APPLICATION NOTE No. 469

Second Generation Material – First Class Performance: Comparison of Safety Parameters and Leaching Levels of Eppendorf Tubes® BioBased and Eppendorf Tubes® Standard

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Abstract

One of the most important and still rare approaches to improve sustainable properties of plastics in the lab is to produce those using recycled and renewable feedstocks. Eppendorf for the first time offers a generation of Eppendorf Tubes® made of ISCC PLUS certified polypropylene based on recycled and renewable feedstocks: Here, the key safety parameters and leachable levels of Eppendorf Tubes BioBased 50 mL were tested under array of strict and application-relevant conditions. The Eppendorf Tubes BioBased showed exactly the same performance as Eppendorf Tubes made of standard polypropylene (fossil-based), both with respect to lid tightness performance and centrifugation stability.
The comparative leachables analysis revealed similarly low levels of UV-active substances migrating into samples incubated both in Eppendorf Tubes Standard and Bio-based. Leaching levels observed for Tubes of other manufacturers were considerably higher.
Overall, the comparative evaluation of the Eppendorf Tubes BioBased 50 mL demonstrates their high safety performance and minimal levels of leachables virtually identical to comparable standard variants. This confirms that the bio-based material regarding its physical and chemical properties may be regarded equal to fossil-based polypropylene. This offers a major improvement of renewable properties of lab plastics, making them more sustainable without compromising product quality and performance.

Introduction

Plastic laboratory consumables are indispensable in laboratories around the world, providing quality standards needed in progressively demanding research. Though versatile and necessary, lab plastics pose increasingly growing challenge in respect to sustainability. One of the key, and until now unfortunately still rare approaches to enhance sustainable

properties of plastics in the lab is to produce those using recycled and renewable feedstocks.
Eppendorf not only focuses on the development of new products but also on new, more environmentally friendly manufacturing materials. For the first time, Eppendorf is in a position to offer a generation of Eppendorf Tubes with screw caps in 5.0 mL, 15 mL, 25 mL and 50 mL formats made from 90% »bio-circular« renewable-based feedstock (recycled e.g., from food oil wastes and residues) plus 10% fossil-based feedstock (applying ISCC mass balance approach)* [1, 2].
Conical tubes with screw cap belong to the most commonly used laboratory vessels and have to withstand a great variety of conditions often regarded as extreme: temperatures between -86 °C and 100 °C, high centrifugal forces, aggressive chemicals or solvents and many others. Under such broad range of conditions, the tube must remain stable and the screw cap tightly sealed ensuring protection of samples, user and equipment. Furthermore, as recent scientific evidence suggests, plastic consumables can release various substances added during their production process and hamper many experimental procedures [3]. Absence or very low levels of such substances in laboratory tubes may therefore be of pivotal importance for most applications and increase data quality and process safety in general.
In this Application Note we investigated the performance of Eppendorf Tubes BioBased made of ISCC PLUS certified bio-based material and compared key safety parameters (centrifugation stability and lid tightness) with standard, fossil-based polypropylene Eppendorf Tubes. In addition, leaching levels were investigated and compared with conical tubes of main competitors.

* The screw caps are actual fossil-based material. The material switch will be made to bio-based in 2023.

Materials and Methods

Materials

The following polypropylene conical tubes 50 mL were evaluated:
> Eppendorf Tubes® BioBased 50 mL, Sterile, (Order no. 0030 122 542)
> Eppendorf Tubes® 50 mL, Sterile, (Order no. 0030 122 178)

For leaching test apart from Eppendorf Tubes BioBased and Standard 50 mL, also following tubes from other manufacturers were evaluated:
> Manufacturer Fi, Conical Centrifuge Tube 50 mL, Sterile, Polypropylene
> Manufacturer Fa, Conical Centrifuge Tube 50 mL, Sterile, Polypropylene
> Manufacturer Co, Conical Centrifuge Tube 50 mL, Sterile, Polypropylene

Methods

Lid tightness, centrifugation stability

Following Eppendorf Core Test Lab SOPs were applied to evaluate performance of the bio-based material of Eppendorf Tubes BioBased 50 mL material in comparison to the fossil-based material of Eppendorf Conical Tubes 50 mL. Details on equipment and reagents used in the SOPs are available on request.

SOP Title	SOP N°	Version N°
Screw Cap Tubes Testing – Tightness at high temperature	SOP-CTL242a	0.2.
Screw Cap Tubes Testing – Tightness at low temperature	SOP-CTL242b	0.3.
Screw Cap Tubes Testing – Centrifugation stability	SOP-CTL242c	0.2.
Screw Cap Tubes Testing – Handling test	SOP-CTL242d	0.1.

Table 1: Test SOPs. Eppendorf Core Test Lab SOPs applied to evaluate performance of Eppendorf Tubes BioBased 50 mL in comparison to standard Eppendorf Conical Tubes 50 mL.

Lid Tightness at High Temperature: Steam Tightness
The tubes were filled with Ethanol/water (1:1) solution equivalent to the 40% of the nominal capacity, weighted (4-digit scale) and placed standing in a plastic rack followed by an incubation: 60 minutes at 70 °C in a heating chamber. After incubation, tubes were cooled down at room temperature and weighted again to deduce the evaporation loss. For each tube type 6 replicates were performed.

Lid Tightness at Low Temperature
Method 1: Horizontal storage with Ethanol 96%
Empty tubes were firstly weighted on a 4-digits balance. Subsequently, the tubes were filled with a volume of Ethanol 96% solution equivalent to 90% of the nominal capacity and weighted again. Tubes were then stored horizontally at -86 °C for 24 hours. After storage, tubes were warmed up at room temperature and weighted again to deduce the evaporation loss. For each tube type 10 replicates were performed.

Method 2: Horizontal storage with Triton X-100™
Tubes were filled with a volume of Triton X-100 0.1% solution equivalent to 90% of the nominal capacity. After cap closing, tubes were stored horizontally at -86 °C for 24 hours. A visual check of tubes was performed after storage. For each tube type 24 replicates were performed.

Method 3: Vertical storage with Triton X-100
Tubes were filled with a volume of Triton X-100 0.1% solution equivalent to 90% of the nominal capacity. After cap closing, tubes were stored vertically at -86 °C for 24 hours. A visual check of tubes was performed after storage. For each tube type 9 replicates were performed.

Centrifugation stability
Method 1: The tubes were respectively filled with 32 mL of water : phenol : chloroform solution (2:1:1). After cap closing, tubes were placed in the centrifuge and tubes were centrifuged 30 minutes at 18,000 g at 4 °C, followed by the same centrifugation at 40 °C. A visual check of tubes was performed after each centrifugation step. For each tube type 6 replicates were performed.

Method 2: The tubes were filled with a volume of saturated NaCl solution equivalent to the nominal capacity. After cap closing, tubes were placed in the centrifuge and tubes were centrifuged 90 minutes at 19,500 g at 4 °C, followed by the same centrifugation at 25 °C. A visual check of tubes was performed after each centrifugation step. For each tube type 6 replicates were performed.

Leaching Test

Equipment: Eppendorf ThermoMixer® C, Eppendorf BioSpectrometer® kinetic, Hellma™ Quartz glass half-microcuvette (10 mm). Reagents: Alfa Aesar™, ultrapure water.

Method: the incubates were prepared by filling the tubes with 10 mL per tube of ultrapure water. After closing, the tubes were placed at ThermoMixer and incubated 40 minutes at 95 °C and 600 rpm. After incubation the tubes were cooled down to room temperature and 500µL were taken for absorbance measurement: for each incubate the absorbance at 260 nm was measured and the absorbance spectrum between 200 nm to 800 nm was performed. Absorbance at 260 nm and the factor 50 µg/mL were used to calculate the false DNA concentration derived from UV-absorbing leachables for each sample. For each tube type 10 replicates were performed.

Results and Discussion

The aim of this application note was to comprehensively evaluate all safety-relevant features of the Eppendorf Tubes 50 mL made of bio-based material in comparison with fossil-based Eppendorf Tubes 50 mL. In particular, the lid tightness and centrifugation stability have been assessed under several challenging conditions in order to fully evaluate all safety-relevant parameters and investigate that the performance of the bio-based tubes is exactly the same as of the fossil-based tubes.

Lid Tightness

Tight sealing of the screw cap in the conical tube is the critical prerequisite for sample integrity and necessary to prevent sample loss. Particularly high temperature incubations and long-term storage at very low temperatures may lead to sample loss.
The results of lid steam tightness test are presented in figure 1 and indicate virtually no difference between Eppendorf Tubes fossil-based and Eppendorf Tubes BioBased performance with values of 0.14% and 0.17% sample loss respectively. Noticeably, the values observed for evaporation loss laid well below the test acceptance level (0.28%), which assures very good level of sample and user safety when performing high temperature incubations.
The lid tightness was also tested under extreme low temperature conditions: ethanol samples were stored in horizontal position at -86 °C to increase vapor pressure and lid strain. As shown in figure 2 both Eppendorf Tubes fossil-based and Eppendorf Tubes BioBased showed very comparable und low sample loss values of 0.03% and 0.00% far below the acceptance level for this test.
The lid tightness was also evaluated using two other test conditions: vertical and horizontal storage of Triton X-100 0.1% samples at -86 °C for 24 hours (method 2 and 3). The observed performance of Eppendorf Tubes BioBased was equivalent to the fossil-based tubes (data not shown).

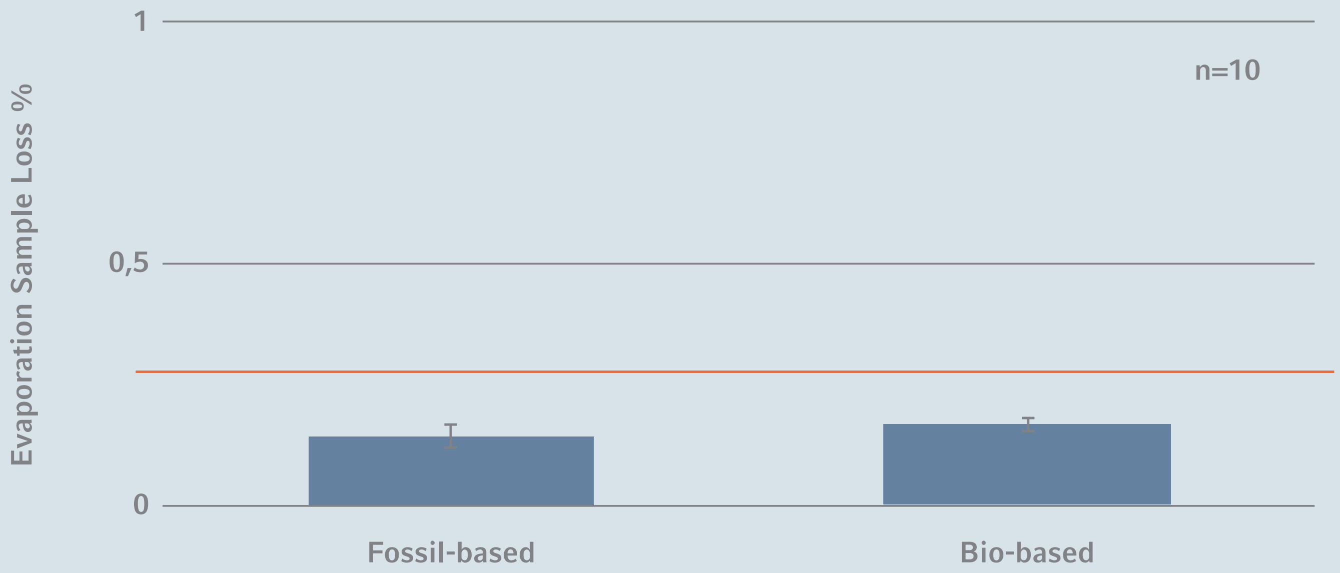

Figure 1: Lid tightness – steam test. Sample loss (%) due to evaporation after incubation of water samples for 60 minutes at 70 °C in Eppendorf Tubes fossil-based and Eppendorf Tubes BioBased. Red line represents the test acceptance level.

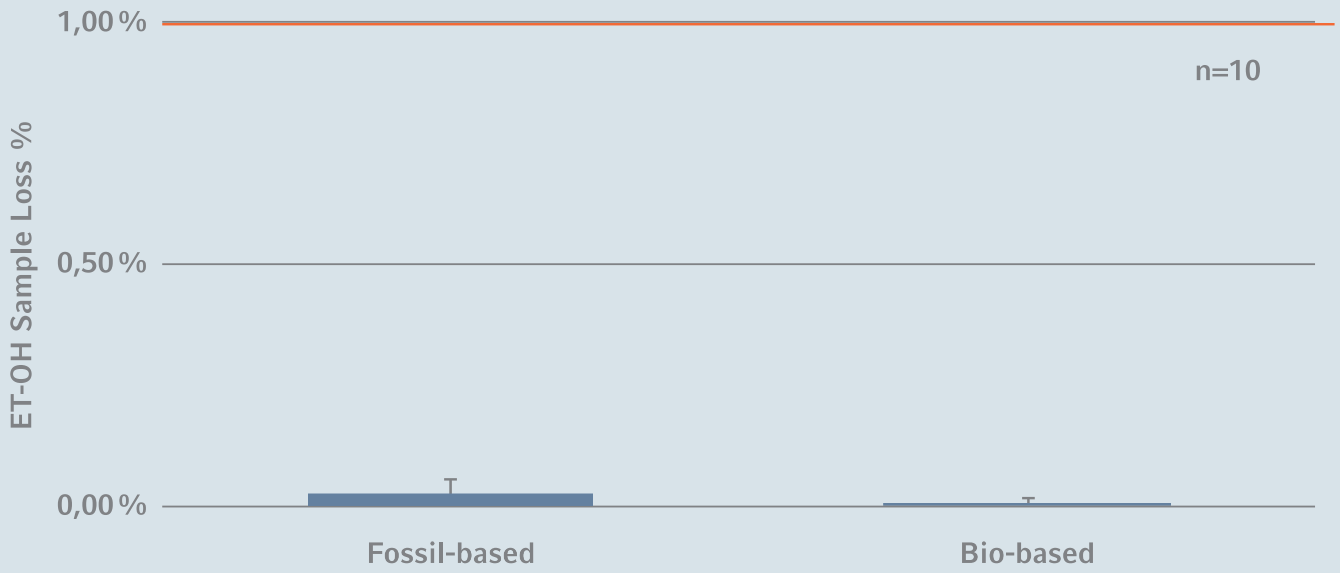

Figure 2: Lid tightness – low temperature test. Sample loss (%) of ethanol samples stored in horizontal position at -86 °C in Eppendorf Tubes fossil-based and Eppendorf Tubes BioBased. Red line represents the test acceptance level.

Centrifugation Stability

Higher speed centrifugation of samples with organic solvents poses considerable challenge for bigger laboratory vessels. Particularly at lower temperatures conical tubes may show deformations or cracks of the tube wall or lid tightness may be compromised, which both may result in sample loss and contamination. In the centrifugation test performed here, a typical application of nucleic acid extraction was simulated by filling the tubes with water : phenol : chloroform (2:1:1) solution and centrifuging the tubes at 18,000 x g at 4° and subsequently, in order to induce further stress to the tubes, at 40 °C.

As shown in Table 2, the ability to resist to high-speed centrifugation is demonstrated, under all conditions tested. Eppendorf BioBased Conical Tubes showed exactly same performance as Eppendorf standard tubes: no damage: liquid loss, broken tubes, distorted lid or white cracks was observed. Centrifugation performance was also tested under regular conditions: saturated NaCl solution, 90 minutes at 19,500 g at 4 °C and subsequently at 25 °C (method 2). Also, in this case, the observed centrifugation performance of Eppendorf Tubes BioBased was equivalent to standard tubes (data not shown): neither tube damage nor sample loss was observed whatsoever.

4 °C					40 °C				
Fossil-based	Liquid loss	Broken tubes	Distorted lid	white cracks	Fossil-based	Liquid loss	Broken tubes	Distorted lid	white cracks
Sample 1	no	no	no	no	Sample 1	no	no	no	no
Sample 2	no	no	no	no	Sample 2	no	no	no	no
Sample 3	no	no	no	no	Sample 3	no	no	no	no
Sample 4	no	no	no	no	Sample 4	no	no	no	no
Sample 5	no	no	no	no	Sample 5	no	no	no	no
Sample 6	no	no	no	no	Sample 6	no	no	no	no
Bio-based	Liquid loss	Broken tubes	Distorted lid	white cracks	Bio-based	Liquid loss	Broken tubes	Distorted lid	white cracks
Sample 1	no	no	no	no	Sample 1	no	no	no	no
Sample 2	no	no	no	no	Sample 2	no	no	no	no
Sample 3	no	no	no	no	Sample 3	no	no	no	no
Sample 4	no	no	no	no	Sample 4	no	no	no	no
Sample 5	no	no	no	no	Sample 5	no	no	no	no
Sample 6	no	no	no	no	Sample 6	no	no	no	no

Table 2: Centrifugation stability. Centrifugation performance of Eppendorf Tubes fossil-based and Eppendorf Tubes BioBased centrifuged with water : phenol : chloroform solution at 18,000 x g at 4° and 40 °C.

Leachables

Recent scientific evidence indisputably shows that various additives used during production process of consumables may be washed out of the plastic material (leach) and negatively influence various experimental systems and falsify the results [3, 4]. To date many such substances have been identified and their effect on various assay systems has been demonstrated – also in routine methods such as absorption spectra and photometric quantification of nucleic acids and proteins [5]. Based on this, a simple test system to evaluate general level of leachables has been established: pure water samples are incubated in given consumable and absorption spectrum measured indicating general amount of UV-absorbing substances leached during the incubation. In this Application Note we comparatively evaluated leachables levels of fossil-based and bio-based Eppendorf Tubes, as well as equivalent tubes from other manufacturers.

Figure 3 shows absorbance values obtained at 260 nm for water samples incubated at 95 °C for 40 min in various conical tubes. The absorbance values obtained at 260 nm translate to dsDNA concentrations and this may yield false elevated results during photometric analyses of molecules such as nucleic acids and proteins which are primarily conducted at 260 nm – 280 nm. The absorbance values obtained for Eppendorf Tubes BioBased and Eppendorf Tubes fossil-based were very low: 0.024 and 0.022 respectively. In contrast, the absorption values of tubes from manufacturers Fi, Fa and Co were considerably higher ranging between 0.072 to 0.103. This high leaching values may potentially have adverse effects on experiments.

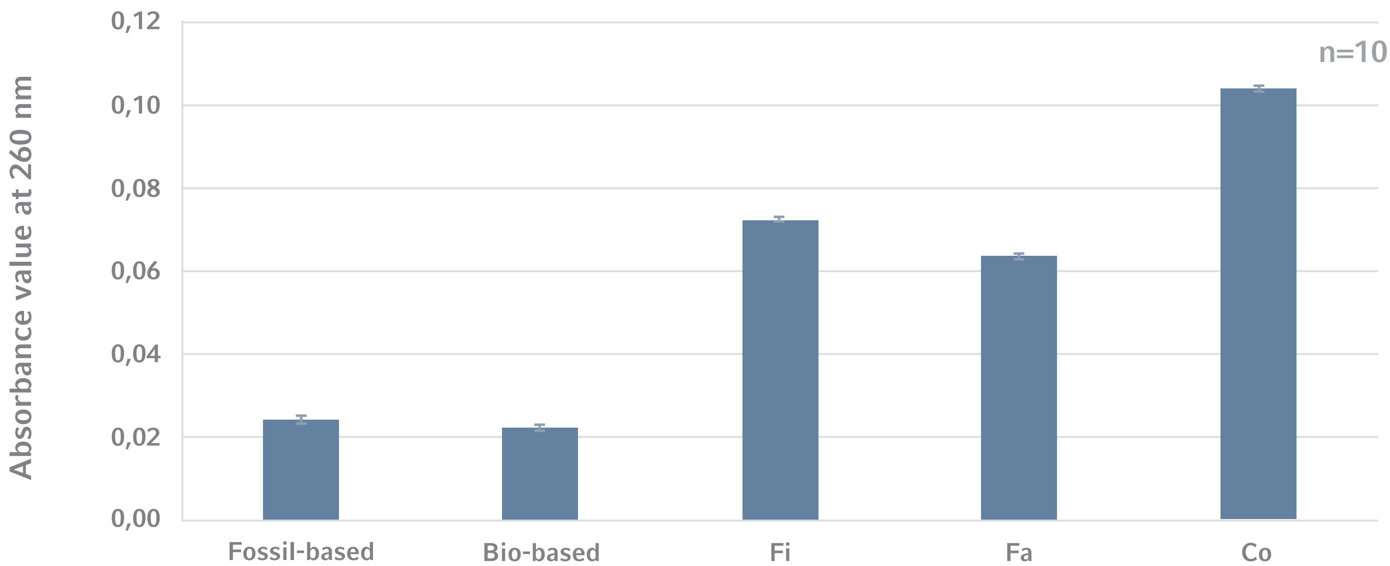

Figure 3: Leachable test. Absorbance value at 260 nm of water samples incubated 40 minutes at 95 °C in Eppendorf Tubes fossil-based and bio-based, as well as equivalent tubes from other manufacturers. (Warning: organic solvents can reduce the mechanical resistance of the tubes – please refer to safety note at the end of this application note.)

Conclusion

In this Application Note we investigated key safety parameters and leachable levels of Eppendorf Conical Tubes 50 mL made of ISCC PLUS certified polypropylene produced using recycled and renewable (bio-based) feedstocks (based on ISCC mass balance approach).

Tested under a range of rigorous and application-relevant conditions, the Eppendorf Tubes BioBased showed exactly same performance as Eppendorf Conical Tubes made of standard polypropylene (fossil-based) both with respect to lid tightness performance and centrifugation stability. Furthermore, comparative analysis revealed consistently low levels of water-soluble compounds (leachables) migrating into samples incubated both in Eppendorf Tubes BioBased and Eppendorf Tubes fossil-based. This indicates excellent properties of bio-based material in respect to leaching and consequently minimizes adverse effects of bio-based Consumables on experiments. Leaching levels observed for tubes of other manufacturers were considerably higher.

In summary, the comparative evaluation of the Eppendorf Tubes BioBased demonstrates their very high safety performance and minimal levels of leachables virtually identical to fossil-based Eppendorf Tubes. This confirms that the bio-based material regarding its physical and chemical properties may be regarded equal to fossil-based polypropylene. Bio-based consumables therefore offer a major improvement of renewable properties of lab plastics, making them more sustainable without compromising product quality and performance.

Literature

[1] For more information on ISCC system please visit: www.iscc-system.org
[2] Hermuth-Kleinschmidt K, Consumables Made of Bioplastics Enter the Lab, Eppendorf White Paper Nr. 78, Eppendorf
[3] McDonald GR, Hudson AL, Dunn SM, You H, Baker GB, Whittal RM, Martin JW, Jha A, Edmondson DE, Holt A. Bioactive contaminants leach from disposable laboratory plasticware. Science 2008; 322:917.
[4] McDonald GR, Kozuska JL, Holt A. Bioactive Leachates from Lab Plastics. G.I.T. Laboratory Journal Europe 2009; 13:24–26.
[5] Lewis LK, Robson M, Vecherkina Y, Ji C, Beall G. Interference with spectrophotometric analysis of nucleic acids and proteins by leaching of chemicals from plastic tubes. BioTechniques 2010; 48(4) 297–302.

Ordering Information

Ordering information

Description	Order no.
Eppendorf Tubes® BioBased screw cap, sterile, 5 mL	0030 122 518
Eppendorf Tubes® BioBased screw cap, sterile, 15 mL	0030 122 526
Eppendorf Tubes® BioBased screw cap, sterile, 25 mL	0030 122 534
Eppendorf Tubes® BioBased screw cap, sterile, 50 mL	0030 122 542

Safety Note

WARNING! Sample loss during centrifugation.

The tubes are subjected to strong forces during centrifugation. These include high g-forces or temperatures above 40 °C in non-refrigerated centrifuges (rotor chamber; rotor; sample). If used improperly (not optimal rotor fit, not properly closed cap), tubes can be destroyed and release the substances they contain.
Close the tube lid properly before centrifugation. Observe the maximum permitted centrifugation temperature. Observe the maximum permitted centrifugation forces. Read the operating manual of the centrifuge used. Please note that organic solvents can reduce the mechanical resistance of the tubes. To determine the maximum centrifugation stability of your applications, perform a test run at a lower g-forces.

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