Two multidimensional chromatographic methods for enantiomeric analysis of *o,p'*-DDT and *o,p'*-DDD in contaminated soil and air in a malaria area of South Africa

Yvette Naudé* and Egmont R. Rohwer

Department of Chemistry, University of Pretoria, Private Bag X20, Hatfield 0028, Pretoria, South Africa

ABSTRACT

In rural parts of South Africa the organochlorine insecticide DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) is still used for malaria vector control where traditional dwellings are sprayed on the inside with small quantities of technical DDT. Since *o,p'*-DDT may show enantioselective oestrogenicity and biodegradability, it is important to analyse enantiomers of *o,p'*-DDT and its chiral degradation product, *o,p'*-DDD, for both health and environmental-forensic considerations. Generally, chiral analysis is performed using heart-cut multidimensional gas chromatography (MDGC) and, more recently, comprehensive two-dimensional gas chromatography (GC x GC). We developed an off-line gas chromatographic fraction collection (heart-cut) procedure for the selective capturing of the appropriate isomers from a first apolar column, followed by reinjection and separation on a second chiral column. Only the *o,p'*-isomers of DDT and DDD fractions from the first dimension complex chromatogram (achiral apolar GC column separation) were selectively collected onto a polydimethylsiloxane (PDMS) multichannel open tubular silicone rubber trap by simply placing the latter device on the

flame tip of an inactivated flame ionisation detector (FID). The multichannel trap containing the *o,p'*-heart-cuts was then thermally desorbed into a GC with time-of-flight mass spectrometry detection (GC-TOFMS) for second dimension enantioselective separation on a chiral column (β-cyclodextrin-based). By selectively capturing only the *o,p'*-isomers from the complex sample chromatogram, ^{1}D separation of ultra-trace level enantiomers could be achieved on the second chiral column without matrix interference. Here, we present solventless concentration techniques for extraction of DDT from contaminated soil and air, and report enantiomeric fraction (EF) values of *o,p'*-DDT and *o,p'*-DDD obtained by a new multidimensional approach for heart-cut gas chromatographic fraction collection for off-line second dimension enantiomeric separation by ^{1}D GC-TOFMS of selected isomers. This multidimensional method is compared to the complementary technique of comprehensive GC x GC-TOFMS using the same enantioselective column, this time as the first dimension of separation.

Keywords: Polydimethylsiloxane (PDMS) sorptive extraction; Environmental forensics; Persistent organic pollutants (POPs); Chiral chromatography; Comprehensive GC x GC; Heart-cutting

*Corresponding author. Tel.: +27 12 420 2517; fax: +27 12 420 4687.

E-mail address: yvette.naude@up.ac.za

Abbreviations

CIS cooled injection system

EF enantiomeric fraction

GCFC gas chromatographic fraction collection

IRS indoor residual spraying

MCT multichannel open tubular silicone rubber trap

MDGC multidimensional gas chromatography

PDMS polydimethylsiloxane

POPs persistent organic pollutants

TDS thermal desorber system

1. Introduction

In risk areas of South Africa the organochlorine insecticide DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) is still used for malaria vector control. The strict Stockholm Convention on Persistent Organic Pollutants (POPs) allows Indoor Residual Spraying (IRS) of traditional dwellings with DDT. In South Africa, IRS with DDT resulted in a 65% decrease in the number of deaths caused by malaria and an 83% decrease in the number of confirmed malaria cases [1]. Technical grade DDT (75% wettable powder) used for IRS in South Arica contains 72-75% of *p,p'*-DDT, the active ingredient, and ~22% of *o,p'*-DDT [2,3]. Technical DDT has oestrogen-like properties, mainly due to *o,p*'-DDT [4,5]. *o,p*'-DDT and its degradation product *o,p*'-DDD are chiral molecules (*o,p*'-DDE is achiral) and both exist as enantiomeric pairs (-)-*o,p*'-DDT and (+)-*o,p*'-DDT, and (-)-*o,p*'-DDD and (+)-*o,p*'-DDD [6,7]. A chiral

compound is produced industrially as a racemic mixture [8]. Fresh treatment with technical DDT will have a chiral signature where enantiomers of *o,p*'-DDT and of *o,p*'-DDD are present as a 1:1 racemic mixture corresponding to an enantiomeric fraction (EF) = 0.5 [9]. In the environment selective breakdown of one enantiomer of a pair can result in non-racemic residues [9] and a deviation of EF from 0.5 in samples may therefore be used to differentiate between recent and past inputs of POPs [7,10,11]. *o,p'*-DDT is reported to show enantioselective oestrogenicity since (-)-*o,p*'-DDT is a weak oestrogen mimic while (+)-*o,p*'-DDT is inactive [5,12,13]. These chiral compounds are separated by using columns with a ß-cyclodextrin stationary phase specifically developed for this purpose [7,9,13-18]. However, coelution of compounds in complex environmental mixtures can make forensic determinations difficult. To overcome the problem of coelution, chiral analysis of POPs is generally performed by heart-cut multidimensional gas chromatography (MDGC) [7,11,15-17,19-22] and comprehensive two-dimensional gas chromatography (GC x GC) [11,15,17,19]. An excellent review of multidimensional chromatography in pesticides analysis can be found in Tuzimski [23]. In the heart-cut MDGC technique two independent GC systems are coupled so that one or more unresolved fractions are transferred directly (on-line) from a first non-enantioselective column (first dimension) to a second enantioselective column (second dimension) where separation of the compounds will occur. In comprehensive GC x GC the entire sample is separated, very fast, on two different columns [16].

Organochlorine pesticides are typically solvent extracted from soil by Soxhlet extraction [9,18,20,21,24,25] and by sonication [7]. Microlitre amounts are injected for analysis. Sensitivity limitations are associated with injection of only a fraction of the final 20 µL to 1 mL solvent extract [26]. In contrast to extraction procedures using solvents, the procedure applied in this study is a novel solvent free sorptive extraction technique where DDT is concentrated from soil using polydimethylsiloxane (PDMS) loops. PDMS functions as a hydrophobic solvent for the analytes [26]. After extraction the loop is inserted into a commercial thermal desorption tube for solventless introduction into a GC. Chiral columns are sensitive to moisture and matrix components, and therefore desorption-injection rather than liquid extract injection is preferable in order to protect the expensive column.

Taking into consideration that DDT residues in soil are emitted into air (soil-air exchange) [9] and that contaminated airborne dust presents a pathway for exposure to DDT, enantiomeric signatures are also determined for indoor air samples in this study. Here, DDT in indoor air is concentrated with a denuder configuration of a PDMS multichannel open tubular silicone rubber trap (MCT) combined with a micro quartz fibre filter for single-step collection of vapour phase and particulate phase DDT [27].

We report the extraction of DDT and its associated environmental pollutants by novel solventless sorptive extraction techniques using PDMS and a new multidimensional approach for heart-cut gas chromatographic fraction collection (GCFC) from a non-enantioselective column for off-line

second dimension ^{1}D isomer selective enantiomeric separation of both isomers, *o,p'*-DDT and *o,p'*- DDD, by GC-TOFMS. We compare this multidimensional procedure to the complementary technique of comprehensive GC x GC-TOFMS using the same enantioselective column, this time as the first dimension of separation.

2. Materials and methods

2.1. Sampling site

Outdoor soil and indoor air samples were collected from a rural village (S 23°02'02.3" E 30°51'33.5") in the Vhembe District, Limpopo Province, South Africa. The village is situated within an intermediate-risk malaria area where indoor DDT-spraying is performed annually [24]. A detailed description of the study area was reported in Van Dyk et al. [24]. The province is a summer rainfall region. Outdoor soil samples (D8, D10, D12) were collected two months after completion of the IRS programme in February 2008 during the summer season [24]. Indoor air samples were collected from traditional round thatch-roof huts (D7 to D10) directly after IRS in November 2007 and details are reported elsewhere by Naudé and Rohwer [27].

2.2 Chemicals and equipment

A certified organochlorine pesticides standard mixture (purity ≥97%) containing *p,p*'-DDT, *o,p'*-DDT, *p,p*'-DDD, *o,p'*-DDD, *p,p*'-DDE and *o,p'*-DDE

and a certified inlet degradation mixture (*p,p'*-DDT and Endrin, purity ≥98%) were purchased from Sigma-Aldrich (Pty) Ltd. Kempton Park, South Africa. Solvents used were of analytical grade. The standard stock solution was diluted in hexane (Merck Chemicals (Pty) Ltd., South Africa) to give working standard solutions of 1 ng μL^{-1} and 10 ng μL^{-1}. The inlet degradation solution (*p,p'*-DDT) was diluted in hexane to give a concentration of 1 ng μL^{-1}.

Compounds from air and soil were concentrated on SIL-TEC™ silicone elastomer medical grade tubing (0.64 mm OD x 0.30 mm ID) (Technical Products, Georgia, United States of America). Thermal desorption tubes (17.8 cm long glass tubes, 4 mm ID, 6 mm OD), thermal desorber systems (TDS 3), cooled injection systems (CIS 4) and empty baffled deactivated glass CIS liners were all from Gerstel™ (Chemetrix, Midrand, South Africa or LECO Africa (Pty) Ltd., Kempton Park, South Africa). The GC-FID used for apolar heart-cutting was an Agilent 6890 GC (Chemetrix, Midrand, South Africa). Chiral separation was performed on a LECO Pegasus 4D Agilent 7890 GC system (LECO Africa (Pty) Ltd., Kempton Park, South Africa. Gases were of ultra high purity grade (Afrox, Gauteng, South Africa).

2.3. Solventless concentration methods for p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD, p,p'-DDE and o,p'-DDE

2.3.1 Soil sampling with PDMS loops

A loop was fashioned by taking a 10.5 cm (0.02 g) length of a silicone elastomer medical grade tubing and joining the ends by inserting a 1 cm piece

of fused silica capillary column (250 µm ID) (Figure 1). A loop arrangement prevents soil from entering the PDMS tube and it facilitates ease of handling [28]. The sorption volume of the loop used for solventless extraction of the soil samples was 26 µL. A 40 mL glass vial, fitted with a solid screw cap coated on the inside with polytetrafluoroethylene (PTFE), was filled with 40 g (heart-cut GCFC) soil or 10 g soil (GC x GC - TOFMS). A PDMS loop was submerged in the soil. The vial was placed in an oven at 50 °C for 90 min. After extraction of the soil the PDMS loop was inserted into a glass desorption tube for thermal desorption into a gas chromatograph – flame ionisation detector (GC-FID) or, alternatively, a gas chromatograph – time-of-flight mass spectrometer (GC-TOFMS).

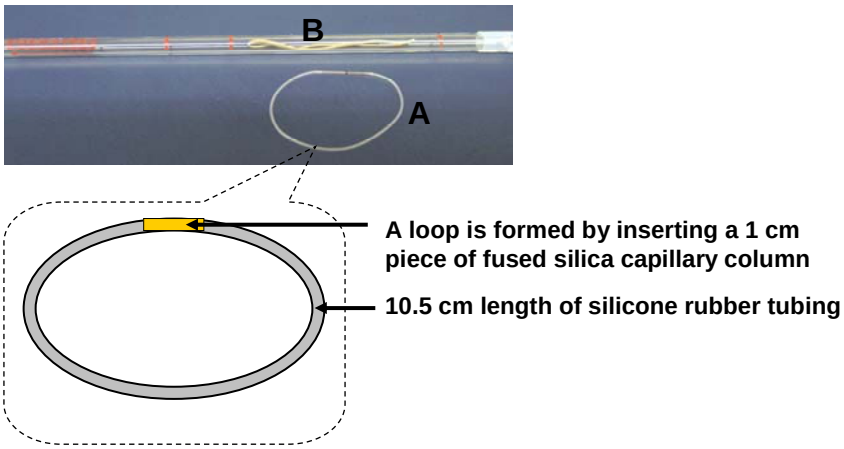

Figure 1. PDMS loop prior to (A) and after extraction (B) of soil. A loop arrangement prevents soil from entering the silicone rubber tubing.

2.3.2 Denuder indoor air sampling

A full description of the methodology is reported elsewhere by Naudé and Rohwer [27]. Briefly, four litre of indoor air was sampled with a denuder device consisting of a multichannel PDMS trap + micro quartz fibre filter + multichannel PDMS trap combination to sample air borne vapour phase DDT (first PDMS trap) and air borne particulate phase DDT (filter and back-up PDMS trap) in a single step. A denuder allows simultaneous collection of molecules and transmission of aerosol particles through the silicone rubber tubes based on the large difference in radial diffusion speeds in the axial laminar flow environment [29].

2.4. Multichannel open tubular PDMS traps (MCTs) for heart-cut collection of GC separated isomer peaks

Multichannel traps containing 0.099±0.02 g silicone, providing a sample enrichment volume of 106 µL PDMS, were prepared based on a technique described by Ortner and Rohwer [30]. The MCT was designed to fit a commercial thermal desorber system. A bundle of twenty eight channels of silicone elastomer medical grade tubing were inserted into glass desorption tubes (Figure 2). The MCT inside the desorption tube was 15 mm long. The ends of the MCT device were capped with glass stoppers during storage. The glass stoppers were secured with tight-fitting PTFE sleeves. The trapped

analytes inside the MCT are not directly exposed to the PTFE sleeves, thereby preventing potential adsorption of analytes onto the Teflon.

2.5. Multidimensional GC techniques

2.5.1. Apolar separation

2.5.1.1. TDS-CIS-GC-FID

p,p'-DDT, *o,p'*-DDT, *p,p*'-DDD, *o,p'*-DDD, *p,p*'-DDE and *o,p'*-DDE were thermally desorbed from the PDMS loops (soil) and PDMS multichannel traps (air) using a TDS installed on a GC-FID. The TDS transfer line temperature was 280 °C. During splitless desorption the PDMS loops were heated from 30 °C (3 min) at 30 °C min^{-1} to 250 °C (20 min) with a desorption flow rate of 50 mL min^{-1} at 75 kPa (hydrogen). The desorbed analytes were cryogenically focused on a CIS at –100 °C using liquid nitrogen. The GC inlet was in the solvent vent mode to achieve a high desorption flow rate whilst the purge valve remained closed during desorption to give a splitless-type injection from the CIS. After desorption, a splitless injection (purge on at 27 min, purge flow 50 mL min^{-1}, solvent vent mode) was performed by heating the CIS from –100 °C at 6 °C s^{-1} to 250 °C and kept there for 27 min. The GC oven temperature programme was 60 °C (5 min) at 10 °C min^{-1} to 280 °C (1 min). A post run was performed by heating the GC oven to 310 °C (10 min). The hydrogen carrier gas flow rate was 2 mL min^{-1} (52 cm s^{-1}) and the column head

pressure was 75 kPa in the constant pressure mode. The GC column was an apolar Zebron ZB-1 30 m x 250 µm ID x 0.25 µm film thickness (df) (Phenomenex, Separations, Randburg, South Africa). The FID was operated at 300 °C. Flow rates of FID gases were hydrogen 40 mL min^{-1}, air 400 mL min^{-1} and nitrogen (make-up gas) 50 mL min^{-1}.

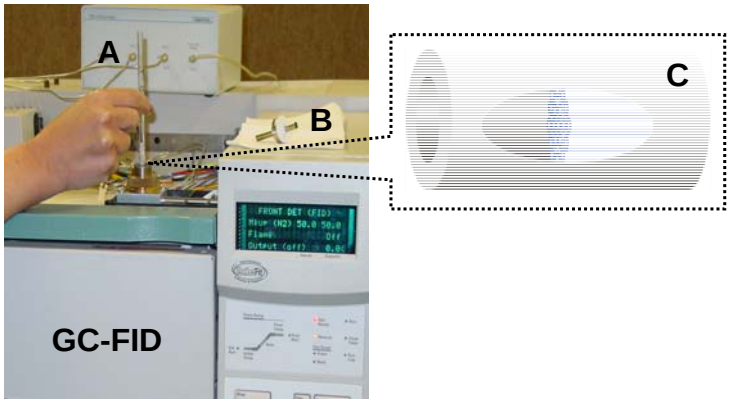

Figure 2. Collection of heart-cuts of *o,p*'-DDD and *o,p'*-DDT from the GC effluent onto a multichannel trap. (A) The multichannel trap is simply placed on an inactivated FID flame tip during selected isomer collection. (B) The FID collector is removed prior to heart-cutting. (C) Schematic diagram of a multichannel trap consisting of silicone rubber tubes arranged in parallel inside a commercial glass desorption tube.

2.5.1.2. Novel gas chromatography fraction collection (GCFC) for heart-cut transfer of selected isomers to another, off-line GC

Chromatographic profiles of authentic chemical standards (*p,p*'-DDT, *o,p'*-DDT, *p,p*'-DDD, *o,p'*-DDD, *p,p*'-DDE and *o,p'*-DDE), contaminated soil and indoor air were first obtained using the conditions described in 2.5.1.1. Integration results of peak start time and peak end time of the chromatograms were then used to establish the heart-cutting event times. In a subsequent run, different sections of the GC-effluent were selectively recaptured onto MCTs on a carefully timed basis (Figure 2). For isomer selected heart-cutting by GCFC the instrumental conditions were as described in 2.5.1.1, the only difference now was the modification of the detector parameters. The electrometer was switched off. The detector top assembly and the collector are easily removed by loosening the knurled brass nut of the detector assembly and by taking out the collector. Single peaks were collected at the end of the GC column by simply placing a MCT on the inactivated FID flame tip and by supporting the MCT in this position by hand. During collection the FID and flame gases (hydrogen and air) were switched off, the make-up gas (nitrogen) plus carrier gas (hydrogen) flow totalled 50 mL min^{-1} and the detector temperature was at 300 °C. After selective capturing of *o,p'*-DDT and *o,p'*-DDD heart-cuts onto MCTs, the MCTs were capped with custom-made glass and Teflon stoppers (section 2.4) and stored for second dimension ^{1}D chiral separation by GC-TOFMS.

2.5.2. *Chiral separation*

2.5.2.1. ^{1}D GC-TOFMS analysis of isomer selected heart-cuts from outdoor soil and indoor air collected on MCTs

The GC-TOFMS used to analyse the isomer selected heart-cuts, *o,p'*-DDT and *o,p'*-DDD, was run in ^{1}D mode. The heart-cut isomers on PDMS multichannel traps were thermally desorbed by heating the traps in a TDS from 30 °C (3 min) at 30 °C min^{-1} to 250 °C (20 min) with a desorption flow rate of 100 mL min^{-1} at a vent pressure of 57 psi (helium). The TDS transfer line temperature was 280 °C. The desorbed analytes were cryogenically focused on a CIS at –100 °C using liquid nitrogen. After desorption, a splitless injection (purge on at 40 min, purge flow 50 mL min^{-1}, solvent vent mode) was performed by heating the CIS from –100 °C at 6 °C s^{-1} to 250 °C and held there for the duration of the GC run.

The primary column (^{1}D) was a β-cyclodextrin-based chiral phase BGB-172 (20% *tert*-butyldimethylsilyl-β-cyclodextrin dissolved in BGB-15 (15% phenyl-, 85% methylpolysiloxane)) 30 m x 0.25 mm ID x 0.25 µm df (BGB Analytik, Switzerland) and the secondary column (^{2}D) was an intermediate polarity Rtx-200 (trifluoropropyl methyl polysiloxane) 1.29 m x 0.18 mm ID x 0.18 µm df (Restek, Bellefonte, PA, USA). The primary oven temperature programme was 120 °C (3 min) at 10 °C min^{-1} to 200 °C (no hold), 1.5 °C min^{-1} to 230 °C (10 min). The GC run time was 41 min. The secondary oven was programmed identical to the primary oven, but offset by + 20 °C. The system was unmodulated, i.e. the separation was essentially

that provided by the primary column (operated in ^{1}D mode with modulation period set at 0 s). The carrier gas (helium) velocity was 54 cm s^{-1} (2 mL min^{-1}) in the constant flow mode.

The MS transfer line temperature was set at 280 °C. The ion source temperature was 200 °C, the electron energy was 70 eV in the electron impact ionization mode (EI+), the data acquisition rate was 10 spectra s^{-1}, the mass acquisition range was 40–360 atomic mass units (amu), and the detector voltage was set at 1700. The order of elution of the enantiomers of *o,p'*-DDT and *o,p'*-DDD was obtained from Buser and Muller [6].

2.5.2.2. GC x GC-TOFMS analysis of outdoor soil and indoor air

The same GC-TOFMS utilised in 2.5.2.1 was now run in GC x GC mode to analyse enantiomers of *o,p'*-DDT and *o,p'*-DDD. The system included a secondary oven and a dual stage modulator. Liquid nitrogen was used for the cold jets and synthetic air for the hot jets. The analytes from contaminated soil (sorbed onto PDMS loops), from vapour phase (on PDMS multichannel trap) and from air borne particulate phase (on a micro quartz fibre filter) contaminants, were thermally desorbed as is described in 2.5.2.1. Columns set-up and GC-TOFMS parameters were as described in 2.5.2.1. The secondary oven was programmed identical to the primary oven, but offset by + 5 °C. The modulator temperature offset was 30 °C. The modulation period was 4 s with a hot pulse time of 1 s. The data acquisition rate was 100 spectra s^{-1}. Identification of *p,p*'-DDT, *o,p'*-DDT, *p,p*'-DDD, *o,p'*-DDD, *p,p*'-

DDE and *o,p'*-DDE was based on comparison of retention times of authentic standards and comparison of mass spectra with a mass spectral library.

2.6 Data analysis

Statistical analyses were performed by using the paired t-test set at a 95% level of confidence. Any difference between results was considered not significant when a probability (two-tailed P value (*p*)) greater than 0.05 was returned by the t-test.

3. Results and discussion

3.1. Solventless extraction and desorption-injection

Typically the method for isolating and analysing POPs from soils and air is solvent extraction of the materials followed by the analysis of microlitre amounts of the diluted final extract. We developed a simple, cheap, non-hazardous solventless extraction technique using PDMS for analyte enrichment from soil and air (Figures 1 and 2) for the introduction of the total amount of sorbed analytes into a GC. A novel multidimensional GC approach was followed where MCTs containing selectively trapped isomers were redesorbed for off-line second dimension separation by ^{1}D GC-TOFMS. For comparison, PDMS loops (outdoor soil samples) and MCTs (indoor air samples) were desorbed for compound separation by the complementary technique of comprehensive GC x GC-TOFMS. For a fixed desorption time

the amount of *o,p'*-DDD and *o,p'*-DDT desorbed from PDMS was dependent on the desorption flow rate of helium. To improve detectability of the target compounds a high desorption flowrate was required. The amount of *o,p'*-DDD and *o,p'*-DDT desorbed from PDMS with a helium vent flow rate of 100 mL min^{-1} was double the amount desorbed at 60 mL min^{-1}. In contrast, hydrogen did not present the higher desorption yields with increased desorption vent flow rate and 50 mL min^{-1} was used in this case (2.5.1). Desorption and programmable temperature vaporisation (PTV) injection were optimised to minimise DDT degradation [27] and inlet liners were replaced regularly with new deactivated liners to keep DDT degradation below 10%.

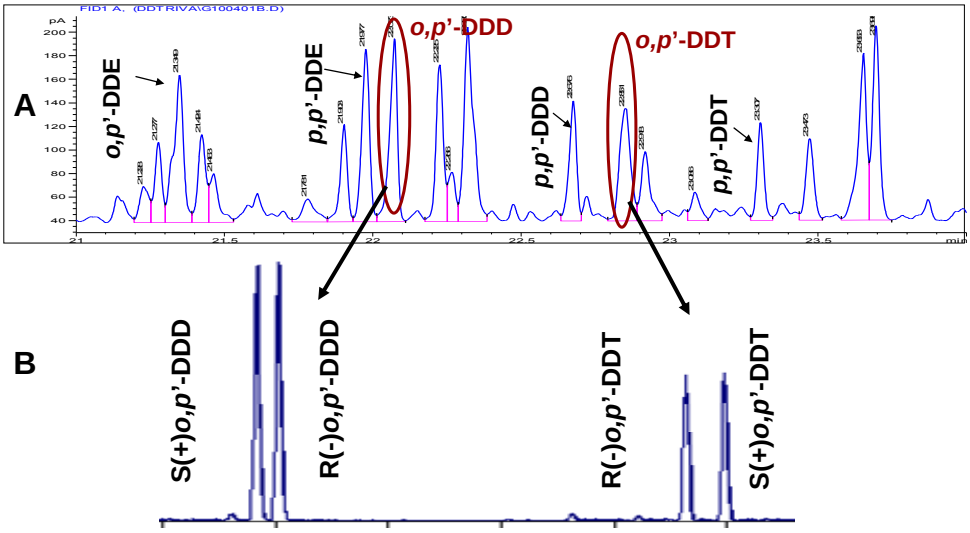

Figure 3. Enantiomeric profiling by off-line GCFC–GC-TOFMS. (A) First dimension non-enantioselective separation. (B) Second dimension ^{1}D enantioselective separation (β-cyclodextrin phase) of the heart-cuts of *o,p'*-DDD and *o,p'*-DDT collected from A.

3.2. Novel isomer selective off-line heart-cutting by GC fraction collection onto PDMS MCTs

Figure 2 depicts selective heart-cutting of the chiral isomers, *o,p*'-DDD and *o,p*'-DDT, from the complex sample achiral chromatogram. Complicated instrumental set-ups, sophisticated equipment or valves were not required. The FID collector was removed prior to heart-cutting. The FID flame gases (hydrogen and air) were switched off. PDMS containing sorbed analytes from soil or from air was desorbed for achiral (apolar phase) first dimension separation of *o,p*'-DDE, *p,p*'-DDE, *o,p*'-DDD, *p,p*'-DDD, *o,p*'-DDT and *p,p*'-DDT (Figure 3A). During this first dimension achiral separation of the complex mixture only *o,p*'-DDD and *o,p*'-DDT were selectively collected from the GC effluent onto a PDMS multichannel trap. The MCT was placed, by hand, on the inactivated flame tip prior to peak elution and removed once the peak had eluted. The two chiral isomers, *o,p'*-DDT and *o,p'*-DDD, were sequentially collected on a single MCT during the same chromatographic run. The MCT with *o,p*'-DDD and *o,p*'-DDT heart-cuts was then desorbed into a GC-TOFMS for second dimension ^{1}D chiral (β-cyclodextrin phase) separation of the enantiomers (Figure 3B).

The open tubular structure of the MCT and low pressure drop associated with multichannel flow [28] are characteristics that are particularly suited to the recapturing of chromatographic fractions from the GC effluent

during a GC run (Figure 2). Most of the effluent of the FID passes through the trap without special sealing arrangement that would otherwise complicate the GCFC procedure. The advantage of a low pressure drop is not offered by conventional packed traps.

3.3. Comparison of chiral separation by one dimensional and multidimensional gas chromatography

Figure 4 shows the comparison of conventional and multidimensional GC techniques for the enantiomeric separation of *o,p*'-DDD and *o,p*'-DDT. During conventional ^{1}D chiral separation the achiral isomer, *p,p'*-DDD, interferes with the enantiomer (-)*o,p'*-DDT (Figure 4A). In this case, EF values for *o,p'*-DDT cannot be calculated with accuracy in the presence of an interfering peak. However, the interfering *p,p'*-DDD peak was successfully eliminated by selective capturing of only the *o,p'*-DDT isomer during the preceding off-line heart-cut GCFC (Figure 4B). Because the complex matrix is purified by heart-cutting of selected isomers from the total chromatogram, second dimension ^{1}D chiral separation of the *o,p'*-DDT enantiomers was thus achieved without interference by *p,p'*-DDD. Using the complementary technique of comprehensive GC x GC-TOFMS *p,p'*-DDD is similarly well separated from (-)*o,p'*-DDT (Figure 4C).

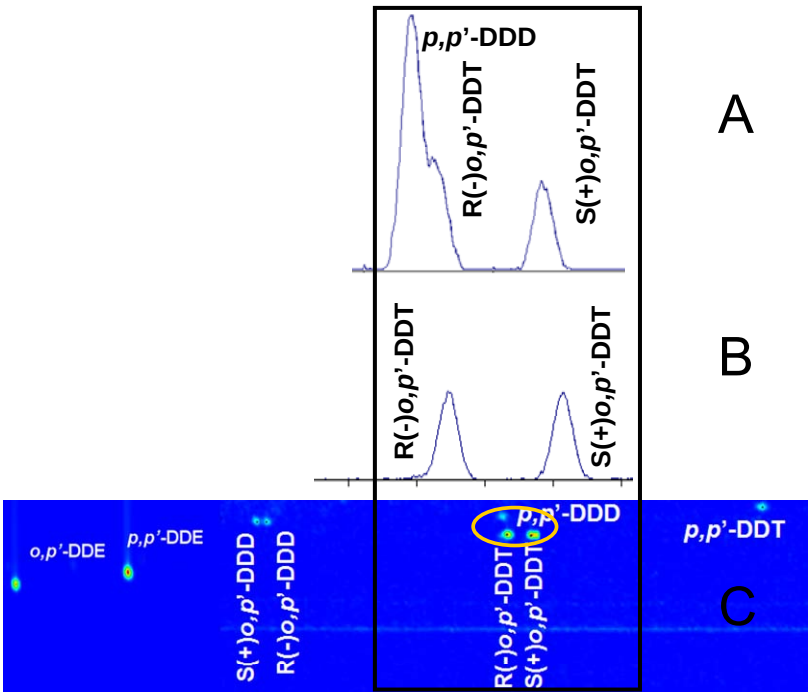

Figure 4. Comparison of conventional and multidimensional GC techniques. (A) *p,p'*-DDD interferes with R(-)*o,p'*-DDT during conventional ^{1}D chiral separation. (B) Second dimension ^{1}D separation of the *o,p'*-DDT enantiomers without interference by *p,p'*-DDD. Off-line heart-cutting eliminated peak interference. (C) The complementary technique of comprehensive GC x GC-TOFMS: *p,p'*-DDD is similarly well separated from R(-)*o,p'*-DDT.

3.4. Multidimensional chromatographic performance

The EF is used as a descriptor of chiral signatures and is defined by: EF = peak area of enantiomer 1/(peak area of enantiomer 1 + peak area of enantiomer 2), where enantiomer 1 is the first eluting enantiomer and enantiomer 2 is the last eluting enantiomer of a pair [8,31]. Thus, EF = 0.5

correlates to a racemic composition of a compound [8,9,31], indicating fresh treatment, whereas a deviation from 0.5 indicates past treatment.

EF values of *o,p*'-DDD and *o,p*'-DDT in a chemical standard, outdoor soil and indoor air samples were determined by ^{1}D heart-cut GCFC–GC-TOFMS and by GC x GC-TOFMS and are given in Table 1. Replicate injections (n=4) of the chemical standard were performed to determine the precision of the EF values obtained with the two multidimensional techniques (Table 1). EF mean values for the chemical standard measured by ^{1}D heart-cut GCFC–GC-TOFMS were 0.494 ± 0.003 (0.69% relative standard deviation (RSD)) for *o,p'*-DDD and 0.498 ± 0.007 (1.38% RSD) for *o,p'*-DDT. The precision of the heart-cut GCFC–GC-TOFMS procedure for replicate injections of the chemical standard, in terms of absolute mass mean values (reported as absolute peak area x 10^{5}), were 55.49 ± 5.21 (9.39% RSD) for S(+)*o,p'*-DDD; 60.03±4.14 (6.89% RSD) for R(-)*o,p'*-DDD; 28.84±1.93 (6.70% RSD) for R(-)*o,p'*-DDT; and 27.61±2.11 (7.66% RSD) for S(+)*o,p'*-DDT. EF mean values for the chemical standard measured by GC x GC-TOFMS were 0.488 ± 0.011 (2.32% RSD) for *o,p'*-DDD and 0.490 ± 0.001 (0.27% RSD) for *o,p'*-DDT. The EF mean values for replicate injections of the chemical standard determined by the two methods were not significantly different from 0.5 ($p > 0.05$) and thus the mixture was considered racemic. Statistical evaluation of the EF mean values for the chemical standard measured by the two multidimensional techniques showed that off-line ^{1}D heart-cut GCFC and GC x GC gave results that do not differ significantly for *o,p'*-DDD ($p = 0.421$) and *o,p'*-DDT ($p = 0.100$).

Table 1
Comparison of enantiomeric fractions (EF) of *o,p*'-DDD and *o,p*'-DDT in outdoor soil and indoor air from a DDT exposed village determined by off-line GCFC–GC-TOFMS and GC x GC-TOFMS

Sample	Isomer	Concentration	$EF_{GCFC–GC\text{-}TOFMS}$	$EF_{GC\ x\ GC\text{-}TOFMS}$
Chemical standard	*o,p*'-DDD	1 ng or 10 ng on	0.494 (10ng, n=4)	0.488 (1ng, n=4)
	o,p'-DDT	PDMS multichannel traps	0.498 (10ng, n=4)	0.490 (1ng, n=4)
Outdoor soil PDMS loops	*o,p*'-DDD	1.0 ng g^{-1} [a]	0.514 (n=3)	0.493 (n=3)
	o,p'-DDT	0.7 ng g^{-1} [a]	0.463 (n=3)	0.508 (n=3)
Indoor air vapour phase on PDMS multichannel traps (IRS 0 h)				
Air D10	*o,p*'-DDD	2.23 µg m^{-3} [b]	0.457	0.491
	o,p'-DDT	2.13 µg m^{-3} [b]	not detected (< 2.13 µg m^{-3})	0.512
Air D8	*o,p*'-DDD		0.523	not determined
	o,p'-DDT		not detected (< 2.13 µg m^{-3})	not determined
Air D7	*o,p*'-DDD		0.518	not determined
	o,p'-DDT		not detected (< 2.13 µg m^{-3})	not determined

[a]Mean concentration values from Van Dyk et al. [24] [b]Mean concentration values for indoor air directly after indoor residual spray (IRS 0 h) from Naudé and Rohwer [27]. Not determined: replicate samples were not available.

3.5. Enantiomeric signatures of outdoor soil and indoor air samples by off-line heart-cut GCFC and comprehensive GC x GC

The EF mean values of 0.493 ± 0.044 for *o,p'*-DDD and 0.508 ± 0.033 for *o,p'*-DDT for three outdoor soil samples by GC x GC-TOFMS (Table 1) did not differ significantly from the EF mean values for the chemical standard ($p_{o,p'\text{-DDD}} = 0.866$, $p_{o,p'\text{-DDT}} = 0.563$). However, the EF values for the three individual soil samples showed variability, ranging from 0.451 to 0.539 for *o,p'*-DDD and from 0.475 to 0.542 for *o,p'*-DDT. The individual EF values for *o,p'*-DDD in soil D12 (EF = 0.490) and *o,p'*-DDT in soil D10 (EF = 0.506) displayed racemic profiles. In contrast, EF values for *o,p'*-DDD in soil D8 (EF = 0.539) and in soil D10 (0.451), and for *o,p'*-DDT in soil D8 (EF = 0.542) and in soil D12 (EF = 0.475), were significantly different from that of the chemical standard ($p < 0.05$). IRS with DDT takes place annually. The outdoor soil samples were collected two months after IRS and therefore the variability in EF profiles of the soils reflects both recent and historic treatment with DDT. EF values determined by off-line ^{1}D heart-cut GCFC–GC-TOFMS were 0.514 for *o,p'*-DDD and 0.463 for *o,p'*-DDT (standard deviations were not calculated since residues were high enough for determination by heart-cut GCFC in only one of the three soil samples). EF values for the soil samples measured by the two multidimensional techniques showed that GC x GC and off-line ^{1}D heart-cut GCFC gave results that do not differ significantly for *o,p'*-DDD ($p = 0.502$) and *o,p'*-DDT ($p = 0.147$).

Directly after IRS, EF values for *o,p'*-DDD in indoor air (vapour phase) by off-line ^{1}D heart-cut GCFC ranged from 0.457 to 0.523 (Table 1). Due to

the low level of *o,p'*-DDT (<2.13 µg m^{-3}) present in indoor air *op'*-DDT was not detected by off-line ^{1}D heart-cut GCFC, and therefore EF values for *op'*-DDT could not be calculated. The enantiomeric mean profiles of indoor air (vapour phase) directly after IRS by off-line ^{1}D heart-cut GCFC displayed compositions that were not significantly different from the racemic profile of the chemical standard ($p_{o,p'\text{-DDD}}$ = 0.953). Replicate sets of the indoor air samples (D7 to D9) were not available and hence EFs were determined by heart-cut GCFC only. Two indoor air vapour phase samples were collected in hut D10 and EFs were determined by both multidimensional procedures: $EF_{GC \times GC}$ was 0.491, while EF_{GCFC} was 0.457 for *op'*-DDD; for *op'*-DDT $EF_{GC \times GC}$ was 0.512, while EF_{GCFC} could not be determined (< 2.13 µg m^{-3}) (Table 1). Due to past DDT treatment non-racemic residues may be emitted from the soil of the floor into the air of traditional huts. IRS is performed annually and chiral signatures of indoor air may therefore be ambivalent due to the presence of both fresh and past DDT treatment.

Table 2
Enantiomeric fractions (EF) of *o,p*'-DDD and *o,p*'-DDT in indoor air vapour phase and indoor air borne particulate phase from a DDT exposed village directly after indoor residual spray (0 h)

Sample	*o,p*'-DDD		*o,p*'-DDT	
	EF	Concentration [a]	EF	Concentration [a]
Indoor air vapour phase $D9_{GCFC}$ on PDMS multichannel trap	0.480	2.23 µg m^{-3}	not detected(< 2.13 µg m^{-3})	
Indoor air borne particulate phase $D9_{GC\ x\ GC}$ on micro-quartz fibre filter	0.583	1.13 µg m^{-3}	0.527	0.28 µg m^{-3}

[a]Mean concentration values for indoor air directly after indoor residual spray from Naudé and Rohwer [27].

Enantiomeric fractions of vapour phase (on PDMS multichannel trap) and air borne particulate phase (on micro quartz fibre filter) *o,p*'-DDD and *o,p*'-DDT in indoor air sample D9 are given in Table 2. Of particular note is that the chiral signature for *o,p'*-DDD in the vapour phase displayed a racemic composition (EF_{GCFC} = 0.480), in contrast to that of *o,p'*-DDD in the air borne particulate phase which showed a non-racemic composition ($EF_{GC \times GC}$ = 0.583) with enrichment of the (+)- enantiomer (Table 2). Also, this $EF_{GC \times GC}$ of 0.583 for *op'*-DDD in indoor airborne particulate phase was significantly different from the enantiomeric profiles of that of outdoor soil, indoor air vapour phase, and of the chemical standard (p = 0.000). Air borne particulate phase pesticide includes dust arising from disturbed surfaces and thus the enantiomeric signature of the particulate phase points to historic DDT treatment. Therefore, two very different enantiomeric profiles are revealed in one sample measurement (single-step denuder sampling distinguishes between vapour phase and particulate phase POPs in air). These first results already indicate the potential importance of investigating EF values of airborne free molecular and particle adsorbed isomers separately.

Compared to off-line GCFC–GC-TOFMS the more sophisticated GC x GC-TOFMS demonstrated enhanced sensitivity of trace level chiral POPs in environmental samples (peaks are narrower in GC x GC). In cases where analytes are present at levels close to detection limits substituting GC-MS (EI+) with negative chemical ionisation (nCI) and selected ion monitoring mode, or with GC-ECD, as a second dimension will greatly improve sensitivity of the off-line heart-cut GCFC method. Muñoz-Arnanz et al. [7] reported

enhancing the limit of detection for *o,p'*-DDT by injection of up to 4 µL solvent extract for enantiomeric separation by traditional heart-cut MDGC-ECD. However, traditional heart-cut MDGC, although successful, is quite complicated involving two independent GC-ECDs, a temperature controlled transfer line and a stream switching system, compared to the simplicity offered by our off-line GCFC technique. The off-line heart-cut method was selective and the interfering *p,p'*-DDD was successfully eliminated, allowing second dimension ^{1}D enantiomeric separation of *o,p'*-DDT in the absence of interference. Furthermore, chiral columns are sensitive to moisture and dirty matrices, and by eliminating the complex matrix the expensive chiral column is protected and its useful lifetime is prolonged.

4. Conclusions

Two multidimensional GC methods, GC x GC-TOFMS and novel off-line GCFC–GC-TOFMS, were evaluated for trace environmental forensic investigations involving solventless sample enrichment with silicone rubber and desorption-injection. Both systems provide sufficient selectivity to perform trace analysis of enantiomers in complex real-life samples. By desorption-injection rather than liquid extract injection, both methods protect the expensive enantioselective column which is sensitive to moisture and matrix components. The off-line GCFC–GC-TOFMS approach provides improved column protection due to injection of only selected isomers from the total complex matrix onto the cyclodextrin column. This procedure has the added advantage of utilising one-dimensional GC and GC-MS equipment

only. The second method using the more sophisticated (and expensive) GC x GC – TOFMS is simpler, providing enhanced sensitivity and fast enantioselective analysis of chiral POPs in environmental samples. First results indicate a significantly different enantiomeric profile for indoor airborne particulate phase compared to the enantiomeric profiles of indoor air vapour phase and of outdoor soil.

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