OSTTHÜRINGISCHE
MATERIALPRÜFGESELLSCHAFT
für Textil und Kunststoffe mbH
Breitscheidstraße 97
07407 Rudolstadt

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GBneuhaus GmbH
Am Herrnberg 10
98724 Neuhaus am Rennweg

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25.07.2017		4 . 5 / B 4 9 / 2 0 1 7	03672 379-521	07.08.2017

TEST REPORT

1 General

Test report - No.:	4 . 5 / B 4 9 / 2 0 1 7 - E N	
Commissioned by:	GBneuhaus GmbH, Mr Bauer	
Objects tested:		**internal number**
Sample 1	Borofloat Referenz (unbeschichtet)	3188
Sample 2	Borofloat mit H2073	3189
Sample 3	Borofloat mit H2081	3190
Sampling:	by customer	
Test:	Biological evaluation of medical devices – Part 5: Test for *in vitro* cytotoxicity (DIN EN ISO 10993-5:2009)	
Date received:	07.08.2017	
Test period:	14. – 16.08.2017	
Processed by:	Ms Mantke, Ms Bauer	
Subcontractors:	none	
Test procedure:	1) DIN EN ISO 10993-5:2009; Deutsche Fassung (Extraktverfahren)/ SOP_4.5.503Bio	
Remarks:	The English version of the test report was issued on January 15th, 2021.	
Pages:	7	
Report copies:	1 copy for client 1 copy for OMPG	

The tests were carried out between the date of receipt and the date of the report. Results of measurements and analyses refer only to the tested samples. This test report is legally valid with the signature of the head of laboratory or his / her representative only. Copies must be done completely. Copies, even in extracts, require the written permission of OMPG ltd.

2 Sample items

Sample No.	Designation acc. customer	Description (material, shape, colour, purpose)
3188	Borofloat Referenz (unbeschichtet)	Glass plate
3189	Borofloat mit H2073	Glass plate
3190	Borofloat mit H2081	Glass plate

3 Test method description

In DIN EN ISO 10993 Part 5, the extraction test method is used to assess the in vitro cytotoxicity of toxic substances that can be extracted from products. The determination of the cell-damaging effects (in-vitro-cytotoxicity) is based on the examination of the cell growth and the cell damage after contact with the test material. The extract is produced in accordance with DIN EN ISO 10993-12. The test material extracts are brought into contact with a cell line over a defined period in accordance with the specifications of DIN EN ISO 10993-5. The cell growth is then determined using a suitable proliferation assay. The assessment of the samples is based on the calculation of the growth of the cells with sample extract in comparison to the control.
The extract method with cell culture medium was chosen for the samples, as both polar and non-polar substances are extracted from the material and thus all soluble components that could cause a toxic effect are recorded.

The investigation for in vitro cytotoxic properties of sample extracts was carried out using the murine fibroblast cell line L-929 as follows:

1. Preparation of the sample extracts:
   - Transferring the sample material into **Petri dishes**
   - **Steam sterilization of the samples for 15 min at 121 ° C**
   - Addition of 1 ml cell culture medium **per 3 cm^2** to the samples **(19 ml per sample = 56 cm^2)**
   - **Seal the Petri dishes with Parafilm**
   - Extraction for 24 h at 37 °C and **70** rpm

2. Pre-incubation of the cells
   - permanent cultivation of the fibroblast cell line L-929 in 75 cm^2-culture flasks at 37 °C and 5 % CO_2
   - Release the cells for passage (after 48 h) with trypsin
   - Adjust the cell density with cell culture medium to $1 \cdot 10^5$ cells/ml
   - Transferring 100 µl of the cell suspension into the wells of a 96-well microtiter plate
   - Incubation of cells for 24 h at 37 °C and 5 % CO_2

3. Incubation with sample extracts
   - Dilution of the extracts with cell culture medium in a 2-fold dilution series
   - Removal of the cell culture medium from the cells
   - Addition of 100 µl of the corresponding extract dilutions in triplicate to the cells
   - Incubation of cells for 24 h at 37 °C and 5 % CO_2

4. Morphological evaluation
   - microscopic examination to assess cell changes with regard to vacuolation, detachment, cell dissolution and cell membrane integrity

5. Measurement of cell vitality
   - Addition of 50 µl XTT reagent (AppliChem GmbH)
   - Incubation for 4 h at 37 °C and 5 % CO_2
   - Measurement of the optical density at 630 nm and 450 nm

Calculation of cell vitality:

$$Cell\ vitality = \frac{OD_{450\ sample} - OD_{630\ sample}}{OD_{450\ BL} - OD_{630\ BL}} * 100\ \%$$

Cell vitality	proportion of living cells compared to the blank
$OD_{450\ sample}$	XTT-signal of cells incubated with sample extracts at 450 nm (optical density)
$OD_{630\ sample}$	XTT-signal of cells incubated with sample extracts at 630 nm (optical density)
$OD_{450\ BL}$	XTT-signal of cells incubated with blank at 450 nm (optical density)
$OD_{630\ BL}$	XTT-signal of cells incubated with blank at 630 nm (optical density)

Assessment criteria:

Cell vitality (XTT-Assay)

Growth	Evaluation
100% - 70%	not cytotoxic
< 70%	cytotoxic

Morphological evaluation:

Scale	Reactivity	Condition of cell culture
0	no	discrete intracytoplasmic granules, no cell dissolution, no reduction in cell growth
1	low	not more than 20 % of the cells are round, loosely adhesive and without intracytoplasmic granules or show changes in the morphology, in some cases dissolved cells are present, only slight growth inhibition is noticeable
2	light	not more than 50 % of the cells are round, free of intracytoplasmic granules, no extensive cell dissolution, no more than 50% growth inhibition noticeable
3	moderate	not more than 70 % of the cell layers contain round cells or are dissolved, cell layers are not completely destroyed, but more than 50% growth inhibition is noticeable
4	strong	almost complete or complete destruction of cell layers

Material and test conductions:

Samples:	BL	Blank control: Cells with culture medium
	PC	Positive control: 2 mg/ml SDS
	NC	Negative control: PP Bormed Slide
	3188	Borofloat Referenz (unbeschichtet)
	3189	Borofloat mit H2073
	3190	Borofloat mit H2081
Sample preparation:	Transfer **56 cm²** of the samples into **Petri dishes**.	
Extraction conditions:	24 h at 37 °C and **70** rpm in DMEM F12 + 5 % FCS + Antibiotica (Batch numbers are documented internally)	
Test cells:	**murine fibroblast cell line L-929** (ATCC No. CCL1, NCTC Clone 929L; CLS Cell Line Service GmbH; the cell line is listed in DIN EN ISO 10993-5 as a suitable cell line and has been used for *in vitro* tests for many years)	
Cell culture medium:	**DMEM F12 + 5 % FCS (fetal calf serum)** (DMEM F12, FCS: Biowest, Batch numbers are documented internally)	
Incubation time of the cells with the extracts:	24 h at 37 °C and 5 % CO_2	
Sample volume / well:	100 µl	
Detection reagent:	XTT (AppliChem GmbH)	

4 Results

The results are shown in Table 1 and in Figure 1.

Table 1: Growth of the murine fibroblast cell line L-929 after incubation with the sample extracts for 24 hours. The calculation was made in % of the blank sample.

Extrakt-Konz.	Zellvitalität	STABW	Bewertung	Morphologie-Beurteilung
BL				
100 %	100,0 ±	5,8 %	not cytotoxic	0 no reactivity
NK				
100 %	96,6 ±	11,3 %	not cytotoxic	0 no reactivity
50 %	102,6 ±	2,1 %	not cytotoxic	0 no reactivity
25 %	100,9 ±	5,0 %	not cytotoxic	0 no reactivity
12,5 %	100,2 ±	3,8 %	not cytotoxic	0 no reactivity
PK				
100 %	6,7 ±	0,2 %	cytotoxic	4 strong reactivity
50 %	7,0 ±	0,1 %	cytotoxic	4 strong reactivity
25 %	7,1 ±	0,1 %	cytotoxic	4 strong reactivity
12,5 %	7,4 ±	0,1 %	cytotoxic	4 strong reactivity
Sample: P1	**3188**	**Borofloat Referenz (unbeschichtet)**		
100 %	97,0 ±	1,8 %	not cytotoxic	0 no reactivity
50 %	104,6 ±	2,2 %	not cytotoxic	0 no reactivity
25 %	100,4 ±	1,8 %	not cytotoxic	0 no reactivity
12,5 %	102,4 ±	1,4 %	not cytotoxic	0 no reactivity
Sample: P2	**3189**	**Borofloat mit H2073**		
100 %	88,9 ±	5,6 %	not cytotoxic	0 no reactivity
50 %	97,7 ±	4,7 %	not cytotoxic	0 no reactivity
25 %	99,4 ±	4,3 %	not cytotoxic	0 no reactivity
12,5 %	86,9 ±	5,2 %	not cytotoxic	0 no reactivity
Sample: P3	**3190**	**Borofloat mit H2081**		
100 %	93,1 ±	0,5 %	not cytotoxic	1 low reactivity
50 %	94,6 ±	5,3 %	not cytotoxic	0 no reactivity
25 %	94,3 ±	7,6 %	not cytotoxic	0 no reactivity
12,5 %	93,6 ±	5,1 %	not cytotoxic	0 no reactivity

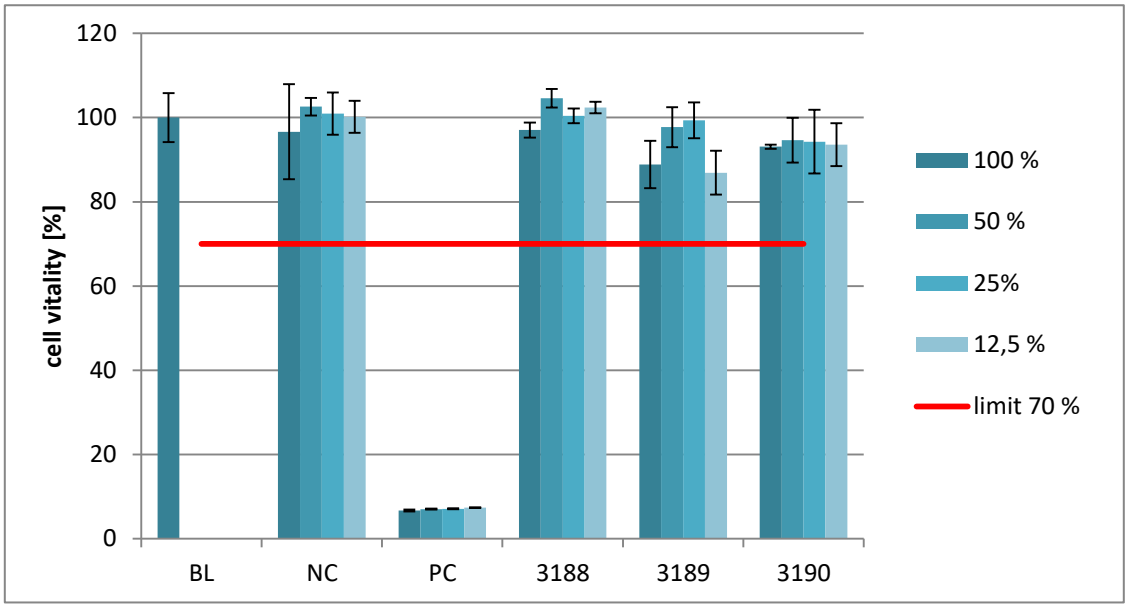

Figure 1: Influence of the sample extracts on the growth of the murine fibroblast cell line L 929. The metabolic activity of the cells was determined with the aid of the XTT proliferation assay. The cell growth is shown as a percentage of the blank sample. BL: blank sample; NC: negative control; PC: positive control.

5 Evaluation

XTT proliferation assay

The samples „Borofloat Referenz (unbeschichtet)“ [3188], „Borofloat mit H2073“ [3189] and „Borofloat mit H2081“ [3190] are not cytotoxic to the mouse fibroblast cell line L-929.

Morphological evaluation

Microscopic examination of the cells confirmed these results. During an incubation of the fibroblasts with the undiluted extract of the samples „Borofloat Referenz (unbeschichtet)“ [3188], „Borofloat mit H2073“ [3189] and „Borofloat mit H2081“ [3190], no morphologically visible cell damage or growth reductions were detectable.

A final assessment of the undiluted sample extracts is summarized in Table 2.

Table 2: Evaluation of the in vitro cytotoxicity of the 100% sample extracts on the murine fibroblast cell line L-929.

Sample		**Designation**	**Evaluation**
BL		Blank control: cells with culture medium	not cytotoxic
PC		Positive control: 2 mg/ml SDS	cytotoxic
NC		Negative control: PP Bormed	not cytotoxic
P1	3188	Borofloat Referenz (unbeschichtet)	not cytotoxic
P2	3189	Borofloat mit H2073	not cytotoxic
P3	3190	Borofloat mit H2081	not cytotoxic

Dr. J. Bauer
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Dr. J. Bauer
Head of the Biology Laboratory
Plastics Research Department