The development and optimization of a modified single-drop microextraction method for organochlorine pesticides determination by gas chromatography-tandem mass spectrometry

Virgínia C. Fernandes & Viswanathan Subramanian &
Nuno Mateus & Valentina F. Domingues &
Cristina Delerue-Matos

Abstract

We have developed a new method for single-drop microextraction (SDME) for the preconcentration of organochlorine pesticides (OCP) from complex matrices. It is based on the use of a silicone ring at the tip of the syringe. A 5 μL drop of n-hexane is applied to an aqueous extract containing the OCP and found to be adequate to preconcentrate the OCPs prior to analysis by GC in combination with tandem mass spectrometry. Fourteen OCP were determined using this technique in combination with programmable temperature vaporization. It is shown to have many advantages over traditional split/splitless injection. The effects of kind of organic solvent, exposure time, agitation and organic drop volume were optimized. Relative recoveries range from 59 to 117 %, with repeatabilities of <15 % (coefficient of variation) were achieved. The limits of detection range from 0.002 to 0.150 μg kg^{-1}. The method was applied to the preconcentration of OCPs in fresh strawberry, strawberry jam, and soil.

Keywords

Organochlorine pesticides · SDME · GC-MS/MS

Introduction

Organochlorine compounds are persistent environmental contaminants that were intensively used in the past as pesticides [1, 2]. Some of the undesirable consequences of pesticide use include the presence of residues in the soil, water, and air; residues in plant and animal tissues and the presence of residues in food [3]. Organochlorine pesticides (OCP) are associated with many chronic diseases [4]. Many problems have been linked to these endocrine disrupters, such as neurological damage, birth defects, respiratory illness, breast cancer, lowered sperm counts and immune system dysfunction [1, 5].

To protect the human health, various guidelines and regulations have been implemented to provide safe food by fixing maximum residue limits (MRL) in various commodities. Innovative sample preparation strategies, particularly that involving enrichment of analytes and advancement in analytical instrumentation may substantially contribute to decrease the limit of the detection (LOD) with acceptable method performance characteristics [6, 7]. In the determination of OCP in different samples, many preparative processes such as liquid–liquid extraction (LLE) [8], microwave assistant extraction (MAE) [9–11], supercritical fluid extraction (SFE) [8, 12], solid-phase extraction (SPE) [13–15] and solid-phase microextraction (SPME) [16–19] have been suggested. Other sophisticated methods for sample preparation such as matrix solid-phase dispersion (MSPD) [20, 21] and QuEChERS method (acronym for quick, easy, cheap, effective, rugged and safe) [22, 23] have also been developed by providing favorable recoveries and also simultaneously lower cost and time of analysis [24].

The solvent microextraction, now called single-drop microextraction (SDME), is also known as liquid–liquid microextraction (LLME) or liquid phase microextraction (LPME). This method is based on the principle of a distribution of analytes between a microdrop of an organic solvent and an aqueous phase [3]. Single-drop microextration (SDME), developed by Jeannot and Cantwell [25] and optimized by Liu and Dasgupta [26] provides an alternative technique, which integrates sampling, extracting and concentrating into a single step [5, 27, 28]. SDME disadvantages include drop volume variation during the process of extraction, which affects parameters such as: drop stability, drop solvent dissolution when using extreme conditions of extraction, such as a high stirring speed, long extraction time, high temperature, and operator experience, which may affect SDME linearity and precision [6, 29]. SDME has several advantages compared to other extraction/preconcentration techniques: uses a negligible amount of organic solvent, offers the freedom to select the most suitable solvent for the target analytes, requires only a short time for analysis, has a high sensitivity and low cost when compared to SPME and SPE, and uses simple equipment [18].

In order to overcome the drawbacks associated with SDME and fully utilize its potential, further studies are necessary. New modifications in microsyringe needle, viz. angle of tip, fabrication of tip and stainless steel material of tip having solvent holding capacity, can improve the performance [6]. Ahmadi et al. employed some modification of the needle tip, causing its cross section to increase and increasing adhesion force between the needle tip and the drop, thereby increasing drop stability and achieving a higher stirrer speed (up to 1,700 rpm) [30].

SDME in pesticide residues analysis has been applied with success in both liquid water [27, 30, 31], juices [32, 33], wine [34, 35], oils [36] and solid samples (vegetables [24, 37, 38], fish [39] by providing low limits of detection and high selectivity as compared with classic robust sample preparation techniques. The novel approachs of SDME were developed by different authors. Liu et al. suspended a much larger volume of drop on the tip of the microsyringe needle with a 3 mm long polytetrafluoroethylene (PTFE) tube [40]. Qian and He developed a novel SDME assisted with a small funnel to determine oganochlorine and pyretroids in tea [41].

The purpose of this work is to prepare a small extraction device for improving the extraction performance of SDME using a technique to enlarge the drop volume and overcome the instability of the drop. This technique is often used for analysis of liquid samples. In this paper we attempt to develop a method for the extraction of OCP from solid samples. The jams are first studied by this method. Therefore, this study aims to investigating the possibility and merits of the improved SDME; optimize, validate, and apply a SDME method to 14 OCP analysis in different solid samples namely soils, strawberries and jams by GC-MS/MS. Other highlights of the method will be described.

Experimental

A total of 14 OCP were selected for this study: α-, β-, γ- and ζ-hexachlorocyclohexanes (lindane) (HCH) , hexachlorobenzene (HCB), o,p′-DDT (1,1,1 trichloro-2,2-bis- (p-chlorophenyl) ethane), p,p′-DDE ([2,2-bis(p-chlorophenyl)-1,1-dichloroethylene]), p,p′-DDD (dichlorodiphenyldichloro-ethane), aldrin, dieldrin, endrin, α-, β-endosulfan, and methoxychlor. Pesticide standards (purity >97.0 %) were obtained from Chemservice (West Chester, PA, USA), Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Sigma-Aldrich Co. A mixed stock solution of 350 μg L^{-1} of each OCP was prepared in n-hexane. For optimization method, a mixed standard solution of 10 μg L^{-1} of each OCP was used, obtained by pipetted a known volume of stock solution and dried with a stream of nitrogen and diluted with water obtained from a Millipore Elix water purification system from Millipore (Interface, Portugal). For the calibration curve a mixed standard working solutions with concentrations of 0.5, 1, 5, 10, 20, and 50 μg L^{-1} were prepared by the same way describe above. The 4,4′-dichlorobenzophenone was purchased from Sigma-Aldrich and used as internal standard (IS) for chromatographic analysis. Chromatographic grade n-hexane, acetonitrile and tetrachloromethane were purchased from Merck (Darmstadt, Germany). Dichloromethane was purchased from AnalaR Normapur.

Strawberry samples and their soils were collected in two types of cultivation: Organic farming (OF) and integrated pest management (IPM) both from Portugal. The sampling period was the first week of May in 2009 and 2010. Strawberry jams were obtained from local food stores.

SDME procedure

The SDME procedure was performed using a 10 μL microsyringe from Thermo® with an addition of a silicone ring (2 mm in length) cutted from a silicone tube and placed on the edge of a cone needle tip (Fig. 1). A glass vial with 20 mL of volume was used in SDME procedure.

The efficiency of SDME was evaluated changing parameters such as extraction time interval (5, 15, 30, 45, 60 min), stirring speed (100, 200, 300 rpm), type of solvent (dichloromethane, n-hexane and tetrachloromethane), microdrop volume (2, 4, 5 μL) and ionic strength (0.5 %, 1 %, 2 % sodium chlorine). The stirrer used is from Multistirred 15, Velp Scientifica. For all the experiments a solution of 10 mL of water contaminated with 10 μg L^{-1} of each OCP was prepared and used to optimize the optimum experimental conditions. The solid and semi-solid samples were homogenized with this pesticide mixture and leaving 30 min at room temperature. Before the SDME, the samples were centrifuged at 10,000 rpm for 10 min (Eppendorf centrifuge 5424, Hamburg, Germany). The microsyringe plunger was depressed to expose the solvent drop to the sample to allow the transfer of analytes from the aqueous phase to the drop. After microextraction, the organic drop was retracted back into the syringe and the needle removed off the vial and immediately injected into the gas chromatograph coupled to a tandem mass spectrometer (total run time of 21 min). The other parameters were tested under the same conditions previously described using n-hexane.

Sample fortification

The recovery experiments were carried out on strawberry jam, soil and strawberry sample by fortifying the samples (2 g) in three replicates with the pesticide mixture separately at three concentration levels (1, 10, 50 μg kg^{-1}) in 10 mL of millipore water. The samples were shaken and left at room temperature during 30 min. Before starting SDME, the samples are centrifuged in order to make the solution more translucent to allow viewing of the drop. The sample recoveries achievement was done by using the peak area of each analyte and the concentration obtained by the calibration curve. Only the IS was added before the SDME extraction to the samples during the sample preparation.

Chromatographic analysis

Analyses were performed on a GC-MS system from Thermo Electron Corporation consisting of a Trace GC Ultra gas chromatograph and a Polaris Q mass spectrometer system operated in the electron impact ionization (EI) at 70 eV controlled by Xcalibur 1.3 software. Chromatographic separation was performed on a 30 m× 0.25 mm ID ZB-XLB capillary with a film thickness of 0.25 μm, operating in large volume injection (LVI) in a PTV Injector. Ultrapure grade helium (He) (Linde Sógas purity ≥99.999 %) was used as the carrier gas at 1.3 mL min^{-1} flow. The GC oven temperature was programmed from an initial temperature of 40 °C (2 min hold), ramped at 30 °C min^{-1} up to 220 °C (5 min hold) and finally at 10 °C min^{-1} to 270 °C with holding for 1 min. This program resulted in a total run time of 21 min. The interface line and ion source temperatures were maintained at 250 °C. The MS/MS conditions were fixed for each compound, trying to select as precursor ion the one with highest m/z ratio and abundance (Table 1). Analyses were performed in the MS/MS mode.

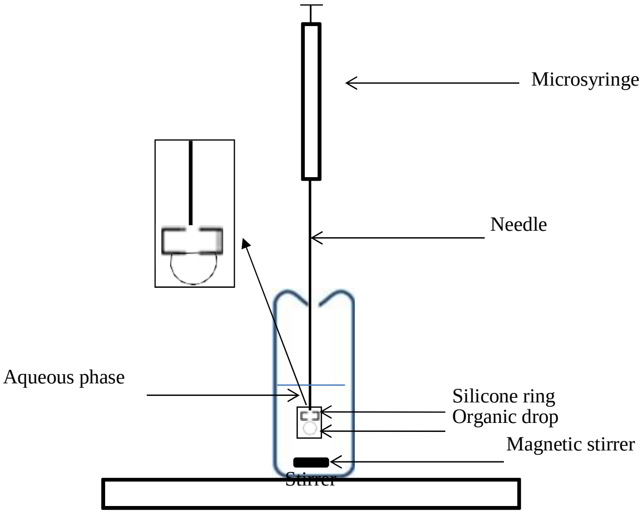

Fig 1 SDME experimental set-up

The LVI-PTV program consisted of four distinct phases: injection, evaporation, analyte transfer to the column, and cleaning. For the injection phase, the initial temperature was kept at 67 °C with pressure set at 50 kPa and He flow at 50 mL min^{-1} with a hold for 0.05 min. The pressure was increased to 125 kPa and temperature was raised to 77 °C at the rate of 5 °C/s with a hold for 0.3 min during the evaporation phase. For the transfer phase, the pressure was increased to 250 kPa and temperature increased to 285 °C at the rate of 14.5 °C s^{-1} with a hold for 1.5 min. Finally, in the cleaning phase, the temperature was increased to 295 °C at the rate of 14.5 °C s^{-1} and held for 10 min, with gas flow maintained at 50 mL.min^{-1}. To attain a low quantification limit, the injection volume was optimized at 5 μL. The injection, evaporation, transfer, and cleaning phases of the LVI-PTV system were optimized to achieve the highest S/N of each individual analyte at the 10 μg L^{-1} level.

Results and discussion

Parameters optimization for SDME technique

It has been well documented that the extraction of analytes from sample solution to organic liquid drop could be influenced by several conditions [4, 24]. Therefore, parameters such as selection of organic solvent, sample agitation rate, extraction time, microdrop volume and ionic strength were optimized for the SDME process. Full details of the optimization for SDME are provided in Supporting Information Figure S1 and S2.

Solvent selection

Different organic solvents, namely toluene, iso-octane, chloroform and n-hexane, are frequently used in SDME, as the acceptor phase, of liquid drop to extract the pesticides from aqueous solution [3, 39]. After three replicate analysis, the chromatographic signals obtained from the analytes using n-hexane as the acceptor phase, are approximately two times higher than the signals obtained with dichloromethane and tetrachloromethane. From the results, n-hexane was used as the acceptor phase for the extraction of OCP from aqueous solution.

Table 1 MS/MS conditions of the analysed pesticides

MS/MS				
Pesticide	Molecular weight (g mol^{-1})	Retention time (min)	Precursor ions (m/z)	Product ions for MS/MS method (m/z)
α-HCH	291	7.85	219	109, 146
β-HCH		8.55		
Lindane		8.70		
δ-HCH		9.06		
HCB	285	7.95	142	141
Aldrin	365	9.80	263	191
α-endosulfan	407	12.10	241	207, 195, 170
p,p′-DDE	318	12.75	318	176, 211
Dieldrin	381	12.94	243	206, 211
Endrin	381	13.60	245	211
β-endosulfan	407	14.44	241	207, 195, 170
p,p′-DDD	320	13.93	235	165, 199
o,p′-DDT	355	14.32	235	165, 199
methoxychlor	346	16.44	227	152, 169

Microdrop volume

The increase in the drop volume results in an increased extraction efficiency [3, 42]. In the analysis of pesticides by SDME, it is common to use 1 μL of organic solvent drop volumes because they form stable drops and thus allow the use of high stirring rates [30, 43]. On the other hand, a drop volume of 1 μL is consistent with GC instruments [42]. In this study, the addition of a silicone ring in the microsyringe needle permitted larger microdrop volumes with stable drops. This approach achieves a better performance in the extraction efficiency. Figure 2 shows the photos of the size of the microdrop obtained with the different volumes used in these tests. Without silicone ring is impossible to increase the drop volume because the microdrops always go up, while with the ring, they remain stable in the surface of the silicone. In photos captured of the different extractant volumes, seemingly there are no big differences between the drops, however the volume of 5 μL the drop size is more evident. The signal area of the 2 μL drop chromatogram was lower than the 5 μL drop. The results suggest that the drop size of 5 μL could be selected for SDME process, because it was stable at this volume and at the same time the efficiency of the extraction was increased. The extraction efficiency increased significantly between 4 and 5 μL, maybe because, as seen in the Fig. 2 the exposed area actually changes more significantly in 5 μL drop.

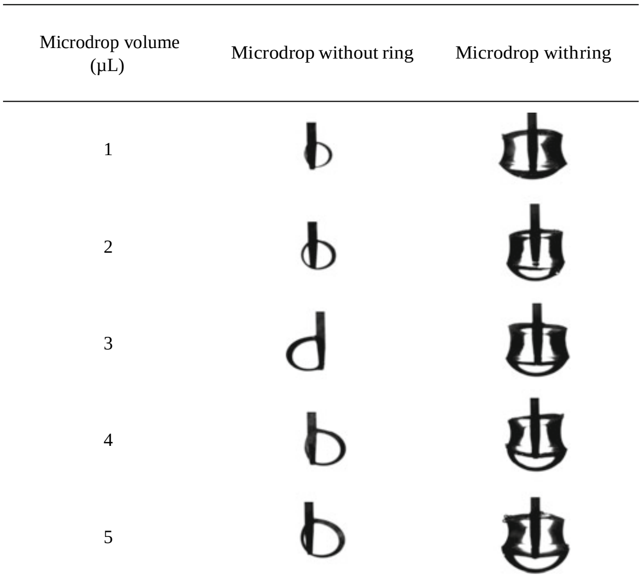

Fig 2 Photos of the different sizes of the microdrops

Sample agitation

Innovative approaches have been employed to achieve higher stirring speed while maintaining the drop stability [3, 6]. In the extraction, the increase of the stirring rate reduced the thermodynamic equilibrium time and increases extraction efficiency since a suitable stirring rate makes a continuous exposure of the extraction surface to the sample. For SDME, however, the over stirring rate would tend to break up the organic drop. To increase the extraction efficiency, agitation of the sample solution was carried out at varying stirring rates of 100, 200 and 300 rpm. Better signal intensity was obtained with 200 rpm and the stability of the organic liquid drop decreased for 300 rpm.

Influence of extraction time

In general, an increase in the signal is expected on increasing droplet volume; however, larger organic solvent drops sometimes require extended equilibration times because mass transfer inside the drop is only due to diffusion. The extract amount at all the OCP greatly increased with the increase of the extraction time until it reached 45 min, from which time the analytical signal started to decrease. To trade off the analytical speed and the highest extraction efficiency, the extraction time of 45 min was employed for further study. Therefore, 45 min was selected as the optimum extraction time.

Ionic strength effects

The addition of salt has a remarkable effect on the extraction of analytes and this effect was similar for all OCP analytes. The addition of salt can reduce solubility of analytes or increase concentration of analytes in the solution which may be beneficial to improve the response signal [43]. On the other hand, the addition of salt can cause the change of the physical properties of the extraction film, thus reducing the diffusion rates of the analytes into the microdrop [6]. The extraction efficiency decreases from 0.5 % to 2 % NaCl and the signal increases slightly when 0.5 % of NaCl is presented. In order to obtain the best performance, 0.5 % NaCl was employed for further study.

Validation of the SDME analytical method

Pesticides were identified according to their retention times and precursor and selected ions. The parameters used in this study for the validation of the developed analytical method were as follows: linearity, precision, limit of detection (LOD) and recoveries.

Linearity was studied using a pre-concentration of 10 mL of ultra-pure water fortified with a mixture OCP standard solution in the concentration range of 0.5–50 μg kg^{-1}. The result for the coefficient of variation (CV) was obtained with triplicates (*n* 03), using a sample of ultra-pure water that had been spiked to a concentration of 10 μg kg^{-1} for each OCP. The calculated values of CV were in the range of 0.3–14.8 % thus indicating that the developed method is precise. The determination coefficients (R^2) were greater than 0.99 for all the OCP, indicating a good linearity of analytical curve. The LOD was established by the signal/noise ration equal to 3 in an analysis of each sample, spiked at a lowest concentration. The LODs obtained in different matrices were similar between them. The found values of LOD (0.001–0.11 μg kg^{-1}) in strawberry sample are lower than those obtained using QuEChERS with GC-ECD and GC-MS/MS method [44], which demonstrates the high sensitivity of the method. Full details of these parameters (Linearity, CV, LOD) for each one of the pesticides are provided in Supporting Information Table S1.

In fact, the present method is able of detecting and quantifying OCP concentrations below the maximum permitted residue level, according to the European Union for fresh strawberries (soils and jam have no legislation). The use of LVI-PTVallowed the increase of the injection volume from 1 μL with split/splitless inlet to 5 μL or higher with PTV inlet, yielding in greatly enhanced analytical sensitivity for analytes with low concentrations.

Because of simplified extraction procedures (a benefit of the high selectivity of MS/MS detection), many compounds or metabolites, can coelute with the analytes of interest and not show up as interference peaks at the m/z channel of the analytes. Those unseen peaks cause matrix suppression and inconsistent signals. Relative recoveries were obtained using a sample without the analytes which was then contaminated with known quantity of a standard mixture with each OCP. The results for each set of experiments are summarized in Supporting Information in Table S2. The samples were spiked with 10 μg kg^{-1} OCP analytical solution in order to assess possible matrix effects. Seven of the fourteen OCP did not exert matrix effects, considering 70 % as the acceptable lower limit and 120 % as the upper limit [45]. The developed method proved to be accurate for the quantification of 7 OCP in the samples tested, as the achieved recoveries were in the range 70–120 %. However, the other seven organochlorine pesticides, mainly, α-HCH, HCB, lindane, ζ-HCH, p,p′-DDE, Endrin and p,p′-DDD, has a notable matrix effect, especially with jams. The decrease in relative recoveries observed could be attributed to three different phenomena: (i) the possible competitive adsorption of target analytes by matrix; (ii) adsorption by the containers and; (iii) losses during sample evaporation and centrifugation. The method developed was validated for strawberry, strawberry jam and soil matrices based on the SANCO European Guidelines.

The developed method in mostly proved to be accurate, as the achieved recovery was in the range of 59–117 %. The relative recoveries from strawberry sample were 70–98 % for α-HCH and α-endosulfan, respectively, from strawberry jam were 59–94 % for α-HCH and β-endosulfan, and from soil were 63–117 % for HCB and methoxychlor, respectively. The results demonstrate that strawberry jam and soil matrices have a much higher effect on SDME analysis of the studied pesticides.

Application of the developed method

The presented method was applied to 12 strawberries, 9 soils and 9 strawberry jams and the analyses of pesticide residues were determined. Figure 3 displays the GC-MS/MS chromatograms of a strawberry sample that shows the pre-

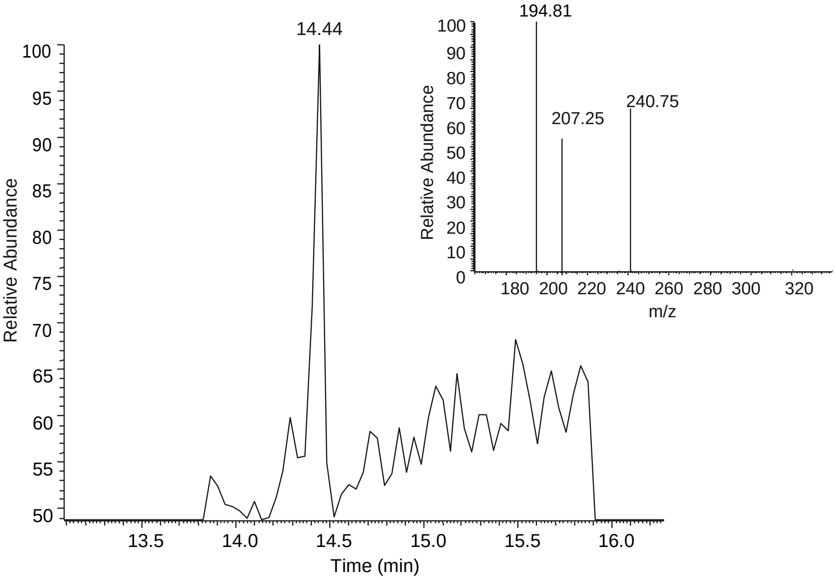

Fig 3 GC-MS/MS chromatogram of strawberry samples with β-endosulfan (7.12 μg kg^{-1})

sence of β-endosulfan below the MRL. In the inset is also shown MS/MS spectra of the ion products of β-endosulfan and then the identification of the residue. Quantification of pesticides was carried out through the calibration curves by GC-MS/MS, obtained in terms of μg kg^{-1} according to the recovery value (Table 2). Five of the investigated strawberries and soils samples showed a presence of lindane at a concentration higher than MRL (10 μg kg^{-1}) in strawberries. The highest concentration found was 27 μg kg^{-1} of lindane. One of these strawberry and soil samples was from organic farming. Nevertheless, despite all of the pesticides detected have been banned for several years in the countries of the European Union; some of them are still present in the environment because of their resistance to the degradation. The results emphasize the need for the maintenance of continuous monitoring of pesticide OCP residues. Among the analyzed pesticides, only lindane and β-endosulfan were found above MRL in strawberries.

Conclusions

A sensitive analytical method comprising SDME coupled with LVI-PTV-GC–MS/MS has been developed to quantify trace levels of 14 OCP in complex samples. Other highlights of the developed method include its ease of use and its requirement of only small volumes of both organic solvent and sample, which makes it suitable for the measurement of OCP levels in different samples. The addition of a silicone ring in the microsyringe needle improved the stability in a larger microdrop volume. The SDME method has been optimized in order to significantly reduce the resources used (time, reagents and experimental work), toxicity and negative environmental effects caused by the solvents used. Sample preparation time was minimized without reducing method sensitivity. This easy-to-handle and cost-effective method represents an attractive alternative to a traditional SDME, as it affords better results.

Table 2 OCP residues detected in the samples analysed by SDME

OCP	MRLs[a] (μg kg^{-1})	Strawberries	Soils	Strawberry jam
		Concentration range (μg kg^{-1})		
HCB	10	nd	nd	1.0–1.3
Lindane	10	3.2–27.1	3.5–21.9	1.0–1.4
ζ-HCH	10	nd	2.1–2.3	nd
Aldrin	10	0.7–1.2	1.0–20.4	0.4–0.5
p,p′-DDE	50	nd	0.1–0.4	nd
Endrin	10	0.2–4.4	0.2–5.2	2.0
β-Endosulfan	50	7.1	1.9–7.4	nd
p,p′-DDD	50	0.1–0.4	0.3–0.4	nd
o,p′-DDT	50	0.1–1.2	0.3–2.1	nd
Methoxychlor	10	0.2–1.9	0.5–2.1	nd

[a] For strawberries

nd not detected

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