

Australian/New Zealand Standard™

Safety of toys

Part 11: Organic chemical compounds—Methods of analysis



AS/NZS 8124.11:2008

This Joint Australian/New Zealand Standard was prepared by Joint Technical Committee CS-018, Safety of Childrens Toys. It was approved on behalf of the Council of Standards Australia on 26 February 2008 and on behalf of the Council of Standards New Zealand on 7 March 2008.
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Australian Competition and Consumer Commission
Australian Consumers' Association
Australian Retailers Association
Australian Toy Association
Consumers Affairs Victoria
Consumers' Federation of Australia
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Australian/New Zealand Standard™

Safety of toys

Part 11: Organic chemical compounds—Methods of analysis

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PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee CS-018, Safety of Childrens Toys.

The objective of this Standard is to specify methods for the analysis of toy and toy material extracts prepared according to the sampling procedures in AS 8124.10, to enable assessment of compliance with the chemical requirements specified in AS 8124.9.

This Standard is identical with, and has been reproduced from EN 71-11:2005, *Safety of toys—Part 11: Organic chemical compounds—Methods of analysis*.

As this Standard is reproduced from an International Standard, the following applies:

- (a) Its number does not appear on each page of text and its identity is shown only on the cover and title page.
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References to International Standards should be replaced by references to Australian or Australian/New Zealand Standards, as follows:

<i>Reference to European Standard</i>	<i>Australian/New Zealand Standard</i>
EN	AS/NZS
71 Safety of toys	8124 Safety of toys
71-9 Part 9: Organic chemical compounds—Requirements	8124.9 Part 9: Organic chemical compounds—Requirements
71-10 Part 10: Organic chemical compounds—Sample preparation and extraction	8124.10 Part 10: Organic chemical compounds—Sample preparation and extraction

Documents not listed have not been adopted as Australian or Australian/New Zealand Standards.

The term ‘informative’ has been used in this Standard to define the application of the annex to which it applies. An ‘informative annex’ is only for information and guidance.

CONTENTS

	<i>Page</i>
1 SCOPE.....	1
2 NORMATIVE REFERENCES.....	1
3 TERMS AND DEFINITIONS.....	1
4 ENVIRONMENT, HEALTH AND SAFETY PRECAUTIONS.....	2
5 METHODS OF ANALYSIS.....	2
5.1 General.....	2
5.2 Flame retardants.....	2
5.3 Colourants.....	5
5.4 Primary aromatic amines.....	11
5.5 Monomers and solvents.....	15
5.6 Wood preservatives.....	33
5.7 Preservatives.....	37
5.8 Plasticisers.....	40
Annex A (Informative) methods of analysis for volatile solvents.....	44
Annex B (Informative) Validation of test methods.....	61
Annex C (Informative) Colourants—conformational analysis.....	62
Annex ZA (Informative) Clauses of this European Standard addressing essential requirements or other provisions of EU Directives.....	64
Bibliography.....	65

Australian/New Zealand Standard
Safety of toys
Part 11: Organic chemical compounds—Methods of analysis

1 Scope

This Part 11 of the European Standard EN 71 for safety of toys specifies methods for the analysis of toy and *toy material* extracts prepared according to the sampling procedures in EN 71-10, to enable assessment of compliance with the chemical requirements specified in EN 71-9.

This European Standard specifies analytical methods for the identification and determination of the following groups of organic chemicals:

- Flame retardants
- Colourants
- Primary aromatic amines
- Monomers and solvents
- Wood preservatives
- Preservatives
- Plasticisers

NOTE 1 Methods for formaldehyde in accessible textile components of toys; accessible paper components of toys; and accessible resin-bonded wood components of toys are specified in EN 71-9.

NOTE 2 The method for free formaldehyde as a preservative is specified in EN 71-10.

2 Normative references

The following referenced documents are indispensable for the application of this European Standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 71-9:2005, *Safety of toys – Part 9: Organic chemical compounds – Requirements*

EN 71-10:2005, *Safety of toys – Part 10: Organic chemical compounds – Sample preparation and extraction*

EN ISO 3696, *Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)*

3 Terms and definitions

For the purposes of this European Standard, the following terms and definitions apply.

3.1**action limit**

routinely-achievable limit of quantification for a particular substance using the specified method of analysis

3.2**aqueous migrate**

liquid obtained after extracting a *toy material* according to the procedure specified in Clause 6 of EN 71-10:2005

3.3**test portion**

portion of the laboratory sample prepared for analysis

3.4**toy material**

material from which toys and toy components are made

NOTE This definition differs from that given in EN 71-3

4 Environmental, health and safety precautions

When preparing this European Standard, consideration was given to the minimisation of environmental impacts caused by the use of the methods of analysis.

It is the users' responsibility to use safe and proper techniques in handling materials in the methods of analysis specified in this European Standard.

- Consult manufacturers for specific details such as material safety data sheets and other recommendations.
- Wear protective goggles and coats in all laboratory areas.
- Be careful about substances, which are toxic and/or human carcinogens.
- A fume cupboard shall be used during preparation of organic solvent solutions.
- Solvents shall be disposed of in accordance with environmental requirements.

5 Methods of analysis

5.1 General

All chemicals used for analysis shall be of analytical grade (pro analysis) or, if unavailable, the best technical grade. Water shall be of grade 3 according to EN ISO 3696 or of a comparable quality, and demonstrably free from analytes of interest.

The precision of volumetric glassware should be grade A.

The analysis of toys and *toy materials* for chemical compounds for which limits are given in Tables 2 A to 2 I of EN 71-9:2005 shall be performed in accordance with the sampling procedures specified in EN 71-10 and the methods of analysis described in this European Standard. Alternative methods of analysis are acceptable only if they are capable of achieving at least the accuracy and precision of the methods described in this European Standard; an adequate sensitivity; and have been validated to show that the results are equivalent to those of these standard methods.

5.2 Flame retardants

NOTE Methods are given for pentabromodiphenyl ether and octabromodiphenyl ether in order to enable compliance with Directive 2003/11/EC of the European Parliament and of the Council to be demonstrated for textile *toy materials*.

5.2.1 Principle

Flame retardants are determined in acetonitrile extracts of *toy materials* by liquid chromatography with diode-array and mass spectrometry detection (LC-DAD-MS) using the external standard method of calibration.

5.2.2 Standards, reagents and solvents

5.2.2.1 Standards

5.2.2.1.1 Pentabromodiphenyl ether^{2*}, CAS No. 32534-81-9

5.2.2.1.2 Octabromodiphenyl ether^{3*}, CAS No. 32536-52-0

5.2.2.1.3 Tri-*o*-cresyl phosphate, CAS No. 78-30-8

5.2.2.1.4 Tris(2-chloroethyl) phosphate, CAS No. 115-96-8

5.2.2.2 Reagents and solvents

5.2.2.2.1 Acetonitrile

5.2.2.2.2 Dichloromethane

5.2.2.2.3 Ammonium acetate, anhydrous

5.2.2.2.4 Acetic acid, glacial

5.2.2.2.5 Ammonium acetate, 10 mmol/l aqueous solution, pH 3,6

Transfer $(0,77 \pm 0,01)$ g ammonium acetate (5.2.2.2.3) into a 1 000-ml volumetric flask, add 980 ml of water, adjust the pH to $3,6 \pm 0,1$ with glacial acetic acid and make up to the mark with water.

5.2.2.3 Stock standard solution (100 mg/l)

Weigh, to the nearest 0,1 mg, (10 ± 1) mg of each flame retardant (5.2.2.1) into a 100-ml volumetric flask. Add 25 ml of acetonitrile (5.2.2.2.1) and mix carefully to dissolve. Place in an ultrasonic bath for 10 min to ensure complete dissolution. Make up to the mark with acetonitrile.

The stability of the mixed stock standard solution should be checked regularly. It should be stable for up to 6 months when stored in the dark at (4 ± 2) °C.

5.2.3 Apparatus

Liquid chromatograph with diode-array and mass spectrometer detectors

The following LC-DAD-MS conditions for flame retardant determination have been found to be suitable:

Column:	C18, 80 Å, 3,5 µm, double endcapped, (Zorbax Eclipse XDB ⁴ , or equivalent) 2,1 mm x 150 mm
Guard column:	C18, 80 Å, 4 mm x 2,0 mm,
Mobile phase A:	Ammonium acetate solution, 10 mmol/l, pH 3,6 (5.2.2.2.5)
Mobile phase B:	Acetonitrile
Gradient:	see Table 1

² This substance is also known as pentabromodiphenyl oxide.

* There are no requirements in EN 71-9 for this substance.

³ This substance is also known as octabromodiphenyl oxide.

⁴ Zorbax Eclipse XDB is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

Injection volume: 5 µl
 Run time: 45 min
 Flow: 0,3 ml/min
 DAD mode: 240 nm ± 20 nm
 DAD range: 200 nm to 800 nm
 Nebulizer: 200 Kpa
 Dry gas: 10 l/min
 MS range: 110 m/z to 500 m/z
 MS mode: Scan positive
 Ionisation: ESI+
 Fragmentor: 80 V

Table 1 – Gradient program

Time min	Mobile phase A %	Mobile phase B %
0	60	40
7	40	60
17	2	98
35	2	98
45	60	40

5.2.4 Procedure

5.2.4.1 Calibration solutions

Prepare a series of mixed flame retardant calibration solutions from the stock standard solution (5.2.2.3) at 1,0 mg/l, 2,0 mg/l, 4,0 mg/l and 8,0 mg/l concentrations in acetonitrile.

5.2.4.2 Determination

Proceed to liquid chromatographic determination using the conditions described in 5.2.3. Inject the calibration solutions (5.2.4.1) and the extract obtained at 8.1.1 of EN 71-10:2005.

5.2.4.3 Identification

For a positive identification, the peak purity factor should achieve a match of at least 85 %.

5.2.5 Calculation of analyte concentration

Determine the concentration of a flame retardant in the acetonitrile extract from a calibration graph produced from the calibration solutions.

Calculate the concentration of a flame retardant in the sample using the following equation:

$$Conc [mg / kg] = \frac{C_{comp, solvent} [mg / l]}{A} \times 10 \quad (1)$$

where

$C_{comp, solvent}$ is the concentration of a flame retardant in acetonitrile extract

A is the mass in grams of the *test portion* taken for analysis (see 8.1.1 of EN 71-10:2005)

5.2.6 Limits and precision

Table 2 – Limits and precision

Component	Action limit mg/kg	RSD % at 5 mg/l (equivalent to 50 mg/kg in sample)	Recovery % at 100 mg/kg from fabric
Pentabromodiphenyl ether (total of 3 isomers)	^a	2,0	103
Octabromodiphenyl ether (total of 4 isomers)	^a	1,2	99
Tri- <i>o</i> -cresyl phosphate	50	2,4	69
Tris(2-chloroethyl) phosphate	50	2,6	102
^a The limit in Directive 2003/11/EC is 0,1 % by mass (1 000 mg/kg)			

Correlation coefficient (*r*): > 0,995

5.2.7 Test report

The test report shall contain the following information:

- description and identification of the product and material tested;
- reference to this European Standard;
- identification of flame retardants in the extract of the *test portion*;
- amount of each flame retardant identified expressed as a concentration (mg/kg) in the *toy material*;
- any deviations from the test procedure specified;
- date of test.

5.3 Colourants

5.3.1 Principle

Colourants are identified and semi-quantified in extracts of *toy materials* by liquid chromatography with diode-array detection (LC-DAD). If a positive identification is obtained, confirmation can be achieved using liquid chromatography with mass spectrometry detection (LC-MS).

5.3.2 Standards, reagents and solvents

NOTE Pure materials for these colourants are not readily available and their composition can vary. A supplier of a suitable colourant is indicated for each analyte.

5.3.2.1 Standards⁵

5.3.2.1.1 Disperse Blue 1, C.I. 64500

e.g. Sigma Aldrich 21 564-3

5.3.2.1.2 Disperse Blue 3, C.I. 61505

e.g. Sigma Aldrich 21 565-1

5.3.2.1.3 Disperse Blue 106

e.g. Fluka 28241

5.3.2.1.4 Disperse Blue 124

e.g. Fluka 21620

5.3.2.1.5 Disperse Yellow 3, C.I. 11855

e.g. Sigma Aldrich 21 568-6

5.3.2.1.6 Disperse Orange 3, C.I. 11005

e.g. Sigma Aldrich 36 479-7

5.3.2.1.7 Disperse Orange 37

e.g. Fluka 21603

5.3.2.1.8 Disperse Red 1, C.I. 11110

e.g. Sigma Aldrich 34 420-6

5.3.2.1.9 Solvent Yellow 1, C.I. 11000

e.g. Sigma Aldrich 18 636-8

5.3.2.1.10 Solvent Yellow 2, C.I. 11020

e.g. Sigma Aldrich 11 449-9

5.3.2.1.11 Solvent Yellow 3, C.I. 11160

e.g. Sigma Aldrich 12 156-8

5.3.2.1.12 Basic Red 9, C.I. 42500

e.g. Sigma Aldrich 21 559-7

⁵ The suppliers of the colourants mentioned in this subclause are examples of suppliers of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN.

5.3.2.1.13 Basic Violet 1, C.I. 42535

e.g. Sigma Aldrich 19 809-9

5.3.2.1.14 Basic Violet 3, C.I.42555

e.g. Sigma Aldrich 86 099-9

5.3.2.1.15 Acid Red 26, C.I.16150

e.g. Sigma Aldrich 19 976-1

5.3.2.1.16 Acid Violet 49, C.I. 42640

e.g. Sigma Aldrich S334294

5.3.2.2 Reagents and solvents**5.3.2.2.1** Tetrabutylammonium hydroxide solution, 40 % in water**5.3.2.2.2** Citric acid**5.3.2.2.3** Ammonium acetate, anhydrous**5.3.2.2.4** Acetonitrile**5.3.2.2.5** Tetrahydrofuran**5.3.2.2.6** Ethanol, absolute**5.3.2.2.7** Ammonium hydroxide, approx. 35 % (V/V)**5.3.2.2.8** Acetic acid, glacial**5.3.2.2.9** Ammonium acetate, 10 mmol/l aqueous solution, pH 3,6

Transfer ($0,77 \pm 0,01$) g ammonium acetate (5.3.2.2.3) into a 1 000-ml volumetric flask, add 980 ml of water, adjust the pH to $3,6 \pm 0,1$ with glacial acetic acid and make up to the mark with water.

5.3.2.2.10 Citrate-buffered tetrabutylammonium hydroxide solution

Transfer ($13,6 \pm 0,1$) g tetrabutylammonium hydroxide solution (5.3.2.2.1) and ($2,8 \pm 0,1$) g citric acid into a 1 000-ml volumetric flask, add 980 ml of water, adjust the pH to $9,0 \pm 0,1$ with ammonium hydroxide (5.3.2.2.7) and make up to the mark with water.

5.3.3 Standard solutions**5.3.3.1 General**

When preparing stock solutions of each colourant, purity values shall be taken into account. Store the stock standard solutions in a refrigerator at $(4 \pm 2) ^\circ\text{C}$.

5.3.3.2 Stock standard solution (50 µg/ml), mix 1

Weigh, to the nearest 0,1 mg, (5 ± 1) mg of each colourant listed below into a 100-ml volumetric flask. Add 50 ml of ethanol (5.3.2.2.6) and mix carefully to dissolve. Place in an ultrasonic bath for 15 min to ensure complete dissolution. Make up to the mark with ethanol.

- Disperse Blue 1
- Disperse Blue 106
- Disperse Blue 124
- Disperse Orange 3
- Disperse Orange 37
- Solvent Yellow 1
- Solvent Yellow 2
- Solvent Yellow 3
- Basic Red 9
- Basic Violet 1
- Basic Violet 3

5.3.3.3 Stock standard solution (50 µg/ml), mix 2

Weigh, to the nearest 0,1 mg, (5 ± 1) mg of each colourant listed below into a 100-ml volumetric flask. Add 50 ml of ethanol and mix carefully to dissolve. Place in an ultrasonic bath for 15 min to ensure complete dissolution. Make up to the mark with ethanol.

- Disperse Blue 3
- Disperse Yellow 3
- Disperse Red 1
- Acid Red 26
- Acid Red 49

5.3.4 Apparatus

5.3.4.1 PTFE membrane filter, 0,45 µm

5.3.4.2 Ultrasonic bath

5.3.4.3 Liquid chromatograph with diode-array detector

The following LC-DAD conditions for colourant analysis have been found to be suitable:

Column: C18, 100 Å, 5 µm, endcapped, (Luna C18(2)⁶, or equivalent), 250 mm x 4,6 mm
 Guard column: 2 x C18, 100 Å, 5 µm, endcapped, (Luna C18(2)⁶, or equivalent)
 Column temperature: 25 °C
 Mobile phase A: Citrate-buffered tetrabutylammonium hydroxide solution (5.3.2.2.10)
 Mobile phase B: Tetrahydrofuran
 Mobile phase C: Acetonitrile
 Gradient: see Table 3
 Run time: 45 min
 Flow rate: 0,8 ml/min
 Injection volume: 5 µl to 50 µl
 Analysis time: 35 min
 Wavelength range: 275 nm to 760 nm
 Resolution factor: 4,8 nm
 Acquisition rate: 1 spectrum/second

Table 3 – Gradient program

Time min	Mobile phase A %	Mobile phase B %	Mobile phase C %
0	80,0	10,0	10,0
2,50	80,0	10,0	10,0
30,0	5,0	48,0	47,0
35,0	5,0	48,0	47,0
45,0	80,0	10,0	10,0

5.3.5 Procedure

5.3.5.1 Calibration solutions

Prepare two series of colourant calibration solutions from the stock standard solutions of mix 1 (5.3.3.2) and mix 2 (5.3.3.3) at 1 mg/l, 2 mg/l, 3 mg/l, 4 mg/l and 5 mg/l concentrations in ethanol.

5.3.5.2 Determination

Proceed to liquid chromatographic determination using the conditions described in 5.3.4.3. Inject the calibration solutions of both mix 1 and mix 2 (5.3.5.1) and the ethanol phase obtained at 8.1.3, 8.2.1, 8.3.1, 8.4.1, 8.5.1, 8.6.1, 8.7.1, 8.8.1 or 8.9.1 of EN 71-10:2005, as appropriate.

5.3.5.3 Identification

For a positive identification, the peak purity factor should achieve a match of at least 85 %.

⁶ Luna C18(2) is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

5.3.6 Calculation of analyte concentration

Determine the concentration of a colourant in the ethanolic extract from a calibration graph produced from the calibration solutions.

Calculate the concentration of a colourant in the sample using the following equation:

$$\text{Conc [mg / kg]} = \frac{C_{\text{comp, solvent}} [\text{mg / l}]}{A} \times D \quad (2)$$

where

$C_{\text{comp, solvent}}$ is the concentration of a colourant in ethanolic extract

A is the mass in grams of the *test portion* taken for analysis (see **8.1.3**, **8.2.1**, **8.3.1**, **8.4.1**, **8.5.1**, **8.6.1**, **8.7.1**, **8.8.1** or **8.9.1** of EN 71-10:2005, as appropriate)

D is the dilution factor; generally 10, but see **8.5** of EN 71-10:2005 for aqueous liquid *toy material* samples.

5.3.7 Limits and precision

Table 4 – Limits and precision

Substance	Action limit mg/kg	RSD % at 5 mg/l (equivalent to 10 mg/kg in a sample)
Disperse Blue 1	10	1,8
Disperse Blue 3	10	4,9
Disperse Blue 106	10	4,4
Disperse Blue 124	10	2,2
Disperse Yellow 3	10	0,3
Disperse Orange 3	10	1,6
Disperse Orange 37	10	2,8
Disperse Red 1	10	1,6
Solvent Yellow 1	10	1,1
Solvent Yellow 2	10	1,1
Solvent Yellow 3	10	1,6
Basic Red 9	10	1,1
Basic Violet 1	10	1,5
Basic Violet 3	10	1,0
Acid Red 26	10	2,1
Acid Violet 49	10	1,4

Correlation coefficient (r): > 0,995

5.3.8 Additional information

5.3.8.1 LC-DAD spectral library

It is advisable to compile a spectral library of all colours listed in **5.3.2.1** using the processing software that is used to operate the liquid chromatograph. Details of retention times and Lambda max of each colourant should be recorded together with peak purity data, if available.

It has been observed that several of the colourants separated into two or more chromatographic peaks. The colourants concerned were C.I. Acid Red 26, C.I. Disperse Blue 3, C.I. Acid Violet 49 and C.I. Basic Violet 1. In an attempt to characterise these peaks, an LC-MS method was developed in tandem with the LC-DAD method. The conditions used for the LC-MS analysis are detailed in Annex C.

5.3.9 Test report

The test report shall contain the following information:

- a) description and identification of the product and material tested;
- b) reference to this European Standard;
- c) identification of colourants in the ethanolic extract of the *test portion*;
- d) amount of each colourant identified expressed as a concentration (mg/kg) in the *toy material*;
- e) whether a confirmation test has been carried out and, if so, the technique used and its result;
- f) any deviations from the test procedure specified;
- g) date of test.

5.4 Primary aromatic amines

5.4.1 Principle

Aromatic amines are determined in extracts of *toy materials* by gas chromatography with mass spectrometry detection (GC-MS) using the external standard method of calibration combined with appropriate internal standards.

5.4.2 Standards, reagents and solvents

5.4.2.1 Standards

5.4.2.1.1 Benzidine

5.4.2.1.2 Aniline

5.4.2.1.3 2-Naphthylamine

5.4.2.1.4 3,3'-Dichlorobenzidine

5.4.2.1.5 3,3'-Dimethoxybenzidine

5.4.2.1.6 3,3'-Dimethylbenzidine

5.4.2.1.7 2-Methoxyaniline

5.4.2.1.8 *o*-Toluidine

5.4.2.1.9 4-Chloroaniline

5.4.2.2 Reagents and solvents

5.4.2.2.1 Acetonitrile

5.4.2.2.2 *tert*-Butyl methyl ether

5.4.2.2.3 *n*-Hexane

5.4.2.2.4 Chromabond XTR (Porous granulated kieselguhr)⁷

5.4.2.3 Stock standard solution (100 mg/l)

Weigh, to the nearest 0,1 mg, (10 ± 1) mg of each aromatic amine (5.4.2.1) into a 100-ml volumetric flask. Add 25 ml of acetonitrile (5.4.2.2.1) and mix carefully to dissolve. Place in an ultrasonic bath for 10 min to ensure complete dissolution. Make up to the mark with acetonitrile.

The stability of the mixed stock standard solution should be checked regularly. It should be stable for up to 6 months when stored in the dark at (4 ± 2) °C.

⁷ Chromabond XTR is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

5.4.3 Apparatus

5.4.3.1 Ultrasonic bath

5.4.3.2 Vortex® shaker

5.4.3.3 Centrifuge

5.4.3.4 Gas chromatograph with mass spectrometer detector

Ensure that the gas chromatograph has been fully cleaned before analysis, as the determination of aromatic amines by this technique is affected by other contaminants. It is recommended that the inlet sleeve is deactivated and an amine-specific column used.

The following GC-MS conditions for primary aromatic amine determination have been found to be suitable:

Injector:
 Mode: Splitless 0,5 min
 Carrier gas: Helium 0,8 ml/min
 Injector temperature: 250 °C
 Injection volume: 2 µl
 Column: 5 % diphenylpolysiloxane / 95 % dimethylpolysiloxane, (RTX-5 Amine⁸, or equivalent), 30 m x 0,25 mm (ID) x 0,25 µm (film thickness)
 Oven program: 60 °C (3 min) – 7 °C/min – 280 °C (4 min) – 10 °C/min – 300 °C (2 min)
 Detector: MSD
 Transfer line temperature: 280 °C
 Detector scan range: 70 m/z to 400 m/z

Quantification ions

Choose the molecular ion as the target ion for each of the aromatic amines followed by two qualifier ions for confirmation.

Table 5 – Target and qualifier ions

Substance	Target ion m/z	Qualifier 1 m/z	Qualifier 2 m/z
o-Toluidine	106	107	77
2-Methoxyaniline	108	123	80
4-Chloroaniline	127	129	92
2-Naphthylamine	143	115	116
Benzidine	184	183	185
Aniline	93	92	94
3,3'-Dimethylbenzidine	212	213	106
3,3'-Dichlorobenzidine	252	254	126
3,3'-Dimethoxybenzidine	244	201	229

⁸ RTX-5 Amine is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

5.4.4 Procedure

5.4.4.1 Calibration solutions

Prepare a series of mixed aromatic amine calibration solutions from the stock standard solution (5.4.2.3) at 1 mg/l, 2,5 mg/l, 5 mg/l, 10 mg/l and 20 mg/l concentrations in *tert*-butyl methyl ether.

The calibration solutions should be freshly prepared on a daily basis.

5.4.4.2 Determination

Proceed to gas chromatographic determination using the conditions described in 5.4.3.4. Inject the calibration solutions (5.4.4.1) and the *tert*-butyl methyl ether phase obtained at 8.1.4, 8.2.2, 8.3.2, 8.4.2, 8.5.2, 8.6.2, 8.7.2, 8.8.2 or 8.9.2 of EN 71-10:2005, as appropriate.

5.4.5 Calculation of analyte concentration

Determine the concentration of a primary aromatic amine in the *tert*-butyl methyl ether extract from a calibration graph produced from the calibration solutions.

Calculate the concentration of a primary aromatic amine in the sample using the following equation:

$$\text{Conc [mg / kg]} = \frac{C_{\text{comp, solvent}} [\text{mg / l}] \times V [\text{ml}]}{A} \quad (3)$$

where:

- $C_{\text{comp, solvent}}$ is the concentration of a primary aromatic amine in *tert*-butyl methyl ether extract;
 V is the volume in ml of *tert*-butyl methyl ether extract;
 A is the mass in grams of the *test portion* taken for analysis (see 8.1.4, 8.2.2, 8.3.2, 8.4.2, 8.5.2, 8.6.2, 8.7.2, 8.8.2 or 8.9.2 of EN 71-10:2005, as appropriate).

5.4.6 Limits and precision

Table 6 – Limits and precision

Substance	Action limit mg/kg	RSD % at 5 mg/l (equivalent to 5 mg/kg in sample)	Recovery % at 2,5 mg/l
<i>o</i> -Toluidine	5	3,7	93
2-Methoxyaniline	5	3,2	95
4-Chloroaniline	5	3,8	87
2-Naphthylamine	5	2,3	84
Benzidine	5	3,2	85
Aniline	5	5,0	102
3,3'-Dimethylbenzidine	5	1,9	82
3,3'-Dichlorobenzidine	5	2,6	81
3,3'-Dimethoxybenzidine	5	3,0	77

Correlation coefficient (r): > 0,995

5.4.7 Additional information

Due to the polar nature of some amines, clean chromatographic conditions are essential. Derivatization of the *toy material* extract using trifluoroacetic anhydride (TFAA) or *N*-methyl-bis(trifluoroacetamide) (MBTFA) may help overcome this problem.

During sample preparation and extraction of a *toy material* for primary aromatic amines, the possibility exists of azo-dyes also being extracted. Such dyes can decompose to aromatic amines during GC analysis. If a result above the action limit for a primary aromatic amine specified in Table 6 is obtained from a *toy material* extract that is coloured, it is necessary to ascertain whether the amine has arisen from the decomposition of an azo-dye. Except for aniline, this could indicate the presence of an azo-dye prohibited from textile and leather toys under European legislation.

NOTE 1 Directive 2002/61/EC of the European Parliament and of the Council refers.

NOTE 2 Primary aromatic amines can also be determined using a suitably validated LC-DAD or LC-MS method.

5.4.8 Test report

The test report shall contain the following information:

- a) description and identification of the product and material tested;
- b) reference to this European Standard;
- c) identification of primary aromatic amines in the *tert*-butyl methyl ether extract of the *test portion*;
- d) amount of each primary aromatic amine identified expressed as a concentration (mg/kg) in the *toy material*;
- e) any deviations from the test procedure specified;
- f) date of test.

5.5 Monomers and solvents

NOTE Monomers and solvents are covered by six methods in this European Standard (5.5.1 to 5.5.6).

5.5.1 Method for acrylamide

5.5.1.1 Principle

Acrylamide is determined in aqueous extracts of *toy materials*, without sample preparation and derivatization, by liquid chromatography with diode-array detection (LC-DAD).

5.5.1.2 Standards

5.5.1.2.1 Acrylamide

5.5.1.2.2 Acrylamide stock standard solution, 1 000 mg/l, in water

5.5.1.3 Apparatus

Liquid chromatograph with diode-array detector

The following LC-DAD conditions for acrylamide analysis have been found to be suitable:

Column: C18, 100 Å; 5 µm, (Nucleosil 100-5 C₁₈⁹, or equivalent), 250 mm x 3 mm
 Column temperature: 25 °C
 Eluent: Deionized water
 Flow: 0,85 ml/min
 Injection volume: 100 µl
 Run time: 10 min
 DAD wavelength: 198 nm

5.5.1.4 Procedure

5.5.1.4.1 Calibration solutions

Prepare a series of acrylamide calibration solutions from the acrylamide stock standard solution (5.5.1.2.2) at 0,02 mg/l, 0,04 mg/l, 0,08 mg/l, 0,2 mg/l and 0,4 mg/l concentrations in water.

5.5.1.4.2 Determination

Transfer a portion of the *aqueous migrate* obtained at 6.4 of EN 71-10:2005 into a 2-ml vial and close with a crimping cap.

Proceed to liquid chromatographic determination using the conditions described in 5.5.1.3. Inject the calibration solutions (5.5.1.4.1) and the *aqueous migrate*.

5.5.1.5 Calculation of analyte concentration

Determine the concentration of acrylamide in the *aqueous migrate* (mg/l) directly from a calibration graph produced from the calibration solutions.

5.5.1.6 Limit and precision

Table 7 – Limit and precision

Substance	Action limit in the <i>aqueous migrate</i> mg/l	RSD % at 0,02 mg/l
Acrylamide	0,02	0,6

— Correlation coefficient (*r*): > 0,995

⁹ Nucleosil 100-5 C₁₈ is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

5.5.1.7 Test report

The test report shall contain the following information:

- a) description and identification of the product and material tested;
- b) reference to this European Standard;
- c) result of the analysis for acrylamide expressed as a concentration (mg/l) in the *aqueous migrate* of the *toy material*;
- d) any deviations from the test procedure specified;
- e) date of test.

5.5.2 Method for phenol and bisphenol A

5.5.2.1 Principle

Phenol and bisphenol A are determined in aqueous extracts of *toy materials* by liquid chromatography with diode-array and fluorescence detection (LC-DAD-FLD).

5.5.2.2 Standards, reagents and solvents

5.5.2.2.1 Standards

5.5.2.2.1.1 Phenol

NOTE Phenol should be colourless or bright yellow. If the colour is pink, the substance should not be used.

5.5.2.2.1.2 Bisphenol A

5.5.2.2.2 Solvents

Methanol

5.5.2.2.3 Standard solutions

5.5.2.2.3.1 Phenol stock standard solution (1 000 mg/l)

Weigh to the nearest mg, (100 ± 10) mg of phenol into a 100-ml volumetric flask. Dissolve and make up to the mark with methanol.

5.5.2.2.3.2 Bisphenol A stock standard solution (1 000 mg/l)

Weigh to the nearest mg, (100 ± 10) mg of bisphenol A into a 100-ml volumetric flask. Dissolve and make up to the mark with methanol.

5.5.2.3 Apparatus

Liquid chromatograph with diode-array and fluorescence detector (FLD)

The following LC-DAD-FLD conditions for phenol and bisphenol A analysis have been found to be suitable:

Column:	C18, 100 Å; 5 µm, (Nucleosil 100-5 C ₁₈ ¹⁰ , or equivalent), 250 mm x 4 mm
Column temperature:	20 °C
Mobile phase:	Methanol : water = 65 : 35; isocratic
Flow:	0,8 ml/min
Injection volume:	40 µl
Detectors:	
For phenol: DAD:	274 nm
For bisphenol A: FLD:	Excitation wavelength, Ex = 275 nm Emission wavelength, Em = 313 nm

NOTE Both detectors are connected in series, the DAD first.

5.5.2.4 Procedure

5.5.2.4.1 Calibration solutions

NOTE Aqueous solutions of phenol and bisphenol A should be stable for a period of up to 3 weeks when stored in the dark at (4 ± 2) °C.

5.5.2.4.1.1 Phenol

Prepare a series of phenol calibration solutions from the phenol stock standard solution (5.5.2.3.1) at 1 mg/l, 3 mg/l, 7,5 mg/l, 15 mg/l and 45 mg/l concentrations in water.

5.5.2.4.1.2 Bisphenol A

Prepare a series of bisphenol A calibration solutions from the bisphenol A stock standard solution (5.5.2.3.2) at 0,01 mg/l, 0,05 mg/l, 0,1 mg/l, 0,2 mg/l and 0,5 mg/l concentrations in water.

5.5.2.4.2 Determination

Transfer a portion of the *aqueous migrate* obtained at **6.4** of EN 71-10:2005 or the extract obtained at **8.2.3**, **8.5.3**, **8.7.3** or **8.9.3** of EN 71-10:2005, as appropriate, to a 2-ml vial and close with a crimping cap.

Proceed to liquid chromatographic determination using the conditions described in **5.5.2.3**. Inject the calibration solutions (5.5.2.4.1) and the *aqueous migrate*.

5.5.2.5 Calculation of analyte concentration

Determine the concentration of phenol and bisphenol A in the *aqueous migrate* (mg/l), or the concentration of phenol (as a preservative) in the extract (mg/l), directly from a calibration graph produced from the calibration solutions.

The mass concentration of phenol (as a preservative) in a sample can be calculated using the following equation:

¹⁰ Nucleosil 100-5 C₁₈ is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.