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APPLICATION NOTE No. 488

# Biocompatibility Studies on CHO Cell Culture in X-ray Irradiated BioBLU® Single-Use Bioreactors according to the DECHEMA Recommendation for Leachables Studies

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## Abstract

The potential leakage of toxic or inhibitory chemicals from plastic materials from single-use bioreactors is increasingly known within cell culture. Leachables are particularly problematic, as they can migrate from the plastic into the process fluid under process conditions and can inhibit cell growth or affect the final product quality. In 2016, the Eppendorf Application Note 308 demonstrated the absence of negative impacts caused by leachable from the E-beam sterilized BioBLU® Single-Use Bioreactor material on cell parameters. Due to the recent change of vessel sterilization method to X-ray irradiation, a new leachable study based also on the standardized cell-based assay published by the DECHEMA® Society for Chemical Engineering and Biotechnology was needed to prove the safe use of these vessels in the mammalian cell culture model. This test is based on a genetically modified model CHO cell line and provides a basis for comparison of different plastic materials as well as general recommendations. By strictly adopting the "DECHEMA Recommendation for Leachables Studies - Standardized cell culture test for the early identification of critical films", this new Application Note demonstrates the absence of negative impacts caused by leachable compounds from the X-ray irradiated material on growth, viability, and metabolic profile of the CHO cell line tested.

## Introduction

BioBLU Single-Use Bioreactors are widely used in biopharmaceutical research and development as well as the manufacture of bioproducts. Although they allow for shortening the time of production and reducing the risk of contamination, a potential problem of a single-use system is the release of chemical compounds into the culture medium which is in contact with the cell suspension. In 2016, a leachable study based on a standardized cell culture test developed by the DECHEMA working group [1] was performed on mammalian cells cultured in E-beam sterilized BioBLU Single-Use Bioreactors. This study was conducted to test whether leachable compounds could affect cell culture condition and performance. It demonstrated the absence of negative impacts caused by leachable compounds from the BioBLU Single-Use Bioreactor material on growth, viability, and metabolic profile of two cell lines tested (CHO and Vero cells). The results obtained were described in the Eppendorf Application Note 308 [2].

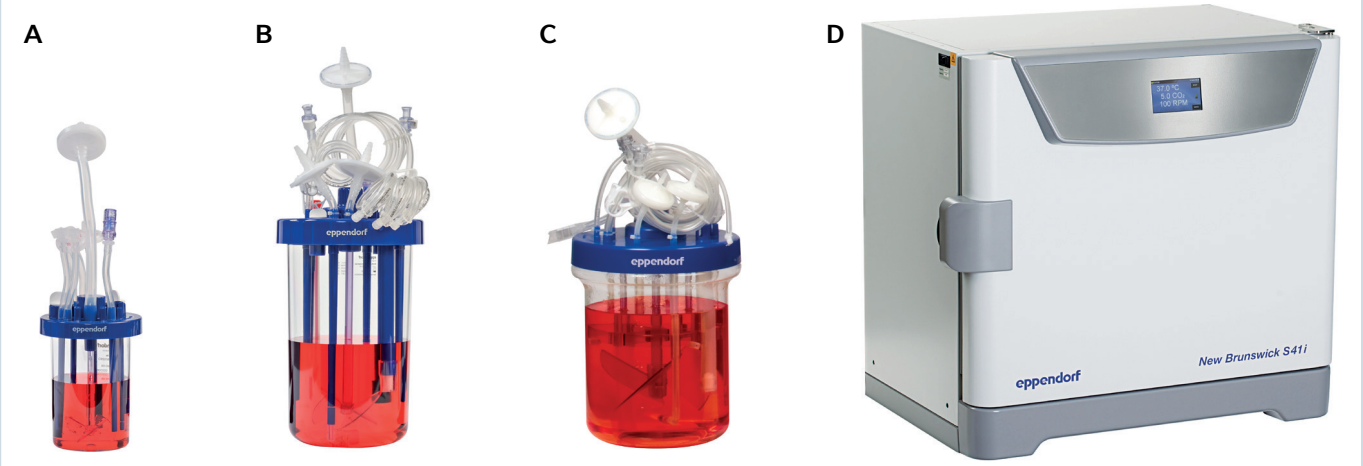


**Figure** 1: For this leachable studies three different BioBLU Single-Use Bioreactor models were employed, namely the BioBLU **(A)** 0.3c, **(B)** 3c, and **(C)** 5c. The extraction procedure was carried out in a **(D)** S41i $CO_2$ incubator shaker.

Learn more about the BioBLU Single-Use Bioreactors at: www.eppendorf.group/biobluc

As a result of the recent change in the methodology of sterilization of the BioBLU Single-Use Bioreactors from E-Beam sterilization to X-ray irradiation, generation of new leachable data based on the same DECHEMA guideline recommendations but performed with X-ray irradiated BioBLU Single-Use Bioreactors were necessary.

This Application note has evaluated the potential impact of leachable molecules on a CHO XM 111-10 cell culture. For that, the cells were cultured in medium previously incubated in one of three BioBLU Single-Use Bioreactor models (BioBLU 0.3c, 3c, or 5c) or prepared from water for injection (WFI) previously incubated in the BioBLU Single-Use Bioreactors. Results demonstrate the absence of negative impacts caused by leachable from the X-ray irradiated material on the growth, viability, and metabolism of the tested CHO XM 111-10 cell line.

## Material and Methods

### CHO cell culture

Following the supplier recommendations and after being thawed, cells were transferred to a 75 $cm^2$ T-flask with FMX-8 medium (Cell Culture Technologies LLC). The maintenance culture was kept in a T75 flask using FMX-8 medium at 37°C and 5 % $CO_2$ during three passages. At the fourth passage, the expanded culture was transferred to a sterile Erlenmeyer cell culture flask (Corning®) and was supplemented with an equal volume of fresh ChoMaster® HP-1 medium (Cell Culture Technologies). The cells were incubated at 37°C and 5 % $CO_2$ for two to three hours. After decantation, the supernatant medium was removed, and 50 mL of fresh ChoMaster HP-1 medium were added.

### Extraction

To extract potential leachables, the DECHEMA guideline recommends two procedures of extraction: one performed with cell culture medium and one with highly purified water for injection (WFI) which was used afterwards for the preparation of ChoMaster HP-1 medium. According to these guidelines, BioBLU Single-Use Bioreactors were filled either with fresh cell culture medium or WFI. The volume added

was calculated to obtain the surface-to-volume ratio (S/V) recommended by DECHEMA (between 0.5 and 2 $cm^2$/mL). As a negative control, the cell culture medium or the WFI was incubated under the same conditions in a polycarbonate shake-flask. BioBLU Single-Use Bioreactors and control were maintained for three days in a dark, $CO_2$-free environment at a temperature of 37°C without agitation in a S41i Incubator Shaker. Each extraction was performed in triplicates.

**Toxicity study**

The various extracts (samples and negative control) previously generated were first transferred into shake flasks. Flasks were then inoculated with 0.25 × $10^6$ CHO XM 111-10 cells/mL with the medium supplemented with PLURONIC® F-68 0.2 % (Thermo Fisher Scientific®) and tetracyclin 2.5 mg/L (Merck®). Then, the cells were cultured for five days at 37°C, 125 rpm, and 7.5 % $CO_2$. A sample from each culture was taken every 24 hours to determine the cell number and viability by using the Vi-CELL® XR Cell Viability Analyzer (Beckman Coulter®). Additionally, the cell metabolism was established by measuring the concentrations of glucose, glutamine, lactate (Ankersmid) using the 2900 Biochemistry Analyzer (YSI®), and ammonium ($NH_3$) by an Enzymatic UV 340 nm test.

## Results and Discussion

**Toxicity study with X-ray irradiated BioBLU Single-Use Bioreactors**

To verify the absence of cytotoxicity induced by molecules leaching from the BioBLU Single-Use Bioreactors sterilized with the new sterilization method, three models of X-ray irradiated bioreactors were used for the leachable study: BioBLU 0.3c, 3c, and 5c. To evaluate the cytotoxicity of potential leachables, three criteria were regularly measured during the culture period: cell viability, cell number, and cell metabolism. Results showed in Figure 2 demonstrate that during the entire culture time the viability of CHO XM 111-10 cells was comparable, regardless of the

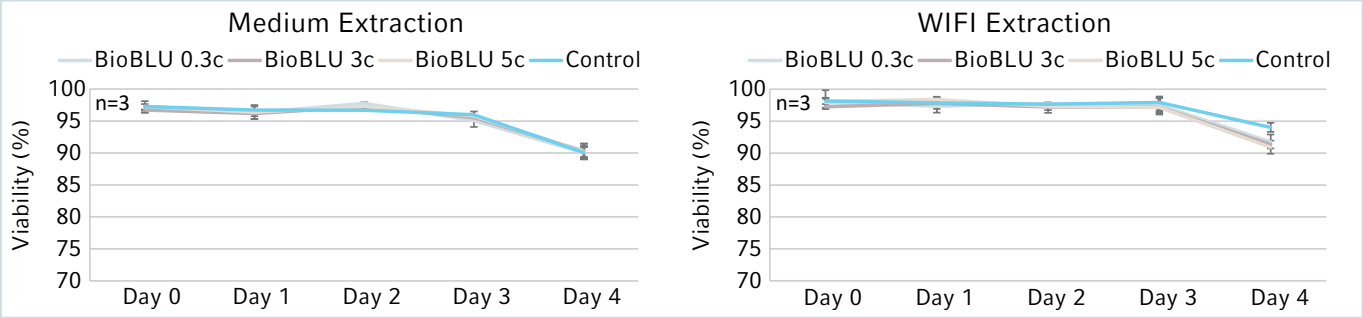


**Figure 2**: Cell Viability – CHO XM 111-10 cells were cultivated in extraction liquids from X-ray irradiated BioBLU Single-Use Bioreactors or in control medium from polycarbonate shake flasks (n = 3).

culture medium (extraction medium generated from X-ray irradiated BioBLU Single-Use Bioreactors or control medium incubated in parallel). In the experiment based on the WFI extraction, a slight deviation between the control and the three bioreactor models was only observed on the last day of culture (Day 4).

As indicated in Figure 3, the cell number measurement correlates with the cell viability result. In the medium

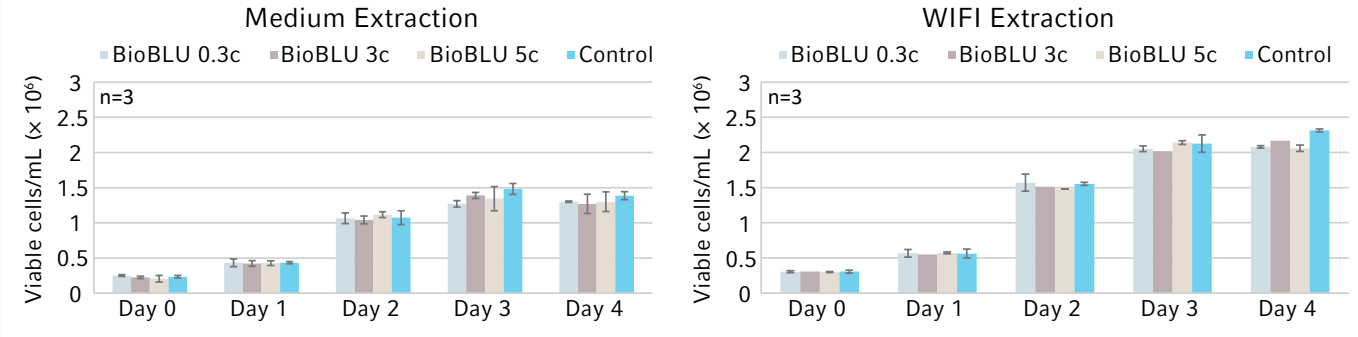


**Figure 3**: Viable cell density (cells/mL) – CHO XM 111-10 cells were cultivated in extraction liquids from X-ray irradiated BioBLU Single-Use Bioreactors or in control medium from polycarbonate shake flasks (n = 3).

extraction experiment, the cell number obtained is comparable between the control and the three bioreactor models tested for the whole culture period. Cell growth studies performed after the WFI extraction on the other hand exhibited a slight deviation between tests and control, but only on day 4.

Monitoring of substrate and metabolite concentrations during the 5-day cell culture period allowed comparisons

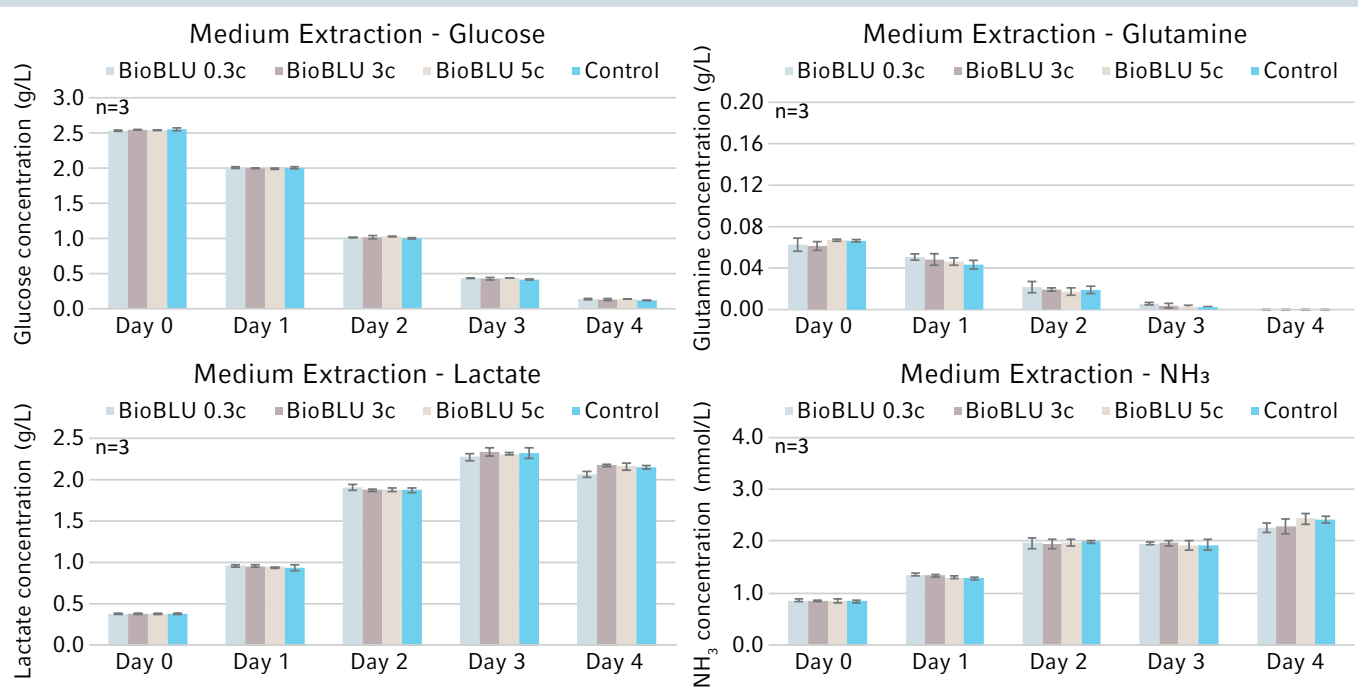


**Figure 4**: Cell metabolism in medium extraction liquid – CHO XM 111-10 cells were cultivated in medium extraction liquids from X-ray irradiated BioBLU Single-Use Bioreactorsor in control medium from polycarbonate shake flasks (n = 3).

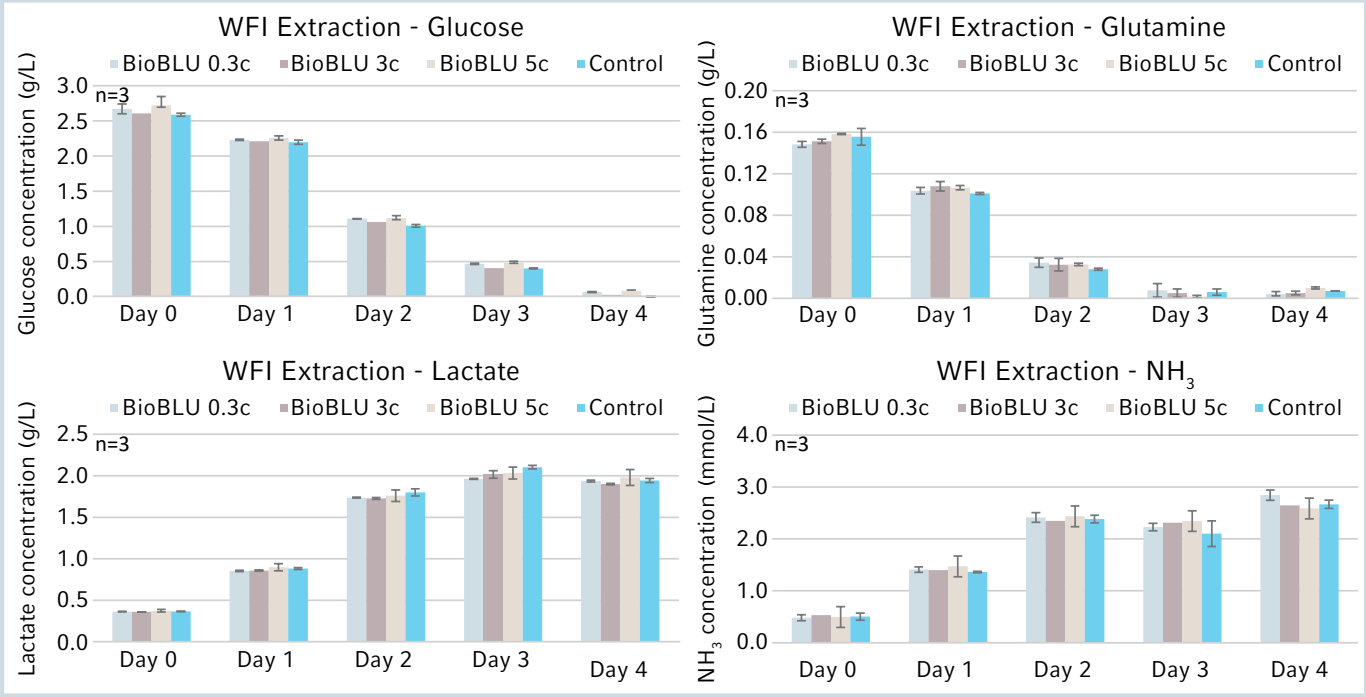


**Figure 5**: Cell metabolism in WFI extraction liquid – CHO XM 111-10 cells were cultivated in WFI extraction liquids from X-ray irradiated BioBLU Single-Use Bioreactorsor in control medium from polycarbonate shake flasks (n = 3).

between the different culture mediums in terms of cellular metabolic activity. As shown in Figure 4 and 5, the metabolic activity of CHO XM 111-10 cells cultured in different extraction liquids is comparable for all X-ray irradiated bioreactor models and the control, as indicated by similar profiles of glucose, glutamine, lactate and $NH_3$.

To evaluate the results of the experiments, it is recommended to normalize data with the reference at 100 %. Therefore, the results for cell density, viability, substrate and metabolite concentrations are expressed as percentages compared to the reference data.

**Table 1:** Matrix to evaluate the potential impact of molecules leaching from three different BioBLU Single-Use Bioreactor models (BioBLU 0.3c, 3c, and 5c). Material can be considered as "non-critical" with regard to leachables if at least two of the three criteria can be designated as "green" (criterion met). % : negative percental deviation from control | + : devitaion from control < 10% | - : devitaion from control ≥ 10%

| | Max. cell density | | Viability | | Metabolic activity | | Criterion | | | Result |
|---|---|---|---|---|---|---|---|---|---|---|
| | Medium extraction | WFI extraction | Medium extraction | WFI extraction | Medium extraction | WFI extraction | 1 | 2 | 3 | |
| **BioBLU 0.3c** | + | 10% | + | + | + | + | | | | Non-critical |
| **BioBLU 3c** | + | + | + | + | + | + | | | | Non-critical |
| **BioBLU 5c** | + | 10% | + | + | + | + | | | | Non-critical |

criterion met criterion not met

Moreover, results obtained with both extraction procedures have to be considered together. As shown in the evaluation matrix presented in Table 1, the final status attributed to the material evaluated is based on the evaluation of three criteria.

The first criterion is met (meaning that the cytotoxicity due to leachables is considered negligible) if there is not ≥ 10% deviation from the control in more than one of the extraction methods. In this study, only one deviation equal to 10% was observed on day 4 with water used as extraction liquid in the BioBLU Single-Use Bioreactors 0.3c and 5c. Therefore, the first criterion is consequently met for the three bioreactor models tested.

To meet the second criterion of negligible cytotoxicity, a deviation of ≥10 % in terms of the cell density can only occur for the water extraction but not the medium extraction method. In this study, this case was never observed, hence the second criterion is met for all bioreactor models.

Finally, the third criterion is met if there are no deviations ≥ 10 % in more than two criteria observed, with either medium or WFI extraction. In this study, this case was never observed, hence the third criterion is met for all bioreactor models.

As a material is considered as "non-critical" if at least two of the three criteria are fullfilled, all X-ray irradiated BioBLU Single-Use bioreactor models tested in this study can consequently be classified as "non-critical".

## Conclusion

The leachable studies in this application note were performed in compliance with the DECHEMA guideline. The potential impact of molecules leaching from the three models of X-ray irradiated BioBLU Single-Use Bioreactors (BioBLU 0.3c, 3c, and 5c) was evaluated based on CHO XM 111-10 cell culture performed in cell culture medium or WFI previously incubated in the BioBLU Single-Use Bioreactors. The result classify these bioreactors as non-critical in terms of leachable substances, as demonstrate by the absence of negative impact from the X-ray irradiated material on growth, viability, and metabolic activity of the tested CHO XM 111-10 cell line.

## Literature

[1] DECHEMA Expert Group. Recommendation for Leachables Studies - Standardized cell culture test for the early identification of critical films. http://dechema.de/en/papers-path-1,123212.html. https://dechema.de/dechema_media/Downloads/Positionspapiere/SingleUse_Empfehlung_Leachables_2014-called_by-dechema-original_page-124930-original_site-dechema_eV-view_image-1.pdf. Published online (2014).

[2] Becken U, Sha M. Leachable Studies on Mammalian Cell Culture in BioBLU® Single-Use Vessels. Eppendorf Application Note 308; https://www.eppendorf.com/product-media/doc/en/193413/Fermentors-Bioreactors_Application-Note_308_BioBLU-c-Single-Vessels_Leachable-Studies-Mammalian-Cell-Culture-BioBLU-Single-Vessels.pdf

**Ordering information**

| Description | Order no. |
|---|---|
| **BioBLU® 0.3c Single-Use Bioreactor**, cell culture, open pipe, 1 pitched-blade impeller, no pH, sterile, 4 pieces | 1386 100 200 |
| **BioBLU® 3c Single-Use Bioreactor**, cell culture, microsparger, 2 pitched-blade impellers, optical pH, sterile, 1 piece | 1386 120 000 |
| **BioBLU® 5c Single-Use Bioreactor**, cell culture, macrosparger, 1 pitched-blade impeller, optical pH, sterile, 1 piece | M1363-0121 |
| **New Brunswick S41i Incubator Shaker** | Inquire* |

*Inquire the part number for your country

**Your local distributor: www.eppendorf.com/contact**
Eppendorf SE · Barkhausenweg 1 · 22339 Hamburg · Germany
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