Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

# Identification of an exposure risk to heavy metals from pharmaceutical-grade rubber stoppers

*Xianghui Li* [a,b,*], *Pingping Qian* [c]

[a] *School of Pharmacy, Second Military Medical University, Shanghai 200433, China*
[b] *Shanghai Food and Drug Packaging Material Control Center, Shanghai 201203, China*
[c] *Department of Nutrition, Number 411 Hospital of the CPLA, Shanghai 200081, China*

ARTICLE INFO

*Article history:*
Received 16 May 2016
Received in revised form
9 July 2016
Accepted 28 July 2016
Available online 2 November 2016

*Keywords:*
heavy metals
metal ion chelating agent
ICP-MS
pharmaceutical closures

ABSTRACT

Exposure to low concentrations of heavy metals and metalloids represents a well-documented risk to animal and human health. However, current standards (European Pharmacopeia [EP], United States Pharmacopoeia [USP], International Organization for Standardization [ISO], YBB concerned with rubber closures) only require testing for Zn in pharmaceutical-grade rubber stoppers and then using only pure water as a solvent. We extracted and quantified heavy metals and trace elements from pharmaceutical-grade rubber stoppers under conditions that might occur during the preparation of drugs. Pure water, saline, 10% glucose, 3% acetic acid (w/v), 0.1 mol/L hydrochloric acid, and diethylenetriaminepentaacetic acid (4 mg/mL, 0.4 mg/mL, and 0.04 mg/mL) were used as extraction agents. We quantified the extracted arsenic, lead, antimony, iron, magnesium, aluminum, and zinc using inductively coupled plasma mass spectrometry. The concentration of extracted metals varied depending on the different extraction solutions used and between the different rubber stopper manufacturers. Rubber stoppers are ubiquitously used in the pharmaceutical industry for the storage and preparation of drugs. Extraction of heavy metals during the manufacturing and preparation of drugs represents a significant risk, suggesting a need for industry standards to focus on heavy metal migration from rubber stoppers.



## 1. Introduction

Rubber products are regularly used as closures, gaskets, and joints in the production of pharmaceutical agents. The fabrication of rubber is very complex and typically requires the use of agents that may be contaminated with heavy metals such as MgO, ZnO [1], and kaolin [2]. Kaolin, in particular, is known to contain various kinds of heavy metals [2–4]. Therefore, there is a potential risk of contaminants, such as heavy metals, migrating from the rubber products to pharmaceutical agents during drug preparation and storage [5,6].

Studies examining pharmaceutical glass products have shown results similar to those reported here. In particular,

* *Corresponding author*. School of Pharmacy, Second Military Medical University, Shanghai Food and Drug Packaging Material Control Center, Shanghai 200433, China.
E-mail address: xianghuili2004@163.com (X. Li).
http://dx.doi.org/10.1016/j.jfda.2016.07.008

significant amounts of silicate, borate, sodium, and aluminum were released from heated glass, and the use of acidic or glucose-containing solutions resulted in extraction of high levels of aluminum, copper, and lead [7]. Arsenic has also been detected in glass ampules containing intravenous nutrition formulas [8]. However, to our knowledge, there have been no studies examining the potential for heavy metals to be extracted from pharmaceutical-grade rubber stoppers. Here, we used an inductively coupled plasma-mass spectrometry (ICP-MS) method to evaluate if potentially toxic heavy metals were extractable from commonly used pharmaceutical-grade rubber stoppers under various conditions.

Exposure to heavy metals is known to result in serious adverse health effects. Although there is no full consensus on the definition of heavy metals, they are commonly defined as high-density chemicals that can be highly toxic [9]. The mechanisms of heavy metal toxicity are often due to a bond formation of the metals with thiol groups of proteins [10]. When heavy metals enter the cell, they can alter biochemical pathways that may ultimately lead to death or illness [11,12]. Recent data suggest that exposure to lead (Pb) resulting in blood concentrations under 10 μg/dL can have negative effects on children's cognition [13,14]. High Pb exposures in pregnant women can cause low infant birth weight, prematurity, miscarriage, or stillbirth [15]. Exposure to aluminum (Al) can damage various systems of the body including the hematopoietic, renal, and skeletal systems together with the central nervous system being its primary target. Al has also been proposed to be involved in the pathophysiology of neurodegenerative disorders (Parkinsonism dementia, Alzheimer's disease, etc.), although this is still controversial [16,17]. Similarly, arsenic (As) is a known toxic agent and carcinogen [18]. Although iron (Fe), zinc (Zn), and magnesium (Mg) are essential for living organisms at low concentrations, exposure to higher concentrations can lead to toxic effects [19], particularly in susceptible subpopulations (infants, individuals with altered renal function, elderly, etc.).

Use of ICP-MS was considered a most advantageous testing method for the determination of metals, with high precision, wide range, low disturbance, high accuracy, high speed, and the ability to quantify multiple metals simultaneously [20,21]. Therefore, ICP-MS was used to quantify the metals in all experiments.

## 2. Methods

### 2.1. Sample preparation

We used 10 batches of rubber stoppers from five different manufacturers (five groups were randomly selected from every batch and analyzed in duplicate). The whole, uncut stoppers were put into suitable polypropylene containers and macerated in the extraction solution using the conventional ratio (1 $dm^2$:100 mL) according to EN 1186-1:2002 and European Union (EU) 10-2011. The samples were then heated in an autoclave to reach a temperature of 121 ± 2°C within 20–30 minutes and maintained at this temperature for 60 minutes, which conforms to the test conditions of USP APPENDIX 660. Samples were allowed to cool to room temperature over a period of about 30 minutes, then mixed and decanted immediately. A blank solution was prepared in the same manner.

### 2.2. Preparation of extraction solutions

We used water purified with a Milli-Q system (Millipore, Billerica, MA, USA). Saline and 10% glucose (common transfusion preparations) were purchased from Baxter (China Shanghai). The 3% acetic acid (w/v) (as the acid simulation according to the EU 10-2011), 0.1 mol/L hydrochloric acid (HCl; as the extraction for plastic in EP APPENDIX 3.1.3), and diethylenetriaminepentaacetic acid (DTPA; 4 mg/mL, 0.4 mg/mL, and 0.04 mg/mL) (a metal ion chelating agent preparation) were diluted or dissolved in water using an ultrasonic water bath. All chemicals were at least of analytical grade.

### 2.3. Quantification of heavy metals

Measurements were made using an Agilent Technologies 7500ce ICP-MS system (Agilent Technologies, Wilmington, DE, USA) equipped with an octopole collision/reaction cell, Agilent 7500 ICP-MS ChemStation software, a Babington nebulizer, a Peltier cooled (2°C) quartz Scott-type double pass spray chamber, and an Agilent I-AS integrated autosampler. We also used a Metler-AE240 electronic balance and MLS-3780 pressure steam sterilizer for extraction purposes. Standard solutions of Pb, antimony (Sb), Fe, Mg, Al, and Zn (1000 μg/mL) were purchased from Shanghai Institute of Measurement and Testing Technology. Standard solutions of As (1000 pg/mL) were purchased from the National Standard Material Center. Once the proper mass isotope was selected, bismuth (Bi) was used as the internal standard for Pb, and scandium (Sc) was used as the internal standard for Fe, Mg, and Al.

### 2.4. Statistical analysis

All statistical analyses were done with SPSS 16.0 statistical software. Differences among groups were analyzed by variance tests. A $p$ value $< 0.05$ was considered significant.

## 3. Results

### 3.1. Extraction of heavy metals using different solvents

Using pure water, saline, or 10% glucose, we measured very low or undetectable concentrations of As, Sb, Pb, Fe, and Al. However, under acidic conditions using acetic acid or HCl and in the presence of DPTA, we observed a significant increase in the extraction of most metals tested (see Tables 1–7).

Concentrations of As did not reach detectable levels when water and saline were used as solvents for most samples; however, detectable levels were measured using 10% glucose. We observed a significant increase in the detectable As concentrations when using 3% acetic acid (w/v), 0.1M HCl, and DTPA as solvents (Table 1).

We found detectable levels of Sb in the extract regardless of the solvent used (Table 2). However, when using DTPA as a solvent, the increase in Sb was significant.