A candidate reference material '*Mya arenaria*' for the monitoring of butyltin compounds in mollusks in the marine environment†

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Most analytical techniques used for the speciation analysis of butyltin compounds in biological tissues usually contain multiple procedures (e.g., extraction, derivatization, separation, preconcentration, determination) which may increase the risk of systematic errors. The use of reference materials (RM) makes it possible to correct most of these errors and improve the validity of quality control. In this study, a new candidate RM for butyltin compounds in mollusks was developed. It was prepared with a mollusk '*Mya arenaria*' directly bought from the market. The preparation of this candidate RM, the homogeneity and stability studies, the value assignment and the evaluation of uncertainty were described. The certified values of 0.33 ± 0.08 , 0.62 ± 0.09 , $2.64 \pm 0.20 \mu\text{g Sn g}^{-1}$ (based on dry mass) for monobutyltin (MBT, $[\text{Sn}(\text{C}_4\text{H}_9)^3]^+$), dibutyltin (DBT, $[\text{Sn}(\text{C}_4\text{H}_9)_2^{2+}]$) and tributyltin (TBT, $[\text{Sn}(\text{C}_4\text{H}_9)_3^+]$) respectively have been assigned by inter-laboratory comparison. This work will be of great support to the monitoring of butyltin compounds in biota, as well as meeting the need for implementing international treaties in China.

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1 Introduction

Tributyltin (TBT) has been introduced into the environment in large amounts during the last century due to its wide application in antifouling paints on ship hulls, marine platforms and fishing nets.^{1,2} The environmental and health impacts of TBT and its degradation products (monobutyltin (MBT), dibutyltin (DBT)) to aquatic life have therefore been of high concern. TBT can cause chronic and acute poisoning to the most sensitive aquatic organisms, such as algae, zooplankton, mollusks and the larval stage of some fish even at very low concentrations.³ Physiological abnormalities such as growth reduction in marine microalgae,⁴ shell thickening and spat failure in oysters,^{5,6} and imposex in gastropods, whelks and *Mya arenaria*^{7–10} induced by butyltin pollution have been reported. The toxic mechanism of TBT has also been extensively studied.¹¹ Although MBT and DBT are generally less toxic than TBT to aquatic organisms, they are also of high environmental concern due to their higher solubility in water. These mono- and di-alkylated compounds may originate from the dealkylation of TBT caused by microbial activity and/or photochemical reactions, or be directly discharged to the environment by leaching from PVC materials.¹

Measures limiting the usage of butyltin compounds (BTs) have been adopted by many developed countries and international organizations.¹ A total ban of TBT came into effect in January 2008 as proposed by the International Maritime Organization (IMO).¹² TBT was also added into a global trade “watch list” by the United Nations Environment Programme (UNEP) and Food and Agriculture Organization of the United Nations after the ‘Rotterdam Convention’ in November 2008.¹³ However, there is no restriction on the use of organotins in China.¹⁴ Serious contamination of BTs in Chinese aquatic ecosystem has been evidenced by several studies.^{15–18} Thus, routine monitoring and analysis of BTs are indispensable to underpin control of environmental contamination in China.

Many analytical techniques have been developed for the speciation of organotin compounds.^{1,2} However, these methods involve various tedious analytical procedures, such as extraction, derivatization, separation and final determination which each can have analytical errors and can contribute to higher unreliability of the data.² Therefore, quality control of the experimental procedure is important to validate the results. Use of reference materials (RM) is one of the solutions for this problem. Nevertheless, RMs for organotin compounds in mollusks, especially for butyltin compounds in marine environments have seldom been developed. Although the Community Bureau of Reference (BCR) introduced the ERM-CE 477 material,¹⁹ this was not considered to be sufficient to meet the demand. Therefore, a new candidate RM for BTs in mollusks was developed in this study as part of a project for developing monitoring methods for priority pollutants within

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the framework of the National High Technology Research and Development Program of China. This paper presented the preparation, the homogeneity and stabilization test, the value assignment and the evaluation of uncertainty for a new candidate RM based on the mollusk '*Mya arenaria*'.

2 Materials and methods

2.1 Materials

All reagents were obtained commercially and used without further purification. Mussel tissue reference material ERM-CE 477 was obtained from the Community Bureau of Reference, Commission of the European Communities (Belgium). Tetrahydrofuran anhydrous (THF, 99.9%, free of inhibitors) was purchased from Sigma Aldrich (USA). MBT chloride was purchased from Dr Ehrenstorfer GmbH (Germany). Chinese national primary certified reference materials (CRM) 'dibutyltin in methanol' (GBW08711) and 'tributyltin in methanol' (GBW08710) were obtained from the National Institute of Metrology (China). Tetrabutyltin (TeBT) was purchased from Acros Organics (USA). Distilled deionized water (DDW) (18 MΩ cm) was obtained from Milli-Q Advantage A10 system, Millipore (USA). Tropolone (98%) was purchased from Acros Organics Inc. (USA) and *n*-hexane (Pesticide Grade) was bought from Dikma Technologies Inc. (USA).

The stock solutions of MBT and TeBT were obtained by dissolving an accurate weight of corresponding reagents into methanol to adjust the concentrations to 1 mg Sn g⁻¹. The pH was adjusted to 2 by concentrated hydrochloric acid (HCl) to ensure the stability of the stock solutions. Working standard solutions were obtained by gradually diluting the stock solutions and the CRM of GBW08710 and GBW08711 to the desired concentrations. All solutions were stored at 4 °C in a refrigerator. The Grignard reagent of *n*-propylmagnesium bromide (*n*-PrMgBr, 2.0 mol L⁻¹, in ether) was synthesized in the laboratory according to the methods presented in ref. 20. All other reagents and solvents used in this study were of analytical grade or better. All glassware was rinsed with deionized water, decontaminated overnight in 50% (v/v) nitric acid solution and rinsed again with ultra pure water.

Except during the value assignment section, all sample manipulations were performed in the State Key Laboratory of Environmental Chemistry & Ecotoxicology (SKLECE), Research Center for Eco-Environmental Sciences (RCEES), Chinese Academy of Sciences (CAS) to minimize the risk of possible exogenous contamination of the target compounds.

2.2 Instruments

A balance from Mettler Toledo (AL204, Max = 210 g, *d* = 0.0001 g, Shanghai, China) was used for weighing subsamples for analysis. A Shimadzu GC-2010 gas chromatograph equipped with a split/splitless injector was used during the homogeneity and stability test. Separation of MBT, DBT and TBT was conducted on a capillary column (Rtx-5 ms, 30 m × 0.25 mm × 0.25 μm) coated with 95% methyl silicone and 5% phenyl silicone. A flame photometric detector (FPD) with a tin filter was used for the

qualitative and quantitative analysis. High purity He (≥99.999%) was used as carrier gas. The column flow was set at 1.6 mL min⁻¹, and the purge flow at 3.0 mL min⁻¹. The injector temperature was held at 250 °C with a 1 : 10 split injection mode. The oven temperature was initially held at 50 °C for 1 min, then increased at 20 °C min⁻¹ to the final temperature of 250 °C and held for 3 min. The temperature of FPD was set at 250 °C. The air and the hydrogen flows of the detector were both set at 70 mL min⁻¹. Signal collection and processing were conducted by the Shimadzu GC solution software.

2.3 Selection, collection and preparation of the candidate RM

In recent years, several monitoring campaigns have been carried out in the Chinese Bohai coastal region to evaluate the extent of contamination and potential adverse effects on mollusks.^{9,15,17,18,21} Relatively high levels and low elimination rate of BTs were found in *Mya arenaria* compared with other species investigated as a consequence of maritime activities.^{9,21} In addition, *Mya arenaria* was widely present in the coastal environment of China and other countries. These suggest that *Mya arenaria* could be suitable as a candidate RM for the monitoring of BTs in coastal environments.

A batch of 30 kg of *Mya arenaria* was purchased directly from the market in areas around the Bohai Sea. After collection, the samples, stored in a butyltin-free ice-cooled box, were transported to laboratory in RCEES, CAS. The samples were immediately shelled using special mussel knives. The edible part was cleaned and washed with fresh water to eliminate extraneous impurities, and then ground using a homogenizer. The samples were then freeze-dried for more than 48 hours. The freeze-dried material was further ground in a ball-mill, taking additional precautions to avoid contamination. It was then sieved through a 400 μm mesh and mixed for 15 days under an argon atmosphere in a special polyethylene-lined mixing drum. Finally, the material was bottled under an argon atmosphere in brown Pyrex-glass bottles.

2.4 Pretreatment procedure

The same pretreatment procedure was applied in the organizer's laboratory throughout the studies on homogeneity, stability and value assignment. Briefly, about 0.3 g sample was weighed exactly for the analysis of BTs. After mixing with a suitable amount of internal standard (TeBT), 10 mL of HCl-THF (1 : 20) solution was added and then extracted with 25 mL 0.01% (m/v) tropolone-hexane solution under vigorous shaking in a mechanical shaker for 40 min. After decanting the extract, another 10 mL of hexane was added to the original homogenate, and the mixture was again shaken for 10 min. The combined extract was concentrated on a rotary evaporator to about 1–2 mL and subjected to Grignard propylation for derivatization. The derivatized analytes were purified using a glass column packed with anhydrous Na₂SO₄ (2 g), silica gel (2 g) and Florisil (2 g) from top to bottom. The final volume was adjusted to 1.0 mL under a gentle stream of pure nitrogen. The method applied in ref. 17, 21, 22, which has been confirmed by the determination

of the certified reference material (ERM-CE 477), was optimized for the determination of butyltin compounds in this work. The same or very similar pretreatment procedures were used by the participating laboratories during the value assignment campaign.

In addition, an ethylation pretreatment procedure was also applied by one of the participating laboratories. Briefly, about 1.0 g sample was weighed exactly for the analysis of BTs. After addition with a suitable amount of internal standard, tripropyltin (TPtT), 10 mL of HCl-CH₃OH (1 : 120, v/v) solution was added and then extracted in an ultrasonic bath for 1 h. After centrifugation, the supernatant was transferred and the residue was re-extracted with 5 mL of HCl-CH₃OH. 1.5 mL of the combined extractant was transferred into a 50 mL glass tube. 30 mL of acetic acid-sodium acetate (HAc-NaAc) buffer (0.2 mol L⁻¹, pH 5.0), 100 µL sodium tetraethyl borate (NaBEt₄) solutions (5%, m/v) and 1 mL hexane were added into the tube. The derivatization and extraction were carried out under vigorous shaking. After centrifugation, the upper layer was purified using a commercial Florisil SPE column. The extractant was adjusted to 1.0 mL under a gentle stream of pure nitrogen for GC analysis.

2.5 Homogeneity

A homogeneity study was carried out according to the Technical Norm of Primary Reference Material (JJG1006-1994) of China. A total of 16 bottles in the overall bottling period were randomly selected according to a random number table for homogeneity testing between bottles. Three subsamples of 300 mg from each bottle were assayed for homogeneity testing within the bottle. Each bottle was shaken manually for 5 min before sampling to eliminate the segregation of particles which might have occurred during transport and storage. The results were evaluated through the *F*-test statistic method.²³ If the *F* values in the homogeneity test were smaller than those of the critical values at a significance level of 0.05, then the candidate RM could be considered homogeneous.

2.6 Stability

Stability tests were performed for determining the suitability of this material as a reference material. Two types of stability studies were performed for the candidate RM: short-term stability was studied to evaluate the effect of transport conditions and long-term stability was studied to evaluate the effect of storage conditions. In the study of short-term stability, temperature was investigated as the main factor affecting the stability of the candidate RM. Influences of light and moisture were not considered due to appropriate packaging of materials during transport. The candidate RM was investigated at time intervals of 0, 1, 3, 7, 15 days under 4 °C, 25 °C and 40 °C, respectively. Four subsamples were determined at each short-term stability test. In the study of long-term stability, changes in values of the candidate RM were monitored for two years. Samples were determined at the beginning of the storage period and after 1, 3, 6, 12, 15, 18 and 24 months. Considering the similarity of the mollusk matrix to ERM-CE477, -20 °C was

selected as the storage temperature as used in ref. 19. A set of 3 bottles of sample was selected at random and 2 portions from each bottle were studied by determining the concentration of BTs. The results were evaluated through the *t*-test statistical method.²³ The candidate RM could be considered stable when the *t* values of stability test were smaller than those of the critical values at a significance level of 0.05.

2.7 Value assignment

Inter-laboratory certification study was carried out for the value assignment of the candidate RM '*Mya arenaria*'. Large bias or errors usually occur in inter-laboratory certification due to different laboratory personnel, different methods and different laboratory levels. Therefore, similar basic principles for measurements are critical for the participating labs to avoid these errors. Four principles were established in this work as follows:^{24,25}

(1) Highly specialized operatives: the organizer should ensure that high quality of the specialized operatives from all participating laboratories is met through professional training. The laboratories involved should have advanced training and experience of butyltin analysis.

(2) Uniform organotin calibrants and derivative reagent: standards used for calibration have their own uncertainties. Important bias usually occurs when various standards are used by different labs. Various derivation reagents have different derivatization efficiencies which may also lead to large bias. The organizer should therefore provide a set of standards for direct use or verifying standards used by other labs and uniform derivative reagents in order to guarantee the same efficiency of derivatization.

(3) Accurate and reliable analytical methods: the nominal concentrations should be measured gravimetrically. This provides measurement traceability to the results. The used methods should be corrected by determining the concentrations of ERM-CE477.

(4) All participating laboratories should determine various bottles on different days. At least 3 portions were taken from each bottle for analysis and 9 results were submitted.

The results of value assignment campaign were calculated statistically according to ref. 23. The average concentrations obtained from the statistical analysis were assigned to the candidate RM.

2.8 Dry mass correction

Since moisture content in the candidate RM can vary with ambient humidity and temperature due to inappropriate storage, it is recommended that the water content is checked prior to analysis. To avoid the effect from increased moisture, the selected samples should be made by taking a separate portion of 100 mg and drying it in an oven at (105 ± 2) °C for 3–4 h.²⁴

2.9 Quality control and quality assurance

A solvent blank with and without internal standard were determined simultaneously with every batch of 10 samples for

monitoring of contamination of glassware and instruments and the dealkylation of internal standard. ERM-CE477 was used for validation of the analytical procedures.

3 Results and discussion

3.1 Homogeneity

The concentrations of MBT, DBT and TBT during the homogeneity test are displayed in Table S1, ESI.† These results were statistically inspected by an *F*-test. The method of the *F*-test can be expressed by eqn (1):

$$F = \frac{s_1^2}{s_2^2} \quad (1)$$

where s_1 and s_2 are 'between-bottle variability' and 'within-bottle variability', respectively. The results from the *F*-test for MBT, DBT and TBT and the critical value F_α at a significance level of 0.05 are shown in Table 1. The results showed that DBT and TBT in the candidate RM displayed homogeneity at 300 mg levels of sample and is in conformity with the requirement of homogeneity by the regulation (JJG 1006-1994). However, the results revealed significant difference between the between- and within-bottle variances for MBT in the candidate RM at the same amount of sample.

3.2 Stability

The results from short-term and long-term stability tests are shown in Tables S2 and S3, ESI† and Fig. 1 and 2, respectively. A *t*-test was carried out for the statistics of these data. This method can be expressed by eqn (2):

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\frac{(n_1 - 1)s_3^2 + (n_2 - 1)s_4^2}{n_1 + n_2 - 2} \sqrt{\frac{n_1}{n_1 - 1} + \frac{n_2}{n_2 - 1}}} \quad (2)$$

where $\bar{x}_1$, s_3 , n_1 represent mean value, standard deviation, determination times determined at time t_1 , and $\bar{x}_2$, s_4 , n_2 being represent mean value, standard deviation, determination times determined at time t_2 , respectively.

The *t*-test results from the short-term stability are shown in Table 2. All the values were less than the critical value t_α at a significance level of 0.05 ($t_\alpha = 2.45$). No significant changes were observed in the candidate RM under simulant transport conditions of two weeks.

The *t*-test results from the long-term stability of MBT, DBT and TBT and the critical value t_α at a significance level of 0.05 ($t_\alpha = 2.23$) at a storage temperature of -20°C are shown in Table 3. All *t* values for DBT and TBT were below the critical value. As a result, DBT and TBT in the candidate RM were considered very stable over a period of two years and under a storage temperature of -20°C . This was also in corroboration

Fig. 1 Results of short-term stability tests in a two week period, with error bars representing the standard deviations at 4 °C, 25 °C and 40 °C, respectively.

with the requirement of stability for the primary RM in the regulation (JJG 1006-1994). Although the *t* values for MBT at month 1 and 12 exceeded the critical value, *t* values at other time periods were satisfactory. Therefore, the conclusion of instability should be drawn with caution. The stability of MBT in the candidate RM should be further studied in future work.

3.3 Value assignment

Five other laboratories were invited to participate in the value assignment. The method accuracy used by each lab participating was validated by determining the concentrations of ERM-CE477. Concentrations of ERM-CE477 determined by participating labs are shown in Table S4, ESI.† The

Table 1 Results of *F*-test for homogeneity inspection of MBT, DBT and TBT in the candidate RM

Species	MBT	DBT	TBT	F_α
<i>F</i> value	2.48	0.94	1.73	1.97

Fig. 2 Results of long-stability tests in a two year period, with error bars representing the standard deviations.

Table 2 Results of *t*-test for short-term stability inspection of MBT, DBT and TBT in the candidate RM

Temperature	Species	Day			
		1	3	7	15
4 °C	MBT	0.13	1.39	0.44	0.59
	DBT	0.40	1.09	1.35	0.42
	TBT	0.33	0.99	0.78	0.60
25 °C	MBT	1.61	0.86	0.97	0.54
	DBT	0.20	0.38	0.63	0.02
	TBT	0.83	0.54	0.40	0.32
40 °C	MBT	0.26	0.82	0.83	0.51
	DBT	0.24	0.12	0.19	1.26
	TBT	0.04	0.92	0.37	0.97

Table 3 Results of *t*-test for long-term stability evaluation of MBT, DBT and TBT in the candidate RM

Month	1	3	6	12	15	18	24
MBT	3.43	1.64	0.21	3.00	0.88	0.04	1.52
DBT	2.14	0.22	0.49	1.70	0.11	1.13	0.07
TBT	1.09	0.67	1.03	0.47	0.52	1.12	1.54

concentration values of ERM-CE477 determined by methods used in the value assignment procedure all agreed well with the reference values and the results are shown in Table S5, ESI.† The method, the mean and the standard deviation of each lab are displayed in Fig. 3. The datasets were thereafter subjected to the following statistical tests:²³

- (1) Dixon test to detect 'outlying' values in results of each laboratory;
 - (2) D'Agostino test to assess the conformity of the distributions of all individual results to normal distributions;
 - (3) Dixon test to detect 'outlying' values in laboratory means;
 - (4) Cochran test to verify the equal precision of each data set.
- When all the sets of data have equal precision, the average and standard deviation were calculated.

Except for one discarded 'outlying' value for TBT in data set from lab 5 inspected by Dixon tests, other values displayed

Fig. 3 Results of value assignment for MBT, DBT and TBT of the candidate RM with the methods used displayed on the right hand side. The dot in the centre of the box represents mean concentrations and the lower and upper edges of the box mark the standard deviations. The whiskers extending from the box show the highest and lowest values.

normal distributions. In the following Dixon test for laboratory means, all the reserved values were proved verified values. However, in the Cochran test for equal precision among the data of each laboratory, the results for MBT determined by ethylation-GC-MS/MS submitted by lab 3 proved to have unequal precision with other data, and these data were therefore discarded. The means and standard deviations of the remaining values were calculated with low relative standard deviations (Table 4). The mean certified concentrations of MBT, DBT and TBT in the candidate RM are 0.33, 0.62 and 2.64 $\mu\text{g Sn g}^{-1}$, respectively.

Table 4 Mean of data presented by each laboratory; means, standard deviations and relative standard deviations of the whole preserved data ($\mu\text{g Sn g}^{-1}$)

Lab no.	MBT	DBT	TBT
Lab1	0.32	0.62	2.67
Lab2	0.33	0.64	2.54
Lab3-1 ^a	0.39	0.56	2.65
Lab3-2 ^b	0.40	0.70	2.72
Lab4	0.30	0.62	2.7
Lab5	0.35	0.58	2.49
Lab6	0.30	0.63	2.7
Means	0.33	0.62	2.64
Standard deviations	0.04	0.04	0.09
Relative standard deviations (%)	12.1	7.0	3.4

^a Data determined by ethylation. ^b Data determined by propylation.

3.4 Evaluation of uncertainty

3.4.1 Uncertainty sources. In this work, four main factors were assumed to contribute to the uncertainty of the values: the uncertainty from the heterogeneity of materials, the uncertainty from the instability of the materials, the uncertainty from purity of standard agents used in the campaign of value assignments and the uncertainty originating from the process of value assignments for the candidate RM.

3.4.2 Quantification of the uncertainty

(1) *The standard uncertainty from homogeneity test.* Usually, this type of uncertainty can be recorded as s_H and calculated according to eqn (3):

$$s_H = \sqrt{\frac{1}{n}(s_1^2 - s_2^2)} \quad (3)$$

where n is the number of replicates in each group.

However, in certain cases, the variability of the measurements can mask the variation between bottles, *i.e.*, $s_2 > s_1$. Then, the uncertainty can be calculated by eqn (4):

$$s'_H = \sqrt{s_2^2/n + 2/\nu_2} \quad (4)$$

where ν_2 is the degrees of freedom of s_2 , n is the same as in eqn (3). As an estimate of the uncertainty related to possible between-bottle variation, the largest values of s_H and s'_H are used.

(2) *The standard uncertainty from stability test.* The uncertainty from long-term stability can be recorded as s_T and calculated according to eqn (5):

$$s_T = \sqrt{\frac{1}{n}(s_5^2 - s_6^2)} \quad (5)$$

where s_5 and s_6 are variability among and within groups of data obtained from different points in time, respectively; n is the number of replicates each time.

When the results of long-term stability test passed the t -test, the uncertainty would be calculated through a linear regression analysis. The linear regression curves were generated with time as the x -coordinate and the concentrations as the y -coordinate. The slopes of the regression curve (b_1) were compared with the statistical value $t_{0.95, n-2}s(b_1)$, where $s(b_1)$ is the standard error of

the slope and $t_{0.95, n-2}$ is the student distribution factor for freedom degree of $n - 2$ and $P = 0.95$ (95% confidence level). If $|b_1| < t_{0.95, n-2}s(b_1)$, the slope is considered to be insignificant. The uncertainty can be expressed by eqn (6):

$$s'_T = s(b_1)t \quad (6)$$

where t is the number of months for period of validity.

The uncertainty from short-term stability can be recorded as s_{sts} and calculated using the same method as for long-term stability. The maximum value of s_{sts} calculated under different temperatures was selected as the final uncertainty from short-term stability.

(3) *The standard uncertainty from purity of the standard agents.* The standard agents used in the campaign of value assignments have their own uncertainty. Because the same standard agents were offered to all participants, this part of uncertainty from all participants is the same and could be calculated according to eqn (7):

$$s_P = \frac{P}{\sqrt{3}} \quad (7)$$

where P is the uncertainty of the certified reference material used in the campaign of value assignments.

(4) *The standard uncertainty from the assignment of certified values.* This uncertainty can be recorded as $s_{\bar{x}}$ and calculated according to eqn (8):

$$s_{\bar{x}} = \sqrt{\frac{1}{N(N-1)} \sum_{i=1}^N (\bar{x}_i - \bar{\bar{x}})^2} \quad (8)$$

where N means the numbers of participants, $\bar{x}_i$, $\bar{\bar{x}}$ are the means of each laboratory and the means of the whole dataset, respectively.

(5) *The combination of standard uncertainty.* The combined standard uncertainty s_c associated with the four parts can be defined by eqn (9)

$$s_c = \sqrt{s_H^2 + s_T^2 + s_{\text{sts}}^2 + s_{\bar{x}}^2 + s_P^2} \quad (9)$$

(6) *The expanded standard uncertainty.* The expanded uncertainty of the candidate RM was calculated according to a modification of the Guide to the Expression of Uncertainty in Measurement (GUM, EC) and the recommendations given in ISO Guide 35: 2006,^{24,25} using eqn (10):

$$S_c = k s_c \quad (10)$$

where k is coverage factor. We used a coverage factor of 2, to provide a 95% confidence interval.

Each part of standard uncertainties of MBT, DBT and TBT were calculated according to the data from the homogeneity test, stability test and value assignment campaign and the corresponding equations. The standard uncertainties, $s_H(s'_H)$, $s_T(s'_T)$, s_{sts} , s_P , $s_{\bar{x}}$, s_c , S_c divided by the certified values assigned to the candidate RM were transformed to the relative standard uncertainties of μ_H , μ_T , μ_{sts} , μ_P , $\mu_{\bar{x}}$, μ_c , U_c , respectively. The results are shown in Table 5. The final certified values of the

Table 5 Certified values with uncertainties for the concentration of MBT, DBT and TBT in the candidate RMs

Species	Certified values ($\mu\text{g Sn g}^{-1}$)	Relative standard uncertainty (%)						U_c (%)	S_c ($\mu\text{g Sn g}^{-1}$)
		μ_H	μ_T	μ_{sts}	μ_P	$\mu_{\bar{x}}$	μ_c		
MBT	0.33	5.2	7.1	5.3	0.58	4.6	11.3	22.6	0.08
DBT	0.62	1.7	1.3	3.0	0.58	2.7	7.3	14.6	0.09
TBT	2.64	1.4	2.2	1.9	1.7	1.3	3.9	7.8	0.20

candidate RM can be expressed as 0.33 ± 0.08 , 0.62 ± 0.09 , $2.64 \pm 0.20 \mu\text{g Sn g}^{-1}$ for MBT, DBT and TBT, respectively. The relative expanded uncertainties of 22.6%, 14.6%, and 7.8% of certified values for MBT, DBT and TBT in the candidate RM are comparable to those of ERM-CE477 which are 19%, 7.8%, 8.6%, respectively.

4 Conclusions

In this study, a candidate RM '*Mya arenaria*' for the monitoring of BTs in mollusks was developed. Notwithstanding the unsatisfactory results of MBT in the homogeneity and stability tests, it was proven to be sufficiently homogeneous and stable for DBT and TBT. The values were assigned by inter-laboratory certification study. All the methods used were validated by determination of ERM-CE 477. The relative expanded uncertainty values of the certified values for the candidate RMs were estimated by combining uncertainty contributions including uncertainties from the homogeneity test, stability test, and purity of the standard agents and the assignment of certified values. They were found to be comparable with those of ERM-CE 477. The new RM could be very promising for performing method validation and quality assurance in the organotin speciation analysis of mollusks in marine environments.

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