

EUROPEAN CO-ORDINATION OF NOTIFIED BODIES NB-TOYS	**Co-ordination of the Notified Bodies NB-TOYS under the Safety of Toys Directive**	**NB-TOYS/2021-053 January 2022**

FINAL EC-type approval protocol No. 2 Microbiological safety of toys containing aqueous media REV 4

Agreed by:
NB-Toys group on: 14 September 2021
EC-expert group on Toy safety on: 27 October 2021
ADCO group on Toy Safety on: 27 October
Available at: https://ec.europa.eu/growth/sectors/toys/safety/guidance_en

Changes made in rev2:

Paragraph 2:

- Enterococci Faecalis removed from the table
- Note 1 additional explanations
- Notes 2,3,4 are added

Paragraph 3:

- Clause 3.3 h (enumeration of yeast and mould) has been added.

Paragraph 4:

- Clauses 4.4 (on testing for E.Coli) and 4.5 (on the fact that for cosmetics no tests are available for Salmonella and Enterobacteriaceae) have been removed.

Changes made in rev3:

Paragraph 1:

- Clause 1.3 Finger paints: definition is added, originating from EN 71-7 Safety of toys - Part 7: Finger paints - Requirements and test methods.

Paragraph 3:

- Clause 3.2 c (USP 51 Antimicrobial Effectiveness Testing) has been added.
- Clause 3.3 i (EN ISO 11930 Evaluation of the antimicrobial protection of a cosmetic product) has been added.
- Clause 3.3 Note about EN ISO 18415 Detection of specified and non-specified microorganisms has been added.

Changes made in rev4:

Paragraph 2:
Limits: change of the the parameter “Enterobacteriaceae” to “bile-salts tolerant gram-negative bacteria”

Reason: there are 2 different parameters, Enterobacteriaceae are not renamed to “bile-salts tolerant gram-negative bacteria”, but one parameter was exchanged for another.
The Enterobacteriaceae are part of the group “bile-salts tolerant gram-negative bacteria”, but there are a few more in this group.

• EC-type approval protocol No. 2 Microbiological safety of toys containing aqueous media (rev 3)

Introduction

This protocol intends to specify microbiological requirements for toys specified in the scope (see section 1). Micro-organisms are known to proliferate in the presence of water; therefore the primary hazard is related to aqueous media.

If pathogenic micro-organisms are present in toys they may present a risk of microbial infection. As no specific microbiological requirements for toys exist, the Notified Body toys group has developed a harmonised protocol based on requirements that are applicable for cosmetic products.
Relevant exposure routes for microbiological evaluation are:
- skin contact;
- eye, ears or nose contact (mucous membranes);
- ingestion.

If an infection occurs in the body after an intake of microorganisms, the level of infection depends on different factors:
- minimum infection dose of the germ;
- ability of the immune system to ward off germs;
- pH-value and aW-value of the product;
- matrix into which the germs are embedded: fat and protein form a protective colloid around the germs, so that the stomach-intestine-passage remains mostly unscathed;
- amount of the product which possibly enters the body of a child.

1. Scope

1.1 Aqueous liquid
Aqueous liquid: a water containing liquid/paste in a toy, on a toy or accompanying a toy to which the child is likely to become exposed during normal or foreseeable use of the toy (e.g. liquid paints, bubble liquids, ink in pens, liquid provided with toys for squirting, liquid in teethers and pacifiers).

1.2 Modelling clays based on aqueous formulations
Remark: Some clays are not based on water or do not contain any significant quantity of water. This means they have very low water activity and so are not prone to microbiological attack or breakdown. Oven hardening modelling compounds as well as plasticine might be examples of such materials. Therefore the scope is restricted to modelling clays based on aqueous formulations.

1.3 Finger paints
Aqueous semi-solid or liquid, coloured mixture specially designed for children to apply directly to suitable surfaces with the fingers and hands (EN 71-7:2014).

1.4 Gels and semi-liquids based on aqueous formulations
Remark: Some non-aqueous gels are used inside some toys e.g. in place of sand in a timer or a fully encapsulated “slime”. These are exempted as there would be no microbiological hazard if they leaked. Examples would be high viscosity hydrocarbons.

2. Limits

Limits indicating microbiological safety:

Total aerobic microbial count (TAMC) [1]	≤ 1000 cfu/g or ml
Yeast and mould [2]	≤ 100 cfu/g or ml
Staphylococcus aureus	Absent in 1 ml or g
Pseudomonas aeruginosa	Absent in 1 ml or g
Candida albicans	Absent in 1 ml or g
Escherichia Coli [3]	Absent in 1 ml or g
Salmonella spp.	Absent in 1 ml or g
bile-salts tolerant gram-negative bacteria 4	≤ 100 cfu/g or ml

NOTE: [1] Great care is required in using this measure because it is not a good indicator of the risk posed. For example many categories of food such as fresh fruit and vegetables, cooked meats, sandwiches (especially with salad fillings), cream cakes, pastries, cheesecake etc all are considered acceptable to eat if they have a TAMC of 10^5 to 10^7 (10 E5 to 10 E7) c.f.u per gram, especially as testing is performed on finished products and not on raw materials. A TAMC value over 1000 cfu/g or ml shall not necessarily be regarded as a non-compliance and should be reviewed on a case by case basis taking into account the nature of the material and the way it is used in a toy. It is advised to retest values over 1000 cfu/g or ml after storage for 7 days at room temperature. If the second test is decreasing (at least 1 log) with reference to the first test result, the TAMC value can be considered as immaterial. Samples with TAMC over 100 000 cfu/g or ml should be regarded as non-compliant in general.

[2] Guidelines should not be taken alone to prove that the product is unsafe or non-compliant. Further work may be necessary to establish whether sufficient preservation was used and its effectiveness'

[3] E. Coli is used as an indicator of potential contamination by pathogenic bile-salts tolerant gram-negative bacteria

[4] As pathogens other than Escherichia Coli and Salmonella spp are not specified, the limit for bile-salts tolerant gram-negative bacteria is introduced to cover for other pathogens.

3. Test procedures

The following methods can be used:

3.1 Test for microbial contamination European Pharmacopeia EP

a) European Pharmacopeia, ("microbiological examination of non-sterile products") Chapter 2.6.12 (1)
b) European Pharmacopeia, ("microbiological examination of non-sterile products") Chapter 2.6.13 (1)
c) European Pharmacopeia, ("efficacy of antimicrobial preservation") Chapter 5.1.3 (1)

3.2 United States Pharmacopeia USP

a) USP "Microbiological examination of non-sterile products: Microbial enumeration tests", USP 31, chapter 61, latest edition
b) USP "Microbiological examination of non-sterile products: Tests for specific microorganisms", USP 31, chapter 62, latest edition
c) USP 51 Antimicrobial Effectiveness Testing

3.3 The European methods for the microbiological testing of cosmetics

a) EN ISO 18416 Detection of *Candida albicans* (ISO 18416)
b) EN ISO 21148 General instruction for microbiological examination (ISO 21148)
c) EN ISO 21149 Enumeration and detection of aerobic mesophilic bacteria (ISO 21149)
d) EN ISO 21150 Detection of *Escherichia coli* (ISO 21150)
e) EN ISO 22716 Guidelines on Good Manufacturing Practices (ISO 22716)
f) EN ISO 22717 Detection of *Pseudomonas aeruginosa* (ISO 22717)
g) EN ISO 22718 Detection of *Staphylococcus aureus* (ISO 22718)
h) EN ISO 16212 Enumeration of yeast and moulds (ISO 16212)
i) EN ISO 11930 Evaluation of the antimicrobial protection of a cosmetic product

Note: EN ISO 18415 Detection of specified and non-specified microorganisms can be used as an alternative to the standards *EN ISO 18416, EN ISO 21150, EN ISO 22717 and EN ISO 22718.*

3.4 The European methods for the microbiological testing of water and foods

4. General remarks

4.1 If the specification is applied to raw materials then failures could occur which do not actually represent a real risk in the final toy because in the final toy other ingredients may act as biocides or preservatives. So it is inappropriate to expect that the technical dossier should contain microbiological test data for raw materials.

4.2 The limits mentioned in the table are the limits using the European test methods. In case the test method from the United States is used, the test results have to be converted to the European test methods.

4.3 This specification/protocol is inappropriate to apply to products that are consumer complaint returns because there is no way to establish what adverse treatments may have been given to the toy before being returned as a complaint.