Caldas, S.S., Costa, F.P., Gonçalves, F.F., Clementin, R.M., 2010. Development and validation of a method using SPE and LC-ESI-MS-MS for the determination of multiple classes of pesticides and metabolites in water samples. J. Brazil Chem. Soc. 21, 1424–1433.

Elsner, M., Zwank, L., Hunkeler, D., 2005. A new concept linking observable stable isotope fractionation to transformation pathways of organic pollutants. Environ. Sci. Technol. 39, 6896–6916.

Evgenidou, E., Konstantinou, I., Fytianos, K., Albanis, T., 2006. Study of the removal of dichlorvos and dimethoate in a titanium dioxide mediated photocatalytic process through the examination of intermediates and the reaction mechanism. J. Hazard. Mater. 137, 1056–1064.

Farooq, R., Lin, F., Wang, Y., Huang, J., Xu, Z., Shaukat, S.F., 2004. Pressure hydrolysis for degradation of omethoate pesticide in water. J. Shanghai Univ. (Engl. Ed.) 8, 221–226.

Fischer, A., Herklotz, I., Herrmann, S., Thollner, M., Weelink, S.A.B., Stams, A.J.M., Schloemann, M., Richnow, H.H., Vogt, C., 2008. Combined carbon and hydrogen isotope fractionation investigations for elucidating benzene biodegradation pathways. Environ. Sci. Technol. 42, 4356–4363.

Fletcher, K.E., Loffler, F.E., Richnow, H.H., Nijenhuis, I., 2009. Stable carbon isotope fractionation of 1,2-dichloropropane during dichloroelimination by dehalococcoides populations. Environ. Sci. Technol. 43, 6915–6919.

Hirschorn, S.K., Dinglasan, M.J., Elsner, M., Mancini, S.A., Lacrampe Couloume, G., Edwards, E.A., Sherwood Lollar, B., 2004. Pathway dependent isotopic fractionation during aerobic biodegradation of 1,2-dichloroethane. Environ. Sci. Technol. 38, 4775–4781.

Hofstetter, T.B., Berg, M., 2011. Assessing transformation processes of organic contaminants by compound-specific stable isotope analysis. TrAC, Trends Anal. Chem. 30, 618–627.

Jiang, X.L., 1993. Study on screening of the priority monitoring pesticides. Adv. Environ. Sci. 1, 1–15.

Liu, J.L., 2010. Advance on microbial degradation of organphosphorus pesticides pollution. J. Biol. 27, 79–82.

Mancini, S.A., Ulrich, A.C., Lacrampe-Couloume, G., Sleep, B., Edwards, E.A., Lollar, B.S., 2003. Carbon and hydrogen isotopic fractionation during anaerobic biodegradation of benzene. Appl. Environ. Microbiol. 69, 191–198.

Mckelvie, J.R., Mackay, D.M., de Sieyes, N.R., Lacrampe Couloume, G., Sherwood Lollar, B., 2007. Quantifying MTBE biodegradation in the Vandenberg air force base ethanol release study using stable carbon isotopes. J. Contam. Hydrol. 94, 157–165.

Meckenstock, R.U., Morasch, B., Griebler, C., Richnow, H.H., 2004. Stable isotope fractionation analysis as a tool to monitor biodegradation in contaminated acquifers. J. Contam. Hydrol. 75, 215–255.

Nijenhuis, I., Nikolausz, M., Köth, A., Felföldi, T., Weiss, H., Drangmeister, J., Großmann, J., Kästner, M., Richnow, H.H., 2007. Assessment of the natural attenuation of chlorinated ethenes in an anaerobic contaminated aquifer in the Bitterfeld/Wolfen area using stable isotope techniques, microcosm studies and molecular biomarkers. Chemosphere 67, 300–311.

Oncescu, T., Oancea, P., 2007. On the mechanism of dichlorvos hydrolysis. Rev. Chim. 58, 232–235.

Oncescu, T., Stefan, M.I., Oancea, P., 2010. Photocatalytic degradation of dichlorvos in aqueous TiO_2 suspensions. Environ. Sci. Pollut. Res. 17, 1158–1166.

Pappas, C.J., Kyriakidis, N.V., 2003. A comparison of dimethoate degradation in lemons and mandarins on the trees with two GC systems. Food Chem. 80, 23–28.

Penning, H., Sorensen, S.R., Meyer, A.H., Aamand, J., Elsner, M., 2010. C, N, and H isotope fractionation of the herbicide isoproturon reflects different microbial transformation pathways. Environ. Sci. Technol. 44, 2372–2378.

Priya, D.N., Modak, J.M., Trebše, P., Žabar, R., Raichur, A.M., 2011. Photocatalytic degradation of dimethoate using LbL fabricated TiO_2/polymer hybrid films. J. Hazard. Mater. 195, 214–222.

Reinnicke, S., Simonsen, A., Sørensen, S.R., Aamand, J., Elsner, M., 2012. C and N isotope fractionation during biodegradation of the pesticide metabolite 2,6-dichlorobenzamide (BAM): potential for environmental assessments. Environ. Sci. Technol., 1447–1454.

Richnow, H.H., Annweiler, E., Michaelis, W., Meckenstock, R.U., 2003a. Microbial in situ degradation of aromatic hydrocarbons in a contaminated aquifer monitored by carbon isotope fractionation. J. Contam. Hydrol. 65, 101–120.

Richnow, H.H., Meckenstock, R.U., Ask Reitzel, L., Baun, A., Ledin, A., Christensen, T.H., 2003b. In situ biodegradation determined by carbon isotope fractionation of aromatic hydrocarbons in an anaerobic landfill leachate plume (Vejen, Denmark). J. Contam. Hydrol. 64, 59–72.

Rosell, M., Barcelo, D., Rohwerder, T., Breuer, U., Gehre, M., Richnow, H.H., 2007. Variations in $^{13}C/^{12}C$ and D/H enrichment factors of aerobic bacterial fuel oxygenate degradation. Environ. Sci. Technol. 41, 2036–2043.

Rosell, M., Finsterbusch, S., Jechalke, S., Hubschmann, T., Vogt, C., Richnow, H.H., 2010. Evaluation of the effects of low oxygen concentration on stable isotope fractionation during aerobic MTBE biodegradation. Environ. Sci. Technol. 44, 309–315.

Rosell, M., Olmos, R.G., Rohwerder, T., Kopinke, F.D., Richnow, H.H., 2012. Critical evaluation of the 2D-CSIA scheme for distinguishing fuel oxygenate degradation reaction mechanisms. Environ. Sci. Technol. 46, 4757–4766.

Van Breukelen, B.M., Hunkeler, D., Volkering, F., 2005. Quantification of sequential chlorinated ethene degradation by use of a reactive transport model incorporating isotope fractionation. Environ. Sci. Technol. 39, 4189–4197.

Vieth, A., Muller, J., Strauch, G., Kastner, M., Gehre, M., Meckenstock, R.U., Richnow, H.H., 2003. In situ biodegradation of tetrachloroethene and trichloroethene in contaminated aquifers monitored by stable isotope fractionation. Isot. Environ. Health Stud. 39, 113–124.

Wang, D., Chen, J., Su, Y., 2006. Study on photocatalytic degradation of organophosphorous pesticides with nanometer TiO_2. Foshan Ceram. 6, 1–3.

WHO, 2012. Specifications and evaluations for public health: Dimethoate.