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Application of static liquid-phase microextraction to the analysis of organochlorine pesticides in water

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Abstract

Static liquid-phase microextraction, with subsequent analysis by gas chromatography–electron-capture detection, has been applied to extract eight organochlorine pesticides from water. A conventional microsyringe was used to extract analytes from water samples over a concentration range of 0.05–100 μg/l. Factors relevant to the extraction process were investigated. The sensitivity of the method was enhanced with agitation, and increasing the extraction temperature, of the sample solution. Concentration factors of >50-fold were easily achieved within 25 min of extraction. The analytical data exhibited a relative standard deviation (RSD) range of 3.2% (lindane) to 10.7% (methoxychlor) for the eight pesticides; most RSD values were under 7%. Water samples collected from a reservoir, and from tap water in a chemical laboratory were analyzed using the procedure.

Keywords: Water analysis; Environmental analysis; Liquid-phase microextraction; Extraction methods; Organochlorine compounds; Pesticides

1. Introduction

Organochlorine pesticides (OCPs) are one of the most persistent organic pollutants present in the environment. The toxicity, potential bioaccumulation and non-biodegradability of these compounds represent risks to the environment in respect of human health [1]. Hence, evaluation and monitoring of trace levels of these compounds from different environmental matrices are imperative. In order to determine trace level of these pollutants, an extraction and pre-concentration step is necessary.

Conventional liquid–liquid extraction (LLE) [2] and solid-phase extraction (SPE) [3,4] have been commonly used for the extraction of organochlorine pesticides from aqueous matrices. However, LLE has many disadvantages: it requires large amounts of toxic organic solvents, and is time-consuming and tedious. Although SPE is less time-consuming than LLE, it still requires an appreciable amount of toxic solvent for analyte desorption. Solid-phase microextraction (SPME) is a more recent procedure and has been developed for pretreatment of OCPs [5–7]. It has important advantages over conventional extraction techniques because it is solvent-free, fast, portable and easy to use. But SPME also suffers from some drawback: its fiber is fragile and has limited lifetime, and sample carry-over is also a problem [8].

Liquid-phase microextraction (LPME) was developed as a solvent-minimized sample pretreatment

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procedure, which is quick, inexpensive and since very little solvent is used, there is minimal exposure to toxic organic solvents. This novel technique for sample extraction has been described in several papers [9–12]. In this simple technique, a microdrop of solvent is suspended directly at the tip of either a PTFE rod or a microsyringe needle that is immersed in a stirred aqueous sample solution. In the latter case, after exposure for a prescribed time, the microdrop is retracted into the microsyringe, and part or all of the solvent is injected into the gas chromatograph for analysis. One advantage of LPME over other liquid extraction techniques is that only small volumes of organic solvent are required. Due to the extremely small phase ratio (i.e., 3 μl of organic/3 ml of water), this microextraction system has been successfully applied to speciation studies [10]. Another important feature of LPME is the integration of extraction and injection in a single device, i.e., a commonly used microsyringe, which functions as a microseparatory ''funnel'' for extraction as well as a syringe for injection into the gas chromatograph [10–12]. Apart from being inexpensive, LPME requires only common laboratory equipment and does not suffer from carry-over between extractions that may be experienced using SPME.

The use of LPME in OCPs analysis was described previously [13,14]. In the present work, eight semi-volatile OCPs, including several not considered previously, were selected as our target compounds, and factors influential to the LPME procedure were examined and discussed. Using the optimized conditions, the limits of detection (LODs) for most analytes of less than 0.05 μg/l can be achieved. The optimized conditions were also applied to tap water and reservoir water to evaluate the method's application to real samples.

2. Experimental

2.1. Standards and reagents

All pesticides used were purchased from Polyscience (Niles, IL, USA). 1,3,5-Trichlorobenzene (TCB) used as internal standard (I.S.) was obtained from Aldrich (Milwaukee, WI, USA). Pesticide-grade *n*-hexane and HPLC-grade methanol were purchased from BDH (Dorset, UK). The water used was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Each pesticide was dissolved in methanol to obtain a standard stock solution with the concentration of 1.00 mg/ml. They were stored at 4°C. A fresh 10.0 mg/l standard solution containing the eight pesticides was prepared in methanol every week and stored at 4°C.

Natural water from a reservoir was collected for this work. The water was filtered through a Whatman (UK) filter paper and a 0.45-μm membrane (Millipore) to eliminate particulate matter before analysis.

Tap water samples (directly potable) were collected from a laboratory. It was freshly collected, after allowing the water to flow for 4–5 min.

2.2. Instrumentation

Chromatographic analysis was performed on a Hewlett-Packard (Palo Alto, CA, USA) 5890 Series II gas chromatograph equipped with a split/splitless injector, which was used in splitless mode and a ^{63}Ni electron-capture detection (ECD) system. Separations were conducted using a DB-5, 30 m×0.32 mm capillary column with a 0.25 μm stationary phase thickness (J&W, Folsom, CA, USA). The carrier gas was purified nitrogen, at a flow-rate of 1.6 ml/min. The gas chromatography (GC) conditions were as follows: injector temperature 240°C; detector temperature 260°C; initial oven temperature 60°C for 1 min, increased to 150°C at rate of 30°C/min, then maintained at 150°C for 3 min, a second ramp 210°C at rate of 2°C/min, then held at 210°C for 3 min. The total time for one GC run was 40 min.

2.3. Extraction apparatus

A sample vial was placed in a water-jacketed vessel and maintained at a constant temperature (50°C) by a water bath. During an extraction, a 10-μl microsyringe (ITO, Fuji, Japan) was clamped above the sample vial so that the syringe needle tip was below the surface of the aqueous sample. The solution was stirred at 400 rpm. The type of microsyringe employed here has a needle with a 22°C bevel (Fig. 1).

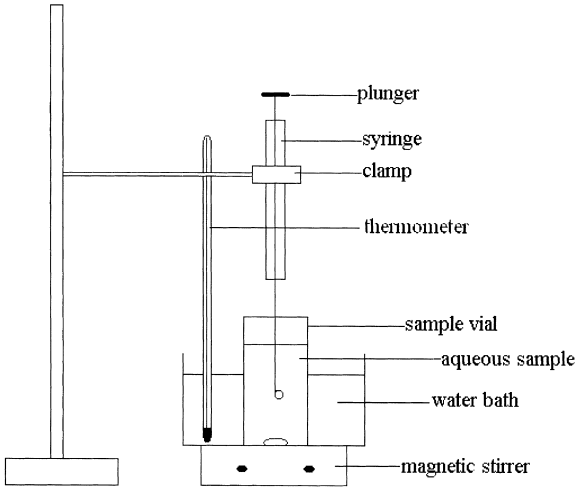

Fig. 1. Schematic of static LPME.

2.4. Static LPME procedure

The static LPME procedure used here has been described in the literature [12]. Briefly, an aliquot (3.5 μl) of *n*-hexane containing 200 μg/l of I.S. was withdrawn into syringe. The syringe plunge was depressed by 0.5 μl to produce a small drop at the tip of syringe needle, which was inserted through the sample vial septum and the needle tip was immersed into the 3-ml stirred sample solution to a depth of 1 cm below the surface. The syringe was held in place by a clamp. The syringe plunge was depressed to expose a 3-μl drop of solvent to the sample. Extraction then occurred between this droplet and sample for 25 min. The drop was retracted into the syringe, which was removed from the sample vial. The plunger was then depressed to the 1-μl position, and the needle tip was cleaned carefully with a tissue to remove possible water contamination. Finally the extract was injected into the gas chromatograph.

3. Results and discussion

3.1. Optimization of static LPME

In order to obtain the optimized extraction conditions and best extraction efficiency, we used the ratio of peak area of analyte and that of internal standard as the GC response to evaluate the extraction efficiency under different conditions. The enrichment factor, defined as the ratio of the GC response after extraction and that before extraction, was also used to evaluate the efficiency.

3.1.1. Solvent selection

Several solvents were evaluated for their suitability. For each solvent, the enrichment factor (for a sample sampling volume of 1 μl) was calculated and is shown in Table 1. The data suggest that using *n*-hexane as the extraction solvent can receive higher extraction efficiency than other solvents. The primary reason is that the low polarity (polarity index, 0.9) of hexane favored towards non-polar compounds that the OCPs are, and its low solubility in water (1.4×10^{-4}, w/w) [15], which results in very

Table 1
Efficiencies of various organic solvents[a]

Pesticide	Enrichment (-fold)			
	n-Hexane	Toluene	*n*-Nonane	Cyclohexane
Lindane	60	60	30	40
Aldrin	6	6	6	4
Methoxychlor	20	20	15	20
Heptachlor	20	10	10	10
Dieldrin	20	20	15	15
p,p′-DDD	25	7	10	10
p,p′-DDT	20	10	10	2
Endosulfan	30	15	10	10

$n=3$.

[a] Water samples at a concentration of 20 μg/l of each compound. Sampling volume = 1 μl. Data were obtained from mean values of three determinations.

little solvent lost through dissolution in water in the extraction procedure.

3.1.2. Organic solvent dropsize

The *n*-hexane drop was exposed to the aqueous sample for a specific period of time prior to injection for GC analysis. We investigated the influence of different dropsizes in the range of 1.5–5 μl. Fig. 2 shows that the GC responses increase with hexane drop volume in the range of 1.5–3 μl and then decrease when the dropsize was increased to 5 μl. When dropsize exceeded 5 μl, the hexane drop became too unstable to be suspended at the needle tip.

In general, LLE involves the distribution of solute between two immiscible liquid phases. Most often, a solute is extracted from an aqueous solution into an immiscible organic solvent. The extraction speed is strongly affected by observed rate constant (s^{-1}) [12,16,17] given by:

$$k = A_i \beta_o \cdot \left(\frac{\kappa}{V_{aq}} + \frac{1}{V_o} \right) \quad (1)$$

where V_o and V_{aq} are the volumes of the organic and aqueous phases, respectively; A_i is the interfacial area, β_o is the overall mass-transfer coefficient with respect to the organic phase, and κ is the distribution coefficient. It is obvious that large A_i and small V_o and V_{aq} are beneficial for fast extraction. However, increasing dropsizes leads to the increase in both interfacial area A_i and organic volume V_o. Hence, the influence of dropsize comes from the integrated influence of two factors. This explains why the GC response increases with increasing dropsize up to a point and decreases thereafter.

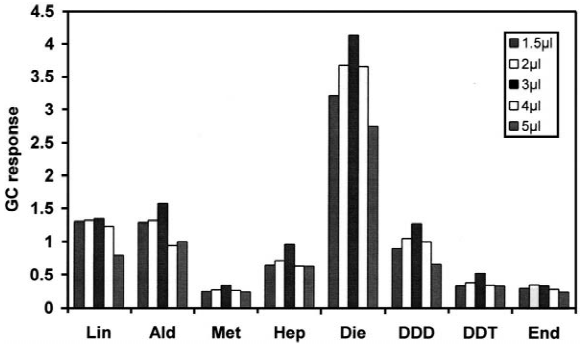

Fig. 2. Effects of different dropsize on the extraction efficiency. Abbreviations: Lin = lindane, Ald = aldrin, Met = methoxychlor, Hep = heptachlor, Die = dieldrin, DDD = *p,p′*-DDD, DDT = *p,p′*-DDT, End = endosulfan.

There is also another influential factor [10]. There must be a small but finite space between the side of the wire plunger and the inner wall of the syringe needle. Furthermore, the length, and thus the volume, of this film increases as the plunger is depressed and decreases as the plunger is retracted. When the solvent drop is drawn back into the needle, it mixes with the *n*-hexane/I.S. solution from the portion of the film that is liberated by the retracting plunger. This represents a dilution of the drop with additional I.S. solution after extraction. One point that should be mentioned here, is that the concentration of I.S. was not diluted as it was prepared directly in the *n*-hexane. The volume of the film liberated by withdrawing the plunger cannot affect the I.S. concentration. This factor also contributes to the decrease of GC response intensities when the solvent dropsize is larger than 3 μl.

3.1.3. Extraction time

The effect of time was examined in the range of 5–50 min at room temperature (23°C) with constant stirring speed. The GC signals generally increased with extraction time until 40 min. After 40 min, the extraction system was basically at a steady state and no dramatic increase was observed with additional extraction time. We select three compounds as representatives of the OCPs, and show their behavior under these extraction conditions in Fig. 3.

Like SPME, static LPME is a process dependent on equilibrium rather than exhaustive extraction. The amount of analyte extracted at a given time depends upon the mass transfer of analyte from the aqueous phase to the organic solvent phase. This procedure requires a period of time for equilibrium to be established. Normally, the time for establishing equilibrium was selected as the extraction time. However, in the present work, drop depletion must be considered in choosing the extraction time. Although *n*-hexane has a very low solubility in water, the effect of drop depletion cannot be entirely negligible in LPME since only a small volume of organic solvent (3 μl) was used in the technique, and

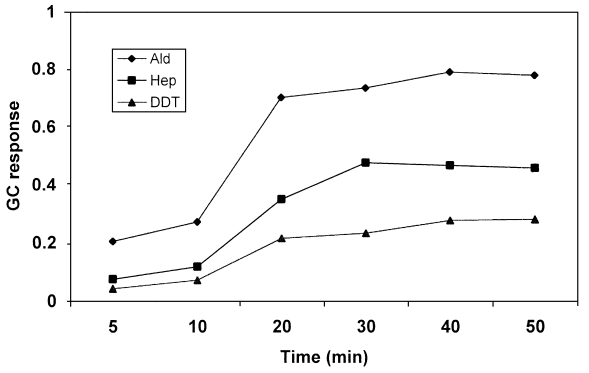

Fig. 3. Time dependence of the equilibration of aldrin, heptachlor and *p,p′*-DDT between the aqueous and the solvent phases. Abbreviations: Ald = aldrin, Hep = heptachlor, DDT = *p,p′*-DDT.

drop depletion would lead to concentration variations of extractants and I.S. in the micro drop. Fig. 4 shows that drop depletion increases with the extension of extraction time.

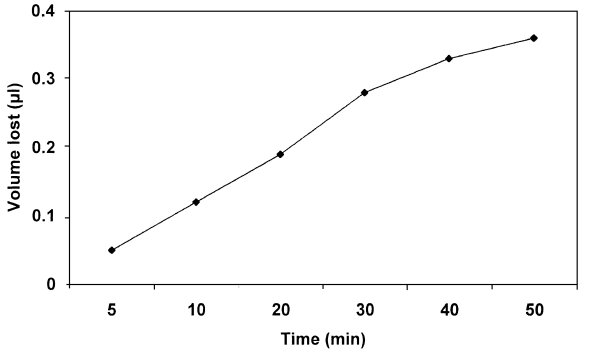

Fig. 4. Time dependence of drop depletion of *n*-hexane/I.S. solution in the aqueous sample.

An exposure time of 25 min was a reasonable compromise for an acceptable extraction time and drop depletion, and was selected for subsequent experiments. Careful attention to consistent extraction operations ensured that quantitative analysis was not compromised.

3.1.4. Stirring rate

The effect of agitation on the extraction of pesticides was studied next. The results, shown in Table 2, indicate that the agitation improved the extraction efficiency significantly.

Based on the film theory of convective-diffusive mass transfer, at steady state, the aqueous phase mass-transfer coefficient β_{aq} is given by:

$$\beta_{aq} = D_{aq}/\delta_{aq} \quad (2)$$

where D_{aq} is the diffusion coefficient in the aqueous phase, δ_{aq} is the thickness of the diffusion film. The film theory of convective-diffusive mass transfer was confirmed to be valid in the LPME system [10]. According to the theory, β_{aq} increases with increasing stirring speed (rpm) because faster stirring speed can decrease the thickness of the diffusion film δ_{aq} in the aqueous phase. As a consequence, agitation produced an enhancement of extraction efficiency. Our results support this explanation.

Nevertheless, although high stirring speed resulted in greater extraction efficiency, it also gave rise to instability of the organic drop. The stability of a micro drop at the tip of the needle depends on the balance of three forces [12]. However, the stability is disrupted by mechanical forces in the sample solution if it is stirred too vigorously. For example, at a stirring speed 600 rpm, it was difficult to maintain

Table 2
Effect of stirring on the extraction efficiency for OCPs (20.0 μg/l) from aqueous samples

Target compound	Relative response			
	No stirring	200 rpm	400 rpm	600 rpm
Lindane	100	470.6	702.7	1002.8
Aldrin	100	243.2	363.7	433.5
Methoxychlor	100	357.9	613.1	1208.5
Heptachlor	100	306.6	436.2	774.5
Dieldrin	100	375.5	463.2	840.9
p,p′-DDD	100	338.1	464.3	820.8
p,p′-DDT	100	354.2	621.7	904.3
Endosulfan	100	365.9	654.9	867.2

the integrity of the solvent drop and nearly 50% of the extractions failed due to losing the solvent drop. Therefore, a stirring speed 400 rpm was used for subsequent experiments.

3.1.5. Effect of temperature

The next step was to optimize the extraction temperature. The study was carried out by varying the temperature in the range of 23–55°C with the other optimized parameters obtained as described above.

From Table 3, it can be seen that the extraction efficiency increases with temperature for all the analytes. We also investigated the effect of drop depletion caused by a higher temperature. Under the same extraction time (25 min), a 3-μl solvent drop lost about 0.28 μl at 23°C after extraction, and other 3-μl drops lost about 0.31 μl at 50°C and 55°C after extraction. Although higher extraction temperature caused some solvent evaporation, the effect was insignificant based on our observed GC responses. In other words, the extraction efficiency was higher at 50°C and 55°C due to increased extraction.

It was observed that at higher temperatures, especially at 55°C, sometimes air bubbles appeared which could significantly affect the stability of the solvent drop. In order to obviate the problem the *n*-hexane/I.S. solution was sonicated before extraction. In any case, as a precautionary measure, 50°C was chosen as the extraction temperature. We believe that no one has previously studied the effect of elevated temperature on LPME.

Fig. 5 shows chromatograms obtained for (a) the standard solution (200.0 μg/l) and (b) a sample extract (spiked with 10.0 μg/l of each compound) obtained under the optimum conditions.

Table 3
Effect of temperature on the extraction efficiency for OCPs (20.0 μg/l) from aqueous samples

Target compound	Relative response			
	23°C	40°C	50°C	55°C
Lindane	100	121.3	160.4	190.6
Aldrin	100	125.7	131.8	117.1
Methoxychlor	100	156.6	231.7	265.3
Heptachlor	100	119.1	162.3	201.8
Dieldrin	100	133.2	208.4	222.5
p,p′-DDD	100	124.3	222.6	224.9
p,p′-DDT	100	111.7	243.1	290.5
Endosulfan	100	143.8	192.3	222.8

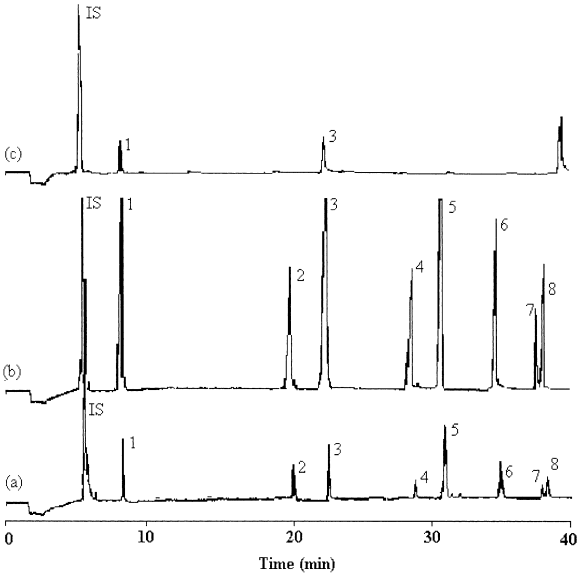

Fig. 5. Analysis of standard solution in hexane (100.0 μg/l) (a); extract of Milli-Q water sample (spiked with 10.0 μg/l of each compound) (b) and extract of reservoir water sample (c) using static LPME with GC–ECD. Peaks: 1=lindane, 2=heptachlor, 3=aldrin, 4=endosulfan, 5=dieldrin, 6=*p,p′*-DDD, 7=methoxychlor, 8=*p,p′*-DDT. GC conditions: see the Experimental section.

3.2. Quantitative analysis

The calibration curves, shown in Table 4, were obtained under the optimized conditions. Linearity was observed over the range 0.05–100 μg/l for most of the analytes. Coefficients of correlation (r^2) ranged from 0.9818 to 0.9934. It should be noted that >50-fold enrichment of pesticides was achieved, except for heptachlor (40-fold) and aldrin (20-fold). Some analytes could be preconcentrated nearly 100-fold.

The reproducibility in peak responses was investigated on six replicate experiments under the optimized conditions. The relative standard deviations (RSDs) of eight pesticides were lower than 7% except for methoxychlor (10.7%) and DDT (9.6%). These RSD values are better than those reported previously for similar work [14], and for SPME [18].

The LODs, based on a signal-to-noise ratio (S/N)

Table 4
Quantitative results of static LPME

Pesticide	RSD (%) ($n=6$)	Enrichment (-fold)	Linearity range (μg/l)	r^2	LOD (μg/l)	EPA method 508 LOD (μg/l)
Lindane	3.2	95	0.05–100	0.9881	0.02	10
Aldrin	3.7	20	0.05–100	0.9894	0.02	75
Methoxychlor	10.7	95	0.5–50	0.9818	0.2	50
Heptachlor	6.9	40	0.05–100	0.9883	0.02	10
Dieldrin	5.7	60	0.05–100	0.9806	0.005	10
p,p′-DDD	5.4	55	0.05–100	0.9934	0.05	2.5
p,p′-DDT	9.6	55	0.5–100	0.9931	0.2	60
Endosulfan	4.6	70	0.5–100	0.9849	0.2	15

of 3, ranged from 0.02 to 0.2 μg/l. When determining the LOD, syringe blanks were carried out every time to confirm that no sample carryover occurred. Elimination of sample carryover is more effectively performed (by repeated rinsing of the syringe with *n*-hexane/I.S. solution) than that in SPME. In comparison with a previously reported work [13,14], we obtained superior LODs for these OCPs. These levels are sufficient to detect these pesticides in aqueous matrices as required by the US Environmental Protection Agency (EPA) Methods 508 and 625 [19,20].

3.3. Real water analysis

Natural water from a reservoir and tap water from a laboratory were extracted using the static LPME method developed and the extracts analyzed by GC–ECD. The results for tap water showed that it was free of OCPs contamination. In the reservoir water samples, lindane and aldrin were detected and they were confirmed by spiking two pesticides into the reservoir water. Fig. 5c shows the chromatograms obtained for reservoir water. The concentrations of lindane and aldrin in the reservoir water were determined to be 0.2 μg/l and 0.5 μg/l, respectively.

Tap water and reservoir water were spiked with pesticide standards at various concentrations to assess matrix effects. Because LPME is a non-exhaustive extraction procedure like SPME, the relative recovery, which is defined as the ratio of GC peak areas of spiked tap water extracts to spiked Milli-Q water extracts, was employed [21]. Results of relative recoveries and RSDs of tap water fortified at the 1.0 μg/l level are shown in Table 5. The data show that for all pesticides, the relative recoveries were higher than 90%. These results demonstrate that the genuine tap and reservoir water matrices, in our present context, had little effect on static LPME.

Table 5
Summary of results from analysis of pesticides in spiked tap water samples

Pesticide	Reservoir water[a]		Tap water[a]	
	Relative recovery[b] (%)	RSD (%) ($n=3$)	Relative recovery[b] (%)	RSD (%) ($n=3$)
Lindane	NC[c]	NC[c]	97.2	4.9
Aldrin	NC[c]	NC[c]	90.9	5.3
Methoxychlor	91.7	11.9	92.7	9.5
Heptachlor	92.3	8.3	94.2	7.8
Dieldrin	95.7	7.1	96.1	6.9
p,p′-DDD	94.2	6.9	98.3	7.4
p,p′-DDT	90.5	11.6	92.6	10.3
Endosulfan	83.3	7.7	90.4	7.2

[a] Water samples containing 1.0 μg/l of each analyte.
[b] $n=3$.
[c] Not considered since both were detected in reservoir water.

4. Conclusion

This paper has outlined the successful development and application of a method based on the static LPME technique for the analysis of organochlorine pesticides from aqueous samples. The method is precise, reproducible and linear over a wide range and requires only small volumes of organic extractant as well as samples. The static LPME method developed has been demonstrated to be viable, rapid and easy to use for the qualitative and quantitative analysis of organochlorine pesticides.

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