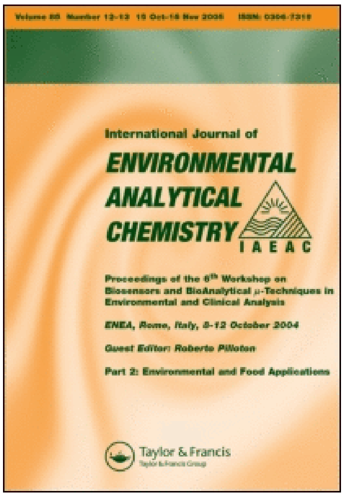

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DETERMINATION OF POLYCHLORINATED BIPHENYLS AND CHLORINATED PESTICIDES IN ENVIRONMENTAL BIOLOGICAL SAMPLES USING FOCUSED MICROWAVE-ASSISTED EXTRACTION

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Organochlorine compounds such as polychlorinated biphenyls (PCBs) and chlorinated pesticides have been analysed in environmental biological samples using focused microwave-assisted extraction (FMW). The analytical procedure is presented and the results for two Standard Reference Materials, a cod liver oil and a freeze-dried mussel tissue, are reported. The average recoveries for the sum of the PCBs are between 93 % and 106 %, and for the sum of the chlorinated pesticides are between 109 to 115 % for all the certified compounds analysed. The developped analytical procedure is highly reproducible with an average standard deviation of 8% for the sum of the PCBs and 9.7% for the sum of the chlorinated pesticides in the two matrices.

Keywords: PCB; chlorinated pesticides; microwave-assisted extraction; biological samples; Standard Reference Materials

INTRODUCTION

Microwave-assisted extraction is a recently developped technique for the extraction of organic compounds [1,2]. This technique extracts organic compounds with an organic solvent from a solid sample which is submitted to a microwave field. This technique has been applied to the analysis of environmental organic contaminants in various matrices, in particular solid samples such as soils and sediments [3–19]. This extraction techniques has appeared as efficient as various other novel extraction methods such as supercritical fluid and accelerated solvent

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extraction [4,20,21]. Some authors have reported the application of microwave-assisted extraction of organic compounds such as essential oils from food and plants [18], and lipids from biological matrices [7]. However most of these studies concern closed vessel systems, which operate at elevated pressure. An open vessel system exists which proceeds at atmospheric pressure and at low temperatures (solvent boiling point). This secure method presents various advantages such as rapid extraction and facility of utilisation. This type of apparatus is often used for the leaching of metals from all kinds of matrices and has recently been used for the analysis of organometallic compounds in marine environmental samples [19]. However this method has been rarely used [3] for the extraction of organic compounds from biological samples.

Environmental biological samples, such as fish and shellfish are regularly analysed for their concentration of organic contaminants in several national monitoring programs such as RNO in France [22] and National Status and Trends Program (NST) in the USA [23]. These environmental programs demand the treatment of numerous samples, and there is a need for a rapid sample preparation procedure to increase sample throughput. Indeed these shellfish samples are complex matrices and necessitate a thorough clean-up procedure to remove lipid and biogenic material before analysis. Several different types of contaminants are analysed by environmental monitoring programs including organochlorine compounds such as polychlorinated biphenyls (PCBs) and chlorinated pesticides, like DDT and its degradation products. These are ubiquitous pollutants in the environment which can be bioaccumulated and have various toxic effects [24–26]. The levels of these compounds are regulated by various national agencies, and are cited by the US Environmental Protection Agency (USEPA) as priority pollutants [27].

This paper presents a procedure for the analysis of organochlorines in environmental biological samples using focused microwave-assisted extraction (FMW). This novel technique is rapid (10 minutes) and uses a reduced volume of solvent (30 ml), these advantages are highly favourable when compared to classical extraction methods like Soxhlet. The extraction proceeds at atmospheric pressure and requires only basic manipulation. The PCBs and organochlorine pesticides are analysed from the same sample extract. The combination of a rapid extraction technique and the increase of the number of analytes from the same sample, reduces the length of the sample preparation, and permits an increased productivity. This procedure is particularly applicable to environmental studies.

This method has been validated with two Standard Reference Materials from the National Institute of Standards and Technology (NIST), SRM 1588a, « Organics in Cod Liver Oil » and SRM 2974 « Organics in Freeze-Dried Mussel Tissue ». These materials present various concentrations of organochlorines and different origins and lipid content. SRM 1588a is a liquid matrix and as such does not strictly need to be extracted, however it allows the validation of the analytical procedure, including purification and analysis, and also the stability of the analytes in a microwave field. The SRM 2974 [28] is a solid matrix which allows the validation of the focused microwave-assisted extraction step. The results of these validations are presented, and the suitability of FMW for environmental biological samples is discussed.

EXPERIMENTAL

Analytical Procedure

The analytical procedure is summarised in the diagram in Figure 1. The matrix was extracted by microwave-assisted extraction (0.5g dry weight of SRM 2974, 0.25g of SRM 1588a) with dichloromethane (Prolabo, Fontenay sous Bois, France, pesticide grade). The FMW apparatus used was a SOXWAVE® 100 from PROLABO (Fontenay-sous-Bois, France). The frequency of the microwaves used was 2450MHz. The apparatus is equipped with a programmable power controller (from 30W to 300W). The extraction was carried out under the following conditions Time = 10 min, Power = 30 W, as presented elsewhere [3]. The internal standards PCB 30, PCB 103, PCB 155, PCB 198 and deuterated d8 4,4′-DDT (Promochem. Molsheim. France) were gravimetrically added to the matrix prior to extraction. The extract was purified by shaking 5 times with 36 N sulfuric acid. The organic and acid phases were separated and the organic extract was neutralised with de-ionised water, and dried with anhydrous sodium sulfate (Merck, Darmstadt, Germany). The extract was concentrated under a gentle flow of nitrogen, and a second purification on a column of silica gel (particle size 0.063–0.2 mm; Merck, Darmstadt, Germany) was performed. The organochlorines were eluted with 15 ml of a mixture of pentane/dichloromethane (90/10 v/v). The pentane of HPLC grade (Scharlau, ICS, St. Médard en Jalles, France) was distilled before use.

To separate the PCBs from the more polar chlorinated pesticides a HPLC step was performed on an aminosilane column (Amino 5μ, length = 250 mm, internal diameter = 4.6 mm, Stagroma, Switzerland). The mobile phase was pentane (HPLC grade Scharlau, ICS, St. Médard en Jalles, France) at a flow rate of 1 ml / min with UV detection at 254 nm. Two fractions were collected; the first contained the PCBs and 2,4′-DDE and 4,4′-DDE, the second fraction contained the more polar pesticides according to Schantz *et al.* [29]. The extract was concen-

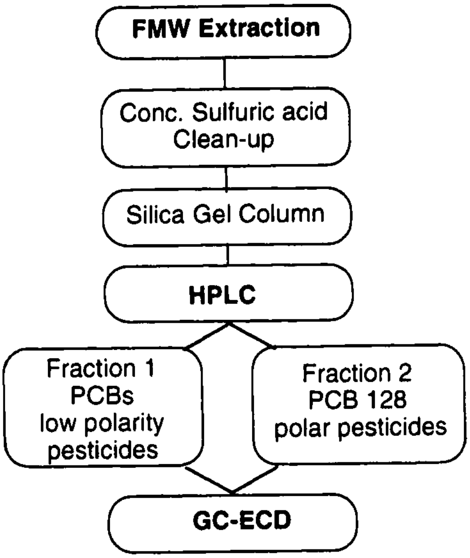

FIGURE 1 Analytical procedure for the determination of organochlorines in biological matrices

trated and transferred to isooctane (Spectroscopy grade: Scharlau, ICS, St. Médard en Jalles, France), and analysed by GC-ECD.

Procedural blanks were regularly performed and all results presented are corrected for blank levels which did not exceed 10% of the measured sample concentrations. All glassware was rigorously cleaned with detergent followed by overnight thermal clean-up at 450°C. The sodium sulfate and silica gel were pre-extracted with dichloromethane in an ultrasonic bath, dried and were rinsed with dichloromethane just before utilisation.

GC-ECD Analyses

Analyses were performed on an HP 5890 series II gas chromatograph (Hewlett-Packard, Avondale, MA) coupled to a ^{63}Ni electron capture detector (ECD) and equipped with an automatic injector. A capillary column HP5 (Hewlett-Packard, Avondale, MA) was used (5% phenyl-methyl polysiloxane, 60m × 0.25 mm internal diameter × 0.25 μm film thickness). The GC conditions are as follows: splitless injection (1ml); injector temperature, 280°C for the PCB fraction and 250°C for the more polar pesticide fraction; detector temperature, 290°C; initial oven temperature, 60°C; held for 2 min, heated to 120°C at

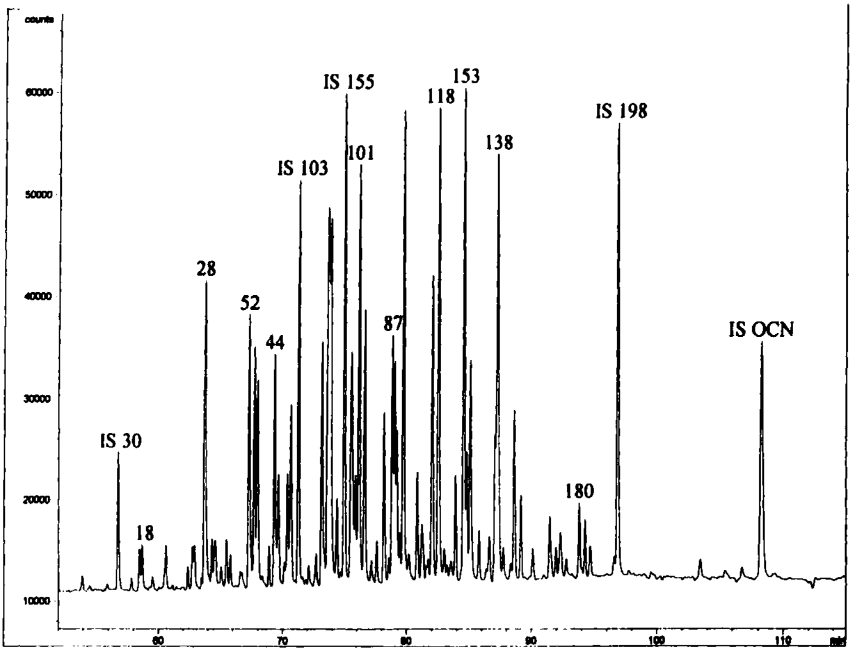

FIGURE 2 GC-ECD Chromatogram of Fraction 1 of a mussel extract, SRM 2974

6°C/min and held for 5 min, then heated to 280°C at 2°C/min and held for 20 min, purge off at injection and on at 1.5 min. Helium was used as the carrier gas at a column head pressure of 140KPa for the PCB fraction, and 190KPa for the pesticide fraction, and the make-up gas was nitrogen at 60ml/min. Helium was used as the anode purge gas at a flow rate of 10 ml/min.

The PCB congeners were analysed individually when possible, though in some cases the concentrations are reported as the sum of the congeners due to coelutions on the GC column. The relative response factors of the different compounds were determined by injecting a standard solution of PCBs (Promochem, Molsheim, France in solution at 99%+ purity) and chlorinated pesticides (SRM 2261 « Pesticides in Cyclohexane », National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA) spiked with the same solution of internal standards PCB 30, PCB 103, PCB 155, PCB 198 and d8 4,4′-DDT. as that used for spiking the samples. Blank injections of isooctane were performed between each injection of a sample to ensure the cleanliness of the injector. Typical GC-ECD Chromatograms of the two fractions obtained by the analytical procedure are shown in Figures 2 and 3.

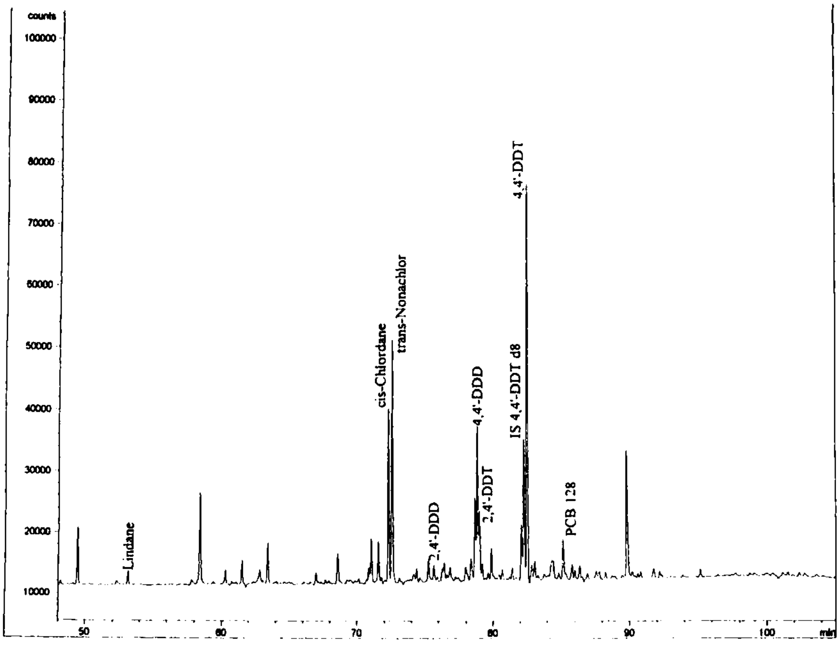

FIGURE 3 GC-ECD Chromatogram of Fraction 2 of a mussel extract, SRM 1588a

RESULTS AND DISCUSSION

Fish and shellfish matrices are complex biological samples which demand intensive purification before analysis by gas chromatography. An acid purification is a standard sample treatment procedure to eliminate interferents in organochlorine analysis [30]. This was the chosen method for the environmental biological samples, as these samples have high amounts of lipids and biogenic material. The acid also destroys many organic compounds which can also interfere in ECD detection. The PCBs are highly resistant to acid, indeed this was one of their advantages in their industrial applications. The recoveries of individual organochlorine compounds congeners have been determined previously [30, 31]. However some of the organochlorine compounds can also be eliminated, in particular certain chlorinated pesticides [28]. All of the studied PCB congeners were recovered in the final purified extracts. Most of the pesticides are also recovered, and only one compound, Dieldrin, is completely lost.

A separation of certain organochlorines is necessary for the analysis by GC-ECD, as some compounds can coelute on the gas chromatographic column.

For example PCB 138 and 4,4′-DDT are closely eluted on this non-polar phase, and as these compounds are usually present in high concentrations and are also priority pollutants their correct determination is important. In order to separate these compounds a HPLC step on a polar phase was performed on the purified extract. This step leads to the collection of two fractions, the first containing the PCBs and the non-polar pesticides such as DDE and HCB, and the second containing the remainder of the pesticides.

Validation of the analytical procedure

SRM 1588a

The procedure was applied to SRM 1588a, Organics in Cod Liver Oil, produced by National Institute of Standards and Technology (NIST). This liquid matrix has recently been recertified [32] and is the same material as SRM 1588. This highly lipidic complex has certified concentrations for 24 PCB congeners and 14 chlorinated pesticides. An extraction step is not necessary for this matrix as it is soluble in organic solvent. However this sample permits the validation of the rest of the procedure, acid purification, adsorption chromatography on silica gel, HPLC and GC-ECD determination. The matrix has been submitted to microwave-assisted extraction to confirm the stability of the analytes under the microwave field.

The results are shown in Figure 4 for the PCBs and Figure 5 for the chlorinated pesticides. Three individual replicates have been performed. Considering the PCBs the average recovery for the sum of the PCB congeners is 106 % and the recoveries range from 77% to 123% except for PCB 187 which has a high recovery of 163% with a relative standard deviation (RSD) of 2.6%. The RSD are low for the majority of the PCBs and similar to those given on the certified values. The higher chlorinated congeners have the lowest RSD values, and in all cases the RSD is close to or less than 10%.

The average recovery of the sum of the chlorinated pesticides is 115 % and the recoveries range from 52 % to 191 % with a RSD of close to or less than 15% for all of the compounds. 2,4′-DDE has a recovery of 191%. This pesticide is co-eluted with an unknown impurity in the GC-ECD analysis as the peak has an unresolved shoulder. Hexachlorobenzene which has a recovery of 130% is co-eluted with PCB 8, which is probably responsible for the high recovery of this compound. γ-hexachlorocyclohexane, or lindane, is the most volatile of the studied chlorinated pesticides, and the low recovery (52%) of this compound indicates losses during reconcentration steps.

Overall the analytical procedure is suitable for the analysis of organochlorines in highly lipidic matrices, although some coelutions seem to persist. 2,4′-DDE

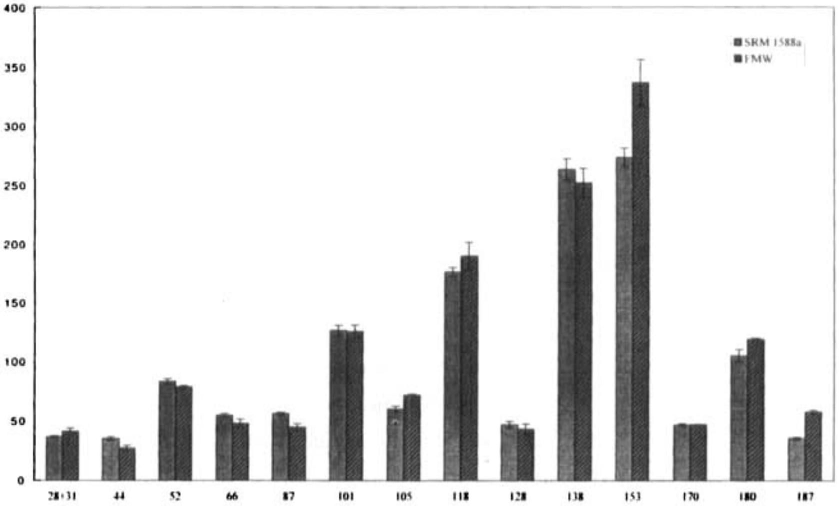

FIGURE 4 Comparison of certified concentrations and obtained concentrations determined using the analytical procedure of polychlorinated biphenyls (ng/g) in SRM 1588a

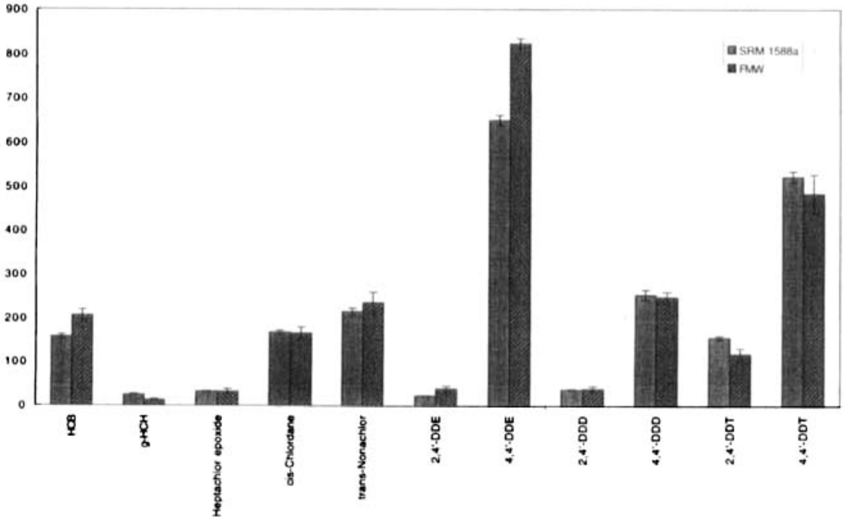

FIGURE 5 Comparison of certified concentrations and obtained concentrations determined using the analytical procedure of chlorinated pesticides (ng/g) in SRM 1588a

and hexachlorobenzene have not been considered in the second validation experiment due to the difficulties in their analysis. The use of GC with mass spectrometric detection would undoutably resolve these coelution problems.

Some high recoveries remain for particular compounds, such as PCB 187. This matrix has been fortified with polychlorodibenzo-p-dioxins and octachlorodibenzofuran at concentrations of 1 ng/g or less. These compounds may have large response factors in ECD, due to their chlorination and oxygen substitution, and may create interferences with the other organochlorines.

SRM 2974

This matrix is a solid sample of freeze-dried whole mussel tissue *Mytilus edulis* from Boston Harbor, MA. 20 PCB congeners and 7 chlorinated pesticides are certified in this matrix. 4 more PCBs and 4 pesticides have reference concentrations. The results of the analysis of this matrix are presented in Tables I and II. This matrix is highly hygroscopic and the certified concentrations are given for the dry weight matrix. The results obtained have been corrected for 8% moisture content as recommended by the analysis certificate of this matrix [33].

TABLE I Concentrations of PCBs (ng/g) in SRM 2974 determined using the analytical procedure (FMW) and compared to certified values given by NIST

Compound	*Certified ng/g*	*FMW ng/g*	*Recovery %*	*RSD %*
PCB 18	26.8 ± 3.3	26.8 ± 7.9	100	29.6
PCB 28/31	155.0 ± 36.0	118.8 ± 17.8	77	15
PCB 44	72.7 ± 7.7	72.6 ± 17.3	100	23.8
PCB 52	115 ± 12	113.2 ± 18.9	98	16.7
PCB 66	101.4 ± 5.4	97.6 ± 14.1	96	14.4
PCB 87	54 ± 14	48.4 ± 3.1	90	6.4
PCB 101	128 ± 10	119.7 ± 7.6	94	6.3
PCB 105	53.0 ± 3.8	51.9 ± 4.7	98	9
PCB 118	130.8 ± 5.3	116.9 ± 7.2	89	6.1
PCB 128	22.0 ± 3.5	18.5 ± 1.1	84	5.7
PCB 138/163/164	133.5 ± 10.3	119.2 ± 2.8	89	2.3
PCB 153	145.2 ± 8.8	132.6 ± 6.8	91	5.1
PCB 170/190	5.5 ± 1.1	4.5 ± 0.4	82	8.9
PCB 180	17.1 ± 3.8	15.5 ± 1.2	91	7.7

TABLE II Concentrations of chlorinated pesticides (ng/g) in SRM 2974 determined using the analytical procedure (FMW) and compared to certified values given by NIST

Compound	*Certified ng/g*	*FMW ng/g*	*Recovery %*	*RSD %*
cis-CHLORDANE	17.2 ± 2.9	17.9 ± 1.3	104	7.3
trans-NONACHLOR	18.0 ± 3.6	16.2 ± 1.3	90	1.9
4,4′-DDE	51.2 ± 5.7	58.9 ± 5.5	115	9.3
4,4′-DDD	43.0 ± 6.4	50.0 ± 6.5	116	13
4,4′-DDT	3.91 ± 0.60	2.4 ± 0.3	62	12.5
2,4′-DDD	13.7 ± 2.8	15.3 ± 2.3	112	15

For the sum of the PCB congeners the average recovery was 93 % with a RSD of 9%, the recoveries range from 75 to 92%, and the RSD from 2.3 to 29.6%. The highest RSD are found for the more volatile lower chlorinated PCBs (PCB 18 and PCB 44). The majority of the congeners have a recovery within the limits of the standard deviations of the certified concentrations. Similar recoveries of approximately 90% for the most concentrated congeners (PCBs 101, 118, 138, 153 and 187) have been reported by other authors for the same matrix [34] using pressurised fluid extraction. SRM 2974 was prepared from a previous certified material SRM 1974, a wet mussel sample. The certified concentrations in SRM 2974 were obtained by combining values for SRM 1974 and the resulting freeze-dried sample. There has possibly been a loss of the more volatile PCB congeners during freeze-drying which modifies the concentrations actually present in the freeze-dried matrix.

Of the 7 certified pesticides 5 have been determined and the results are shown in Table 4. The recoveries range from 62 to 116%, with an average of 109%. The RSD are equal to or less than 15%. The highest RSD is found for 2,4′-DDD which is not certified in this matrix. The low recovery of 4,4′-DDT is probably due to the relatively low concentration of this compound in this material.

Overall the results are highly satisfactory and show the efficiency of focused microwave-assisted extraction of organic contaminants from biological samples.

CONCLUSION

The utilisation of focused microwave-assisted extraction permits a rapid and efficient extraction of the organochlorinated compounds from the sample in only 10 minutes. FMW is a good alternative to classical extraction techniques and is suitable for environmental studies. This analytical procedure is a good compromise between the sample preparation time and the large number of analytes. The use of GC-MS analysis should resolve the coelution problems for hexachlorobenzene and 2,4′-DDE.

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