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Second edition
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Footwear — Critical substances potentially present in footwear and footwear components — Determination of organotin compounds in footwear materials

Chaussures — Substances critiques potentiellement présentes dans les chaussures et les composants de chaussures — Détermination des composés organostanniques dans les matériaux de chaussures

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 216, *Footwear*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 309, *Footwear*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO/TS 16179:2012), which has been technically revised.

The changes are as follows:

- the technical specification becomes an ISO standard;
- GC-MS/MS technique added in 8.9.3 and in Clause B.2;
- new extraction solvent in Clause 4;
- new Table 1 (certain substances added) in Clause 4;
- change in the safety instructions at the preparation of the sodium tetraethylborate solution in Clause 8;
- sample preparation reference to ISO 21061 in Clause 7;
- new Table 2 (certain substances added) in 8.3.1;
- deletion of the need for duplicate determinations in Clause 7;
- new Annex B for mass spectroscopy;
- deletion of the reference to ISO 22744-1 in the Bibliography.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Certain organotin compounds have been identified as carcinogenic. Thus, several countries have restricted them in articles such as footwear, e.g. in the European Union by commission regulation (EU) 276/2010[1] amending regulation (EC) No 1907/2006[2].

Further organotin compounds are restricted by footwear brands in their restricted substances lists (RSL).

Footwear — Critical substances potentially present in footwear and footwear components — Determination of organotin compounds in footwear materials

1 Scope

This document specifies a test method for the qualification and quantification of organotin compounds by applying gas chromatography coupled with mass spectrometry. This test method is applicable to all types of footwear materials except metal hardware (see ISO/TR 16178).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 4787, *Laboratory glass and plastic ware — Volumetric instruments — Methods for testing of capacity and for use*

ISO 21061, *Footwear — Chemical tests — General principles on the preparation of samples*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle

The organotin substances are extracted from the footwear material with methanol, in a medium-strength acidic condition, using tropolone as a complexing agent.

In the previous version of this test method, the extraction solvent was a mixture of methanol-ethanol. Comparisons have shown that methanol gives equivalent results to methanol-ethanol solution. It is still possible to use extraction with methanol-ethanol solution.

The polar and high-boiling organotin is then converted to the corresponding volatile alkyl derivative, by reaction with sodium tetraethylborate, $NaB(Et)_4$. Finally, it is detected and quantified by using a gas chromatograph fitted with a mass selective detector [gas chromatograph with a single quadrupole mass spectrometer (GC-MS) or gas chromatograph with a triple quadrupole mass spectrometer (GC-MS/MS)].

Table 1 indicates the list of target compounds which can be analysed with the method defined in this document. This document is also applicable for further organotin substances provided that the method is validated with the additional compounds.

Table 1 — List of target compounds and internal standards that can be analysed

Type of compound	Compound	CAS Registry Number® [b]
Monosubstituted	Internal standard: *n*-heptyltin trichloride	59344-47-7
	methyltin trichloride	993-16-8
	n-butyltin trichloride	1118-46-3
	n-octyltin trichloride	3091-25-6
	phenyltin trichloride	1124-19-2
Disubstituted	Internal standard: di-*n*-heptyltin dichloride	74340-12-8
	dimethyltin dichloride	753-73-1
	di-*n*-propyltin dichloride	867-36-7
	di-*n*-butyltin dichloride	683-18-1
	di-*n*-octyltin dichloride	3542-36-7
	diphenyltin dichloride	1135-99-5
Trisubstituted	Internal standard: tri-*n*-pentyltin chloride	3342-67-4
	trimethyltin chloride	1066-45-1
	tri-*n*-propyltin chloride	2279-76-7
	tri-*n*-butyltin chloride[a]	1461-22-9
	tri-*n*-octyltin chloride	2587-76-0
	triphenyltin chloride (or fentin chloride)	639-58-7
	tricyclohexyltin chloride	3091-32-5
Tetrasubstituted	Internal standard: tetra-*n*-propyltin	2176-98-9
	tetra-*n*-ethyltin	597-64-8
	tetra-*n*-butyltin	1461-25-2

[a] If bis(tri-*n*-butyltin) oxide (TBTO), CAS RN® 56-35-9, is present, it is detected as tri-*n*-butyltin.

[b] CAS Registry Number® (CAS RN®) is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

5 Reagents

Unless otherwise specified, use only reagents of recognized analytical grade and certified reference standards.

5.1 Water, grade 3 according to ISO 3696.

5.2 Glacial acetic acid, CAS RN®: 64-19-7.

5.3 Sodium tetraethylborate, CAS RN®: 15523-24-7.

5.4 Tetrahydrofuran (THF), stabilized, CAS RN®: 109-99-9.

5.5 Internal standards

Alternative internal standards (such as deuterated compounds) to those listed may be used.

5.5.1 *n*-heptyltin trichloride, CAS RN®: 59344-47-7 (internal standard).

5.5.2 Di-*n*-heptyltin dichloride, CAS RN®: 74340-12-8 (internal standard).

5.5.3 Tri-*n*-pentyltin chloride, CAS RN®: 3342-67-4 (internal standard).

5.5.4 Tetra-n-propyltin, CAS RN®: 2176-98-9 (internal standard).

5.6 *n*-Hexane, CAS RN®: 110-54-3.

5.7 Inert gas, e.g. nitrogen, helium or argon.

5.8 Tropolone (2-hydroxy-2,4,6-cycloheptatrien-1-one), of laboratory grade, CAS RN®: 533-75-5.

5.9 Methanol, CAS RN®: 67-56-1.

NOTE If methanol-ethanol solution is used, ethanol, GPR grade or industrial methylated spirit (IMS), CAS RN®: 64-17-5.

5.10 Sodium acetate anhydrous, CAS RN®: 127-09-3.

5.11 Organotin compounds listed in Table 1.

6 Apparatus and materials

The usual equipment and laboratory glassware, according to ISO 4787, shall be used, in addition to the following.

6.1 Gas chromatograph with a mass selective detector (GC-MS or GC-MS/MS).

6.2 Analytical balance, with a resolution of 0,1 mg.

6.3 Glass vessel, with screw tops and a volume of, for example, 50 ml.

6.4 Micropipettes, of required volumes.

6.5 Pipettes, of required volumes.

6.6 Calibrated pH-meter with a glass combination electrode and range of 0 to 14.

6.7 Volumetric flasks of required volumes.

6.8 Ultrasonic bath with adjustable temperature suitable for operation at about 60 °C.

6.9 Centrifuge.

6.10 Horizontal shaker, with a minimum frequency of 5 s^{-1}.

NOTE Horizontal shaker with minimum frequency of 5 s^{-1}, path length from 2 cm to 5 cm has been found suitable.

6.11 GC vial with PTFE cap, for example 2 ml.

7 Preparation of the sample

Prepare the test specimen in accordance with ISO 21061.

The test piece consists of a single material taken from the footwear, such as leather, textile, polymer, coated material or other. The preparation of the test piece should involve the removal of the individual materials from the footwear and the preparation of a test piece, which results in particles with a maximum edge length of 4 mm.

NOTE Up to three test specimens (of equal mass) of the same material type can be tested together taking into consideration the limits of detection and quantification.

8 Procedure

SAFETY PRECAUTIONS — Sodium tetraethylborate is air-sensitive and can spontaneously combust in the presence of air. The solution should be prepared in a fume cupboard without the presence of flammable substances or under inert gas atmosphere. Organotins are both toxic and known endocrine system disrupters; therefore, they should be treated with utmost care.

8.1 General

All the chemicals that are stored below room temperature should be allowed to reach room temperature before an aliquot is taken.

8.2 Preparation of the sodium tetraethylborate solution

Weigh about 2 g of sodium tetraethylborate (5.3) into a 10 ml volumetric flask (6.7) and make up to volume with tetrahydrofuran (5.4).

This solution is stable for approximately three months if stored under an inert gas blanket (5.7), in refrigerator at approximately 4 °C and preferably in an amber container.

NOTE Pre-weighed tetraethylborate or commercial solutions are available on the market.

8.3 Preparation of standard solutions

8.3.1 General

The organotin compounds are available on the market under their chloride forms, but the mass concentration for the calibration curve and the result are expressed in mg/kg of organotin cations. Certified standard solutions are available on the market.

EXAMPLE 1 With dibutyltin dichloride, Bu_2SnCl_2 (dibutyltin dichloride) is the chloride form and Bu_2Sn^{2+} is the cation form.

Table 2 gives the amount of organotin chloride and the weighting factor for recalculation of organotin cations (for 100 % purity of the chloride form).

Table 2 — Amount of organotin chloride and weighting factor for recalculation of organotin cations

	Compound	Weighting factor F_w	Amount of organotin chloride required to have a solution of 1 000 mg/l of organotin cation (in a 100 ml flask) mg
Target compounds	methyltin trichloride	0,557	179,5
	n-butyltin trichloride	0,623	160,5
	n-octyltin trichloride	0,686	145,8
	phenyltin trichloride	0,648	154,3
	dimethyltin dichloride	0,677	147,7
	di-*n*-propyl dichloride	0,742	134,8
	di-*n*-butyltin dichloride	0,767	130,4
	di-*n*-octyltin dichloride	0,830	120,5
	diphenyltin dichloride	0,793	126,1
	trimethyltin chloride	0,821	121,8
	tri-*n*-propyltin chloride	0,875	114,3
	tri-*n*-butyltin chloride	0,891	112,2
	tri-*n*-octyltin chloride	0,927	107,9
	triphenyltin chloride	0,908	110,1
	tricyclohexyltin chloride	0,912	109,6
	tetra-*n*-ethyltin[a]	1,000	100,0
	tetra-*n*-butyltin[a]	1,000	100,0
Internal standards	*n*-heptyltin trichloride	0,672	148,8
	di-*n*-heptyltin dichloride	0,817	122,4
	tri-*n*-pentyl chloride	0,906	110,4
	tetra-*n*-propyltin[a]	1,000	100,0

[a] These compounds have no chloride and therefore the weighting factor is 1,000.

EXAMPLE 2 If 160,5 mg of monobutyltin trichloride ($BuSnCl_3$) is weighed, a solution of 1 605 mg/l of monobutyltin trichloride is obtained, which corresponds to a concentration of 1 605 × 0,623 = 1 000 mg/l of monobutyltin cation ($BuSn^{3+}$).

EXAMPLE 3 If 110,4 mg of dioctyltin dichloride ($(C_8H_{17})_2SnCl_2$) is weighed, a solution of 1 104 mg/l of dioctyltin dichloride is obtained, which corresponds to a concentration of 1 104 × 0,830 = 916 mg/l of dioctyltin cation $[(C_8H_{17})_2Sn^{2+}]$.

The concentration of organotin cation is usually calculated using Formula (1):

$$\rho_{Sn} = \rho_{Cl} \times F_W \tag{1}$$

where

ρ_{Sn} is the mass concentration of organotin cation (mg/l);

ρ_{Cl} is the mass concentration of organotin chloride (mg/l);

F_W is the weighting factor (see Table 2).

8.3.2 Internal standards — stock solution (1 000 mg/l of organotin cation)

Internal standards are available commercially as certified solutions, or a solution of internal standards can be made. Four internal standards shall be used as solutions in methanol (5.9).

To prepare an internal standard solution use the analytical balance (6.2) to weigh the appropriate amount of *n*-heptyltin trichloride (5.5.1), di-*n*-heptyltin dichloride (5.5.2), tri-*n*-pentyltin chloride (5.5.3) and tetra-*n*-propyltin (5.5.4). Dissolve them together in methanol (5.9) in a single volumetric flask (6.7) to obtain the mass concentration of 1 000 mg/l for each organotin cation.

Store the standard solutions for a maximum of one year in a refrigerator at approximately 4 °C, when not in use, to minimize evaporation of the solvent.

8.3.3 Internal standards — working solution (10 mg/l of organotin cation)

Use a pipette (6.5) to transfer an appropriate volume of the internal standard stock solutions (8.3.2) together into a volumetric flask (6.7) and fill up to the mark with methanol (5.9) to create a 10 mg/l solution of each organotin cation.

8.3.4 Target compounds — stock solution (1 000 mg/l of organotin cation)

Target compounds are available commercially as certified solutions, or a solution of target compounds may be made.

Use the analytical balance (6.2) to weigh the appropriate amount of each target compound (see Table 2). Dissolve each of them (separately or together) in methanol (5.9) in an appropriately sized volumetric flask (6.7) to obtain the mass concentration of 1 000 mg/l for each organotin cation.

Store the standard solutions for a maximum of one year in a refrigerator at approximately 4 °C, when not in use, to minimize evaporation of the solvent.

8.3.5 Target compounds — working solution (10 mg/l of organotin cation)

Use a pipette (6.5) to transfer an appropriate volume of the target compound stock solutions (8.3.4) together into one volumetric flask (6.7) and fill up to the mark with methanol (5.9) to create a 10 mg/l solution of each organotin cation.

8.4 Preparation of the tropolone solution

Use the analytical balance (6.2) to weigh 0,50 g of tropolone (5.8) into a 100 ml volumetric flask (6.7). Dissolve them in approximately 40 ml methanol (5.9), afterwards fill up to the mark with methanol (5.9).

This solution can be used for up to one month from preparation and stored in a refrigerator at approximately 4 °C.

8.5 Preparation of the buffer solution

Prepare a 0,2 mol/l sodium acetate solution, for example by weighting 16,4 g of sodium acetate (5.10) in 1 l of water (5.1) and adjust the pH to (4,5 ± 0,1) with glacial acetic acid (5.2).

8.6 Preparation of the calibration solutions

8.6.1 As a guide, choose standards for example of concentration 100 µg/l, 200 µg/l, 300 µg/l, 400 µg/l and 500 µg/l.

8.6.2 These are added as 20 µl, 40 µl, 60 µl, 80 µl and 100 µl aliquots by micropipette (6.4) of the target compounds working solution (8.3.5) to individual vessels (6.3) containing 20 ml of methanol (5.9).

8.6.3 Add an appropriate amount, e.g. 100 µl, of internal standard working solution (8.3.3).

8.6.4 Add 8 ml of buffer solution pH 4,5 (8.5).

8.6.5 Add 1 ml of tropolone solution (8.4).

8.6.6 Add 100 µl sodium tetraethylborate solution (8.2) and shake vigorously for 30 min using a horizontal shaker (6.10).

8.6.7 Using a pipette (6.5), transfer 2 ml of hexane (5.6) into the vessel and shake vigorously for 30 min using a horizontal shaker (6.10).

8.6.8 Transfer the hexane phase in a GC vial (6.11) for analysis.

8.7 Sample preparation

8.7.1 Use the analytical balance (6.2) to weigh (1 ± 0,1) g of sample (see Clause 7) into a glass vessel (6.3) and record the mass, m_1, with a precision to 0,1 mg.

8.7.2 Add 20 ml of methanol (5.9).

8.7.3 Add an appropriate amount, e.g. 100 µl, of internal standard working solution (8.3.3).

8.7.4 Add 1 ml of tropolone solution (8.4)

8.7.5 Extract in an ultrasonic bath (6.8) at (60 ± 5) °C for (60 ± 5) min.

8.7.6 If required to properly separate the two liquid phases, centrifuge (6.9) for 5 min and transfer the clear solution into another glass vessel.

8.7.7 Add 8 ml of buffer solution pH 4,5 (8.5).

8.7.8 Add 100 µl sodium tetraethylborate solution (8.2) and shake vigorously for 30 min using a horizontal shaker (6.10).

8.7.9 Transfer 2 ml of hexane (5.6) into the glass vessel and shake vigorously for 30 min using a horizontal shaker (6.10).

If necessary to obtain a clear hexane layer, centrifuge (6.9) for 5 min.

8.7.10 Transfer the hexane phase in a GC vial (6.11) for analysis.

8.8 Preparation of the blank solution

Prepare the blank solution in the same way as the samples (see 8.7.2 to 8.7.10).

8.9 Gas chromatography

8.9.1 General

Determine the organotin compounds of the samples by using GC-MS. An example of the method parameters is given in Annex A. At least five calibration standards should be used.

8.9.2 With MS

Three diagnostic ion groups (one ion group for quantification and the two other groups for qualification) and the full spectra are used for the detection of the target compounds (see Annex B, Table B.1 for the choice of the three diagnostic ion groups). The target compounds shall be quantified with an internal standard with the same degree of substitution (refer to Annex B, Table 1).

Use the mass spectrometer in simultaneous SIM/SCAN mode, or in SIM mode with SCAN confirmation in case of positive results.

8.9.3 With MS/MS

The target compounds shall be quantified with an internal standard with the same degree of substitution (refer to Annex B, Table B.2).

9 Expression of the results

9.1 Determination of the mass fraction of the tin organic compounds

Calculate the total peak areas of the standards, the internal standard, and each detected organotin species in the sample.

NOTE 1 If the recovery of the internal standard is suspected to be lower than the expected value (due to matrix effects or unknown reasons) increasing the amount of sodium tetraethylborate can lead to a better recovery.

NOTE 2 Alternative internal standards (such as deuterated compounds) to those listed in 5.5 can be used if the recovery rate is low.

Using the data from the organotin standards, calculate the detector response factor, D_{RF}, for each tin compound at each tin mass concentration using Formula (2):

$$D_{\mathrm{RF}} = \frac{\rho_{\mathrm{St,Sn}} \times A_{\mathrm{St,is}}}{A_{\mathrm{St,Sn}} \times \rho_{\mathrm{St,is}}} \quad (2)$$

where

$\rho_{\mathrm{St,Sn}}$ is the mass concentration of organotin cation in the standard, in µg/l;

$A_{\mathrm{St,is}}$ is the peak area of the relevant internal standard;

$A_{\mathrm{St,Sn}}$ is the peak area of organotin cation in the standard;

$\rho_{\mathrm{St,is}}$ is the mass concentration of the relevant internal standard, in µg/l.

For each compound, calculate an average D_{RFa} with all the D_{RF} obtained at each calibration level i of mass concentration using Formula (3):

$$D_{\mathrm{RFa}} = \frac{1}{n} \sum_{i=1}^{n} D_{\mathrm{RF}i} \quad (3)$$

Theoretically, the D_{RF} values for a particular tin compound should be exactly the same, but slight differences are seen. Therefore, the average is calculated.

This average D_{RF} value, D_{RFa}, is used to calculate the mass concentration of organotin compounds in the sample using Formula (4):

$$\rho_{\mathrm{Sn}} = \frac{A_{\mathrm{Sn}} \times D_{\mathrm{RFa}} \times \rho_{\mathrm{is}}}{A_{\mathrm{is}}} \quad (4)$$

where

ρ_{Sn} is the mass concentration of organotin cation in the sample, in µg/l;

A_{Sn} is the peak area of organotin;

ρ_{is} is the mass concentration of the corresponding internal standard (500 µg/l);

A_{is} is the peak area of the corresponding internal standard.

Using Formula (5), convert ρ_{Sn}, whose units are expressed in µg/l, into µg/kg:

$$w_{Sn} = \frac{\rho_{Sn} \times V}{m_1} \quad (5)$$

where

w_{Sn} is the mass fraction of organotin cation, in µg/kg;

ρ_{Sn} is the mass concentration of organotin cation in the sample, in µg/l;

V is the volume of hexane aliquot taken in 8.7.9 (2 ml);

m_1 is the mass of the sample obtained in 8.7.1, in g.

Mono-, di-, tri-, and tetraethyltinchloride present in samples are converted to tetraethyltin by sodium tetraethylborate Consequently, there is a risk of false positive results for tetraethyltin. Findings of tetraethyltin shall be confirmed by reprocessing the sample (8.7), but without performing 8.7.8 (i.e. without adding sodium tetraethylborate). The result for the tetraethyltin mass fraction of the sample is the result which is obtained without the use of sodium tetraethylborate.

9.2 Precision of the test method

With this method it is feasible to reach detection limits of 50 µg/kg and quantification limits of 200 µg/kg (see Annex C).

10 Test report

The test report shall include, at least, the following:

a) a reference to this document, ISO 16179:2025;

b) all details necessary for complete identification of the sample tested;

c) date of the test;

d) the test result (for each detected organotin cation) as recorded in 9.1 in µg/kg or mg/kg;

e) any unusual features observed;

f) any deviation, by agreement or otherwise, from the procedure specified.

Annex A
(informative)

Gas chromatography apparatus and conditions for organotin analysis

Equipment: gas chromatograph (GC) with mass-selective detector (MS) or (MSMS)

Column: 5 % phenyl methyl siloxane for MS; length 30 m; 0,25 mm interior diameter and 0,25 µm film thickness.

Carrier gas: helium, flow rate 1 ml/min.

Injector: Split/Splitless Injector 250 °C.

Injection volume: 1,0 µl.

Temperature programme: 50 °C for 1 min.
up to 300 °C at 15 °C/min.
300 °C isothermal for 2 min.

Transfer line: 300 °C.

Annex B
(informative)

Suitable conditions for mass spectroscopy

B.1 Suitable conditions for GC-MS

Ion source: Electronic impact at 230 °C

Quadrupole: 150 °C

Table B.1 — Example of quantifier and qualifier ions of the target and their respective internal standard

Compound (as ethyl derivative)	**Group 1 m/z u**	**Group 2 m/z u**	**Group 3 m/z u**
Internal standard: monoheptyltriethyltin	**277/275**	**179/177**	**151/149**
Methyltriethyltin	193/191	165/163	–
n-Butyltriethyltin	235/233	179/177	151/149
n-Octyltriethyltin	291/289	179/177	151/149
Phenyltriethyltin	255/253	227/225	197/195
Internal standard: di-*n*-heptyldiethyltin	**347/345**	**249/247**	**151/149**
Dimethyldiethyltin	179/177	151/149	–
Di-*n*-propyldiethyltin	235/233	193/191	151/149
Di-*n*-butyldiethyltin	263/261	179/177	151/149
Di-*n*-octyldiethyltin	375/373	263/261	151/149
Diphenyldiethyltin	303/301	275/273	197/195
Internal standard: tri-*n*-pentylmonoethyltin	**333/331**	**291/289**	**221/219**
Trimethylmonoethyltin	165/163	179/177	151/149
Tri-*n*-propylmonoethyltin	249/247	235/233	193/191
Tri-*n*-butylmonoethyltin	291/289	263/261	179/177
Tri-*n*-octylmonoethyltin	459/457	375/373	263/261
Tricyclohexylmonoethyltin	233/231	315/313	369/367
Triphenylmonoethyltin	351/349	197/195	–
Internal standard: tetra-*n*-propyltin	**249/247**	**165/163**	**207/205**
Tetra-*n*-ethyltin	179/177	151/149	207/205
Tetra-*n*-butyltin	291/289	235/233	179/177

NOTE The monosubstituted target compounds are quantified under the monosubstituted internal standard. For example, *n*-butyltriethyltin and *n*-octyltriethyltin appear under the internal standard monoheptyltriethyltin.

B.2 Suitable conditions for GC-MS/MS

Ion source: Electronic impact at 230 °C

Quadrupole: 150 °C

Collision cell: Nitrogen