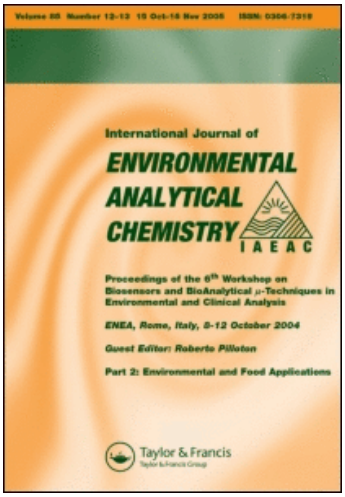


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# Procedure of determination of volatile trihalomethanes in human urine with pervaporation and gas chromatography

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This work presents the development of a novel procedure for the determination of trihalomethanes (THMs) in human urine samples based on: (1) pervaporation (PV) of analytes from urine samples as a convenient analyte isolation/enrichment technique; (2) direct aqueous injection of the extracts onto the column of a gas chromatograph equipped with an electron capture detector (DAI-GC-ECD). Basic parameters of the new PV-DAI-GC-ECD procedure were evaluated. The calibration curves were linear in the concentration range examined. Intermediate precision of the procedure was good, at the same level of about 20% for all analytes. The method detection limits were below $0.10\,\mu g\,L^{-1}$ for all analytes. The enrichment factors did not differ significantly for water and urine samples, indicating little or no matrix effects.

*Keywords:* Biomonitoring; Pervaporation; Trihalomethanes; Validation

## 1. Introduction

The determination of volatile organic compounds in liquid biological samples, especially the most common organohalogens considered to be potentially carcinogenic for humans, constitutes a challenge for analytical chemists owing to the complex and often variable matrix composition of the samples. Typical biological samples usually require special pre-treatment prior to analysis by chromatography or related techniques [1–8]. Special attention has recently been paid to the use of the so-called solvent-free analyte isolation and/or enrichment techniques, which can be attributed to the widespread area of green analytical chemistry [9, 10].

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Membrane techniques play a special role among the solvent-free techniques of sample pre-treatment prior to final determination of analytes [11–13]. In the simplest approach, a membrane can be treated as a selective barrier between two phases. The phase in which mass transfer takes place is called the donor phase (feed), while the other phase is called the acceptor phase (permeate) [14, 15].

One of the most useful techniques in separating trace substances from samples characterized by the complex and often variable matrix composition is pervaporation [16–18]. Pervaporation is a technique in which the liquid sample is in contact with one side of a membrane, whereas the permeate, in a vapour state, is continuously removed from the other side into the vacuum. Pervaporation appears to be promising especially when the preferentially permeable component is present in the sample at low concentration. Therefore, the selective isolation and pre-concentration of low contents of volatile organohalogen compounds (VOXs) from aqueous solutions through polydimethylsiloxane (PDMS) membranes and analysis by direct permeate injection into a thick film, non-polar gas chromatographic column coupled with electron capture detector, presents a large opportunity for the analytical application of pervaporation.

The aim of this study was to evaluate a new procedure of determination of trihalomethanes (THMs) in human urine samples based on the application of:

- a pervaporation technique at the step of isolation and pre-concentration of analytes from human urine;
- direct injection of samples of the permeate to the chromatographic column;
- separation and determination of the analytes by GC-ECD.

The new procedure has been validated, and the full uncertainty budget has been calculated.



## 2. Experimental

### 2.1 *Chemicals*

Standards, $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ (200.00 mg $dm^{-3}$, 5000.00 mg $dm^{-3}$) were from Supelco (Bellefonte, PA). $CH_3OH$ for chromatography was from Merck (Darmstadt, Germany). 'Zero water' (level of total organic carbon 1–4 μg $L^{-1}$ C) was produced using Millipore equipment (Molsheim, France).

### 2.2 *Biological materials*

Samples of urine for the preparation of standard solutions were collected from three volunteers, combined, and divided into five aliquots of equal volume. One of them, used as a reference with the known content of volatile organohalogen compounds, was analysed directly for the content of organohalogen compounds using pervaporation (PV) direct aqueous injection of the extracts onto the column of a gas chromatograph equipped with an electron capture detector (DAI-GC-ECD) procedure. The standard addition method was used for the other four urine samples. Various quantities of the analytes (0.5, 1, 2, and 5 μg $L^{-1}$) were added to each sample, followed by the analysis using the PV-DAI-GC-ECD procedure.

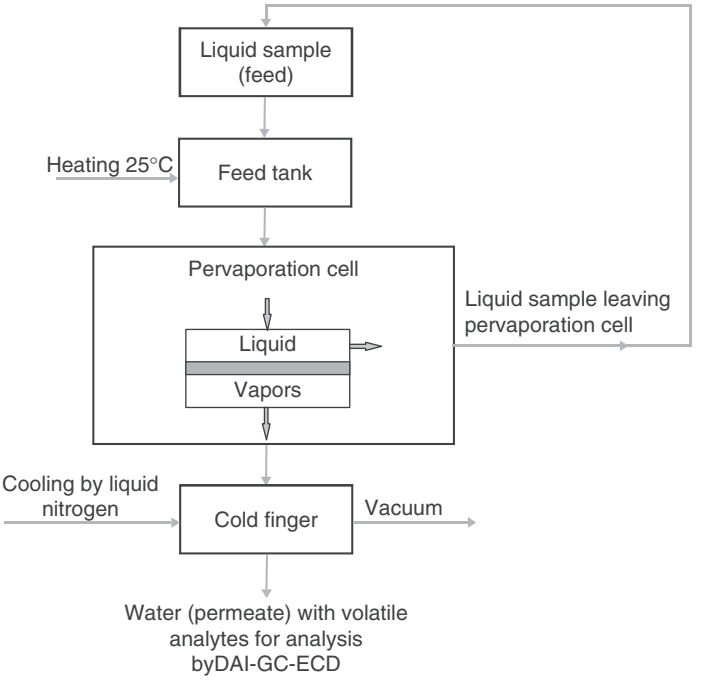


Figure 1. General scheme of the analytical procedure (PV-DAI-GC-ECD) of determination of volatile organohalogen compounds in human urine samples.



### 2.3 *Apparatus*

The pervaporation system and chromatography analysis used a temperature-controlled feed vessel, circulating pump, membrane test cell, cold fingers, and vacuum pump, as indicated in figure 1 (made by the workshop of the Membrane Processes Group, Nicolaus Copernicus University, Toruń, Poland).

### 2.4 *PV-DAI-GC-ECD procedure*

The urine sample (~500 mL) was pumped to a pervaporation cell with a PDMS membrane (area 38.465 $cm^2$). The feed was circulated in the system with a flow rate of 1.7 L $min^{-1}$ and was in direct contact with one side of the membrane, whereas the permeate was removed in a vapour state from the opposite side into a vacuum. The permeate (~300 mg) was collected into cold fingers by liquid nitrogen. During the experiment, the upstream pressure was maintained at the atmospheric pressure, while the downstream pressure was kept below 1 mbar using a vacuum pump. The pervaporation system was operated at 25°C. The permeation rates were determined by weighing the permeate collected over a given period of time in the cold fingers. The permeate was analysed by gas chromatography using direct aqueous injection with electron capture detection (DAI-GC-ECD). The conditions of GC analysis were as follows: GC 8000Top (Carlo Erba Instruments, Milan, Italy); cold on-column injector with secondary cooling; 60 m × 0.32 × 5 μm DB-1 column (J&W Scientific, Folsom, CA); temperature programme: 95–110°C, at 5°C $min^{-1}$, 110°C hold for

Table 1. Parameters of the calibration curves obtained for standard solutions containing volatile organohalogen compounds.

| Analyte | Matrix | Concentration range ($\mu g\,L^{-1}$) | Regression line equation | Regression coefficient $r$ |
|---|---|---|---|---|
| $CHCl_3$ | Urine[a] | 0.5–5.0 | $y = 1.54 \times 10^5 x - 34.0 \times 10^3$ | 0.9969 |
| | Water[a] | 0.5–5.0 | $y = 1.61 \times 10^5 x - 40.1 \times 10^3$ | 0.9937 |
| | Water[b] | 0.1–0.5 | $y = 0.181 \times 10^5 x + 527$ | 0.9979 |
| $CHBrCl_2$ | Urine[a] | 0.5–5.0 | $y = 11.1 \times 10^5 x - 232 \times 10^3$ | 0.9985 |
| | Water[a] | 0.5–5.0 | $y = 11.5 \times 10^5 x - 295 \times 10^3$ | 0.9983 |
| | Water[b] | 0.1–0.5 | $y = 0.448 \times 10^5 x - 620$ | 0.9993 |
| $CHBr_2Cl$ | Urine[a] | 0.5–5.0 | $y = 12.8 \times 10^5 x - 84.2 \times 10^3$ | 0.9992 |
| | Water[a] | 0.5–5.0 | $y = 13.0{\cdot}10^5 x - 73.2 \times 10^3$ | 0.9998 |
| | Water[b] | 0.1–0.5 | $y = 0.443 \times 10^5 x - 98$ | 0.9982 |
| $CHBr_3$ | Urine[a] | 0.5–5.0 | $y = 10.4 \times 10^5 x - 106 \times 10^3$ | 0.9997 |
| | Water[a] | 0.5–5.0 | $y = 13.7 \times 10^5 x - 465 \times 10^3$ | 0.9976 |
| | Water[b] | 0.1–0.5 | $y = 0.235 \times 10^5 x - 1.03 \times 10^3$ | 0.9999 |

[a]PV-DAI-GC-ECD.
[b]DAI-GC-ECD.

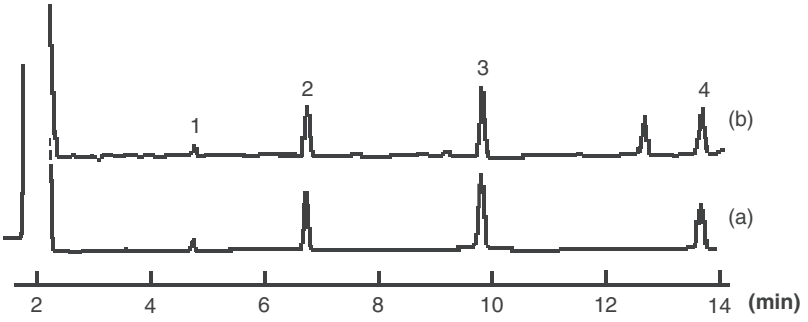


Figure 2. Examples of chromatograms obtained during the analysis of samples of: (a) model water solution of analytes and (b) human urine solution of analytes: 1, $CHCl_3$; 2, $CHCl_2Br$; 3, $CHClBr_2$; 4, $CHBr_3$.



5 min, 110–130°C, at 10°C $min^{-1}$, 130°C hold for 10 min; hydrogen carrier gas (2.2 $cm^3\,min^{-1}$); pressure programme $P_{const} = 130$ kPa; detector temperature 350°C; and nitrogen make-up gas 60 $cm^3\,min^{-1}$.

## 3. Results

### 3.1 *Calibration of the PV-DAI-GC-ECD procedure*

Table 1 lists the parameters of the calibration curves obtained for standard solutions of organochlorine compounds.

Figure 2 presents examples of chromatograms obtained during the analysis of samples prepared in zero water (a) and urine (b), containing 1 $\mu g\,L^{-1}$ of each analyte.

### 3.2 *Validation of the PV-DAI-GC-ECD procedure*

Validation was carried out according to the scheme proposed by Huber [19].

Table 2. Analytical data used for calculation MDL, MQL values of proposed procedure.

| Analyte | Concentrations ($\mu g\,L^{-1}$) | Standard deviation of the intercept $s$ | Slope $b$ | MDL ($\mu g\,L^{-1}$) | MQL ($\mu g\,L^{-1}$) |
|---|---|---|---|---|---|
| $CHCl_3$ | 0.2; 0.3; 0.4 | $1.93 \times 10^3$ | $0.822 \times 10^5$ | 0.08 | 0.23 |
| $CHCl_2Br$ | 0.2; 0.3; 0.4 | $13.6 \times 10^3$ | $4.39 \times 10^5$ | 0.10 | 0.30 |
| $CHClBr_2$ | 0.2; 0.3; 0.4 | $35.7 \times 10^3$ | $15.1 \times 10^5$ | 0.08 | 0.23 |
| $CHBr_3$ | 0.2; 0.3; 0.4 | $29.5 \times 10^3$ | $11.1 \times 10^5$ | 0.09 | 0.26 |

**3.1.1 Linearity.** The linearity of the method was evaluated based on the calibration curves. In general, it was very good in the concentration range examined ($0.1–5\,\mu g\,L^{-1}$), with linear regression coefficients greater than 0.99 in all cases (see table 1).

**3.1.2 Method detection limit (MDL) and method quantitation limit (MQL).** Method detection limits were estimated using the following formula:

$$\mathrm{MDL} = \frac{3.3s}{b},$$

where $b$ is the slope of the calibration curve, and $s$ is the standard deviation of the calibration curve intercept.

Method quantitation limits were estimated using the following formula:

$$\mathrm{MQL} = 3 \times \mathrm{MDL}.$$

The MDL and MQL values and data used to estimate them are listed in table 2.

**3.1.3 Method precision.** Table 3 presents the results of the determination of selected THM concentrations in standard solutions ($2\,\mu g\,L^{-1}$) prepared in water and in urine. Each series concerned seven different samples containing the same quantities of the analytes and analysed using the PV-DAI-GC-ECD procedure, to estimate the repeatability and intermediate precision of the procedure. Repeatability of the results was estimated based on the magnitude of coefficient of variation (CV) in each individual series. Overall intermediate precision was estimated from the magnitude of the $CV_{tot}$ of the pooled results from the two measurement series.

**3.1.4 Uncertainty budget.** The effect of various parameters affecting the uncertainty of the determination of analyte concentration in the samples is illustrated schematically in figure 3.

The expanded uncertainty (%) was calculated according to GUM [20] using the following formula:

$$U = k\sqrt{u(\mathrm{cal})^2 + u(\mathrm{EF})^2 + u(\mathrm{LOD})^2 + \frac{(\mathrm{RSD}_{\mathrm{results}})^2}{n}},$$

Table 3. Results of organochlorine compounds determination in model water samples and human urine solutions for the determination of method repeatability and intermediate precision.

| Analyte | $CHCl_3$ | | $CHCl_2Br$ | | $CHClBr_2$ | | $CHBr_3$ | |
|---|---|---|---|---|---|---|---|---|
| Series | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| *Water* | | | | | | | | |
| Concentration found ($\mu g\,L^{-1}$) | 20.1 | 20.4 | 52.2 | 67.4 | 82.5 | 110 | 71.1 | 100 |
| | 18.7 | 18.1 | 51.9 | 65.3 | 71.8 | 108 | 64.7 | 86.4 |
| | 17.6 | 16.5 | 49.1 | 59.8 | 70.6 | 93.5 | 60.4 | 78.2 |
| | 17.3 | 15.2 | 45.5 | 52.2 | 69.2 | 73.8 | 60.4 | 66.2 |
| | 13.5 | 13 | 37.6 | 43.1 | 57.9 | 67.6 | 59.5 | 57.8 |
| | 12.6 | 10.2 | 36.1 | 42.2 | 50.2 | 57.2 | 50.9 | 56.7 |
| | 12.3 | 10 | 33.3 | 39.2 | 49.5 | 56.6 | 50.9 | 56.2 |
| Average | 16 | 14.8 | 43.7 | 52.7 | 64.5 | 80.8 | 59.7 | 71.6 |
| CV (%) | 19.7 | 26.6 | 18.1 | 22 | 19.1 | 28 | 12.1 | 23.8 |
| Average tot | 15.4 | | 48.2 | | 72.7 | | 65.7 | |
| CVtot (%) | 22.6 | | 22.1 | | 26.8 | | 21.3 | |
| *Urine* | | | | | | | | |
| Concentration found ($\mu g\,L^{-1}$) | 22.1 | 21.7 | 66 | 75.6 | 98.2 | 102 | 101 | 134 |
| | 19.5 | 19.8 | 58.6 | 54 | 85.6 | 84.7 | 87.1 | 86 |
| | 18.4 | 19.4 | 49.7 | 51.8 | 78.6 | 77.5 | 77 | 85.3 |
| | 16.9 | 15.7 | 37.3 | 50.4 | 74.2 | 72.4 | 73.4 | 84.2 |
| | 15.8 | 14 | 46.9 | 42.5 | 74.2 | 63.5 | 69.9 | 71 |
| | 12 | 12.3 | 45.6 | 38.1 | 56.2 | 56 | 57.7 | 70.2 |
| | 10.9 | 11.2 | 33.4 | 39.6 | 44.9 | 51.6 | 48.2 | 60.9 |
| Average | 16.5 | 16.3 | 48.2 | 50.3 | 73.1 | 72.5 | 73.4 | 84.5 |
| CV (%) | 24.3 | 24.8 | 23.5 | 25.4 | 24.3 | 24.1 | 23.8 | 28.1 |
| Average total | 16.4 | | 49.3 | | 72.8 | | 79 | |
| CVtot (%) | 23.6 | | 23.7 | | 23.3 | | 26.4 | |



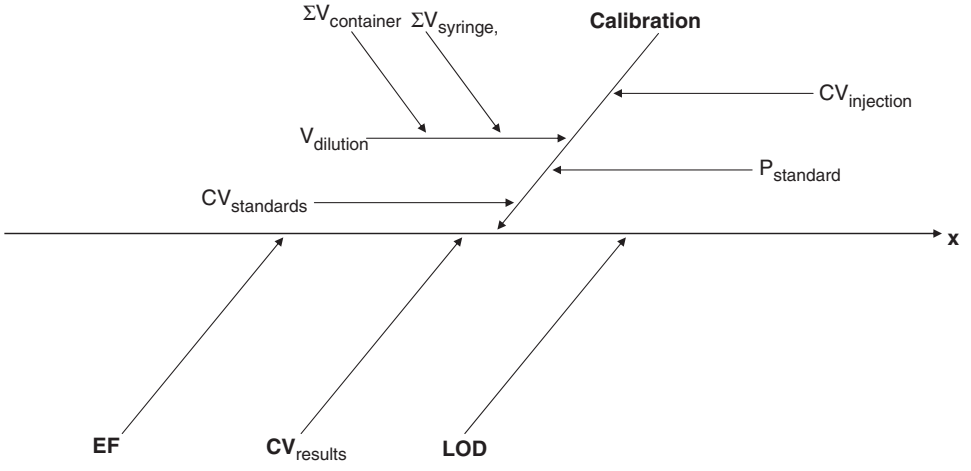


Figure 3. Ishikawa diagram showing uncertainty in the determination of volatile organochlorine compounds in human urine samples using the PV-DAI-GC-ECD procedure.

where $U$ is the expanded uncertainty; $k$ is the coverage factor (usually 2); $u$(cal) is the uncertainty of the calibration step; $u$(EF) is the uncertainty of the enrichment factor; $u$(LOD) is the uncertainty of the limit of detection; $CV_{results}$ is the coefficient of variation of the results; and $n$ is the number of independent determinations.

The values obtained are listed in table 4.

Table 4. Calculated values of standard uncertainties and expanded uncertainties for the analytes.

| | Uncertainty (%) | | | | |
|---|---|---|---|---|---|
| Analyte | $u$ (cal) | $CV_{results}$ | $u$ (LOD) | $U$(EF) | $U(k=2)$ |
| $CHCl_3$ | 3.62 | 23.6 | 0.80 | 1.50 | 15 |
| $CHCl_2Br$ | 3.05 | 23.7 | 1.00 | 3.27 | 15 |
| $CHClBr_2$ | 3.21 | 23.3 | 0.80 | 5.44 | 15 |
| $CHBr_3$ | 1.26 | 26.4 | 0.90 | 4.25 | 15 |

Table 5. Values of analyte enrichment factors (EF) and their extended uncertainties.

| | Water | | Urine | |
|---|---|---|---|---|
| Analyte | EF | $U(k=2)$ | EF | $U(k=2)$ |
| $CHCl_3$ | 6.68 | 0.60 | 6.54 | 0.11 |
| $CHCl_2Br$ | 19.5 | 1.1 | 20.1 | 0.76 |
| $CHClBr_2$ | 27.8 | 1.5 | 27.5 | 1.7 |
| $CHBr_3$ | 29.5 | 3.1 | 30.5 | 1.5 |



**3.1.5 Enrichment factor.** To evaluate the effect of the matrix composition on the final results, the enrichment factors for water and urine standard solutions were statistically compared for all analytes. The values of the enrichment factors (EF) are listed in table 5 together with extended uncertainties ($U(k=2)$) calculated according to GUM [20].

**3.1.6 Matrix effects.** To evaluate the effect of the matrix composition on the final results, the values of enrichment factors for water and urine standard solutions were statistically compared for all analytes.

The precision of the determination of enrichment factors for the two matrices (water and urine) was compared using the Snedecor $F$-test using the following formula:

$$F_{\text{calculated}} = \frac{SD_2^2}{SD_1^2},$$

where $SD_1$ is the standard deviation for water samples, and $SD_2$ is the standard deviation for urine samples.

The results are presented in table 6. The calculated values of the statistic test ($F_{\text{calculated}}$) were in all cases smaller than the critical value at the 95% probability level ($F_{\text{calculated}} < F_{\text{critical}}$), indicating that the precision of the concentration values in urine and water samples did not differ significantly for any of the analytes.

The accuracy of the determination of the enrichment factors for water and urine samples was compared using Student's $t$-test (table 6) by the following formula:

$$T_{\text{calculated}} = \frac{|c_1 - c_2|}{\sqrt{SD_1^2 + SD_2^2}}\sqrt{n},$$

Table 6. Comparison of method precision and accuracy for the analysis of water and urine samples.

| | | Analyte | | | |
|---|---|---|---|---|---|
| | | $CHCl_3$ | $CHCl_2Br$ | $CHClBr_2$ | $CHBr_3$ |
| Water | Average concentration $c_1$ ($\mu g\,L^{-1}$) | 15.4 | 48.2 | 72.7 | 65.7 |
| | No. of results | 14 | | | |
| | Standard deviation $SD_1$ | 3.48 | 10.6 | 19.5 | 14.0 |
| Urine | Average concentration $c_2$ ($\mu g\,L^{-1}$) | 16.4 | 49.3 | 72.8 | 79.0 |
| | No. of results | 14 | | | |
| | Standard deviation $SD_2$ | 3.87 | 11.6 | 16.9 | 20.8 |
| $F_{calculated}$[a] | | 1.24 | 1.20 | 1.33 | 2.21 |
| $F_{crit.}$[b] (95%, 13, 13) | | 2.57 | | | |
| $T_{calculated}$[c] | | 0.72 | 0.26 | 0.01 | 1.98 |
| $T_{crit.}$[d] (95%, 26) | | 2.06 | | | |

[a]$F_{calculated}$: calculated statistic of Snedecor's $F$-test.
[b]$F_{crit}$: critical value of Snedecor's $F$-test.
[c]$T_{calculated}$: calculated statistic of Student's $t$-test.
[d]$T_{crit}$: critical value of Student's $t$-test.

where $c_1$ is the averange concentration for water samples; $c_2$ is the averange concentration for urine samples; and $n$ is the number of results.

The calculated values of $T_{calculated}$ were smaller than $T_{critical}$. This means that no significant differences were observed between concentration values in urine and water samples for any of the analytes.



## 4. Conclusion

This study confirmed the possibility of quantitative determination of trihalomethanes in human urine samples (from exposed and non-exposed persons) by the PV-DAI-GC-ECD procedure. This procedure has several important advantages:

- reduction of use or complete elimination of organic solvents;
- high selectivity;
- possibility of use for both matrix removal and enrichment of microtrace components.

The linearity of the method was very good in the concentration range examined ($0.1–5\,\mu g\,L^{-1}$), with linear regression coefficients greater than 0.99 in all cases and MDL lower than $0.10\,\mu g\,L^{-1}$ for all THM compounds. The repeatability and intermediate precision of the results were similar. The former was estimated from the CV values of the results in an individual measurement series, while the latter was determined from the CV of pooled results from two series. The coefficient of variation of the results obtained for the analysis of water and urine standards did not differ significantly, which indicates that the matrix composition did not affect the method precision. Similarly, the analyte enrichment factors determined for water and urine standard solutions did not differ significantly from each other with respect to their precision and accuracy. Values of the basic validation parameters were at the same level as in the available HS-DAI-GC-ECD procedure [21].

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