# Heavy metal content in cellulosic environmental enrichment for laboratory animals

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## INTRODUCTION

Environmental conditions such as housing and husbandry have a major impact on the laboratory animal throughout its life and will thereby influence the outcome of animal experiments. According to Directive 2010/63/EU of the European Parliament and of the Council, laboratory animals should be maintained in facilities that provide adequate environmental conditions, including free movement, food and water, and proper care to keep them healthy. One possible way to improve the living conditions of laboratory animals equally important as nutrition and veterinary care is environmental enrichment. These include commercially available polycarbonate items (cottages, tubes), cotton, aspen wood and cellulose, as well as “home-made” cellulose tubes of household origin. Available on the market enrichments are characterized by high standards of performance and guarantee of safety, which in the case of "home-made" cellulose tubes is difficult to guarantee, especially when they contain dyes, printing inks or adhesive residue. It is well recognized that trace metallic contaminants play a major role in deterioration of radiopharmaceuticals quality, particularly it can change the biological properties, which may have a negative impact on the quality of the imaging or metabolism.

The **aim** of the study was to investigate if the content of heavy metals in environmental enrichment does not exceed safe limits from Guideline for Elemental Impurities Q3 ICH [2].

## MATERIALS AND METHODS

### PREPARATION OF SAMPLES

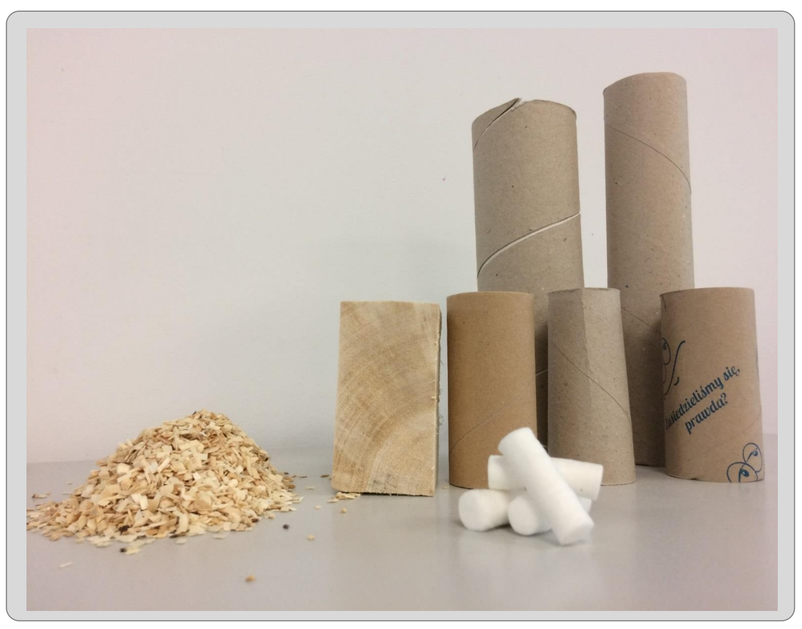
Commercial paper tubes, wooden litter, wooden blocks, cotton cocoons, toilet paper tubes, toilet paper tubes with blue ink, paper towels tubes (rigid and soft) used at animal facility in POLATOM were collected for the comparative study (Picture 1). Six square specimens (1cm x 1cm) were cut out from each paper tube. Samples of wooden blocks and cotton swabs were of similar weight as cellulose samples (ca. 0.5 g).

Samples preparation was carried out using two methods**:**

• *migration test* [3] – dried items were submerged in 3% acetic acid at T=40°C for 48h.

•*mineralization test* [4] – dried items were warmed up in 30% hydrogen peroxide and 60% perchloric acid mixture (1:1) at T=75°C for 2h.

Then the solutions were filtered. Blanc solutions were treated by the same procedures.



**Picture 1**. Examples of examined enrichments.

### ANALYTICAL METHOD

The content of cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), cobalt (Co), arsenic (As) and mercury (Hg) in the examined enrichments were determined by the inductively coupled plasma-optical emission spectrometry (ICP-OES) system - Perkin Elmer Optima 7300 DV. The quantitative determination of each analyte was carried out according to the inner POLATOM procedure for ICP elemental impurities test [5]. Samples were diluted 100-fold using 0,5M nitric acid. Concentration of elements was determined using the calibration curves. Each individual element has defined limit of quantification (LOQ) for the method.

**Table 1. Oral PDE and LOQ.** Oral Permitted Daily Exposures for metal impurities in drug products, drug substances and excipients [ppm/day] according to Guideline for Elemental Impurities Q3 ICH and limit quantification LOQ [ppm].

| | Cd | Cu | Pb | Co | As | Hg |
|---|---|---|---|---|---|---|
| Oral PDE | 5 | 1300 | 5 | 50 | 15 | 40 |
| LOQ | < 0.600 | < 0.869 | < 1.93 | < 0.614 | < 5.64 | < 0.301 |

## RESULTS

The concentration of metal impurities in the tested enrichments and the litter are presented in tables 2-3. In our study we determined also iron content, beside it is not specified in ICH Guideline [5], because of it possible negative impact on radiopharmaceutical in vivo stability.

**Table 2**. Concertation of metal elements [ppm] in enrichment and litter (the samples prepared by the migration test).

| Sample | Cd | Cu | Fe | Pb | Co | As | Hg |
|---|---|---|---|---|---|---|---|
| toilet paper tube | ≤ LOQ | ≤ LOQ | 280,9 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| toilet paper tube, blue ink | ≤ LOQ | ≤ LOQ | 438,9 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| paper towels tube (rigid) | ≤ LOQ | ≤ LOQ | 1127,4 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| paper towels tube (soft) | ≤ LOQ | ≤ LOQ | 837,3 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| wooden litter | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| wooden block | ≤ LOQ | ≤ LOQ | 637,6 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| cotton cocoon | ≤ LOQ | ≤ LOQ | 101,9 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| commercial paper tube | ≤ LOQ | ≤ LOQ | 298,3 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |

**Table 3**. Concertation of metal elements [ppm] in enrichment and litter (the samples prepared in mineralization test).

| Sample | Cd | Cu | Fe | Pb | Co | As | Hg |
|---|---|---|---|---|---|---|---|
| toilet paper tube | ≤ LOQ | ≤ LOQ | 1532,4 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| toilet paper tube, blue ink | ≤ LOQ | ≤ LOQ | 1612,4 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| paper towels tube (rigid) | ≤ LOQ | 1269,7 | 13504,2 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| paper towels tube (soft) | ≤ LOQ | 896,4 | 5910,4 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| wooden litter | ≤ LOQ | ≤ LOQ | 219,6 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| wooden block | ≤ LOQ | ≤ LOQ | 54,5 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| cotton cocoon | ≤ LOQ | ≤ LOQ | 33,7 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |
| commercial paper tube | ≤ LOQ | 452,2 | 4852,2 | ≤ LOQ | ≤ LOQ | ≤ LOQ | ≤ LOQ |

## CONCLUSION

- Either „household” enrichments or commercial enrichments do not contain a substantial amount of heavy metals. Both methods of sample preparation present similar outcome as regards cadmium, lead, cobalt, arsenic and mercury ions.
- The low amount of copper and iron were found in specimens. Even if part of the „toys” are chewed, portion of copper is below Oral PDE. From our observation daily mass loss of enrichment per rodent is about 0,5 g.
- According to Nutrient Requirements of Laboratory Animals [6] iron concentration in rodent diet oscillate between 150 to 250 mg/kg so it seems that content of iron in popular enrichment should not cause physiological changes.
- Nevertheless it should be taken into account that enrichments may contain various kind of toxic substances.

## LITERATURE

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