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Changes in oral bioaccessibility of heavy metals in non-digestive sucking habits due to the formation of complexes between digestive fluid components and metals/metalloids

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ABSTRACT

Humans, especially infants, are exposed to harmful substances through various means, including non-nutritive sucking behaviors. Here, we compared the “one-compartment model” and the “three-compartment model” within the “suck model” to assess the oral bioaccessibility of heavy metals in various products and evaluated whether these models can be employed to assess 12 heavy metals present in consumer products. Several certified reference materials, including plastic, paint, glass, and metals, were employed to ensure sample homogeneity. By comparing the two models, we validated that a considerable amount of complexes were formed between saliva components and the extracted heavy metals and that some of these complexes dissociated during reactions with the gastric/intestinal fluids. Furthermore, we observed that in the cases of Cu and Pb, additional complexes were formed as a result of reactions with gastric/intestinal fluids. We measured the total concentrations of the extracted heavy metals using artificial saliva through acid digestion and found that up to 99.7% of the heavy metals participated in the formation of complexes, depending on the characteristics of the sample (e.g., composition) and the target element. This result indicates that the current suck model may notably underestimate the oral bioaccessibility of heavy metals in products associated with sucking behaviors. Therefore, we propose a more conservative and simpler test method for assessing oral bioaccessibility of heavy metals that involves measuring the total concentrations of heavy metals extracted from consumer products using artificial saliva. By doing so, we can account for potential variations in the digestive milieu (e.g., due to ingested food) and the inconsistency in complex formation-dissociation characteristics.

1. Introduction

The human body is exposed to harmful substances present in various products through ingestion, inhalation, and dermal contact. In most infants, one of the primary means of exposure to harmful substances is non-nutritive sucking habits (Batista et al., 2019; Köhler and Holst,

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Abbreviations: ABS, Acrylonitrile butadiene styrene; BAM, Federal institute for materials research and testing; BSI, British standards institution; CRM, Certified reference materials; DIN, Deutsches institut für normung; ISO, International organization for standardization; IVG, in vitro gastro-intestinal method; IVIVC, in vitro-in vivo; JFE-TEC, JFE Techno-Research Corporation; JRC, Joint research centre; JSAC, Japan society for analytical chemistry; KEITI, Korea environment industry & technology institute; KRISS, Korea research institute of standards and science; KTR, Korea testing and research; LOD, Limits of detection; NIER, National institute of environmental research; NIST, National institute of standards and technology; OC, One-compartment model; PBET, Physiologically based extraction test method; PE, Polyethylene; PET, Polyethylene terephthalate; PVC, Polyvinyl chloride; RIVM, National institute for public health and the environment; TC, Three-compartment model; ST, Total concentration of metals extracted using artificial saliva; UBM, Unified barge method; USEPA, United States environmental protection agency.

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1973; Martens et al., 2020; Modéer et al., 1982; Warren et al., 2000). Köhler and Holst (1973) reported that of 1567 four-year-old children residing in Lund and Dalby, both located in southern Sweden, 1220 children (77.9%) exhibited sucking habits. The National Institute of Environmental Research (NIER) in Korea directly observed the sucking habits of infants at home using cameras (NIER, 2015). The number of hand-to-mouth contacts per hour was found to be 3.92, 1.94, and 4.14 for children of ages 0–2, 3–6, and 7–9, respectively, whereas the number of object-to-mouth contacts was 4.36, 1.69, and 2.10 for each of these age ranges, respectively. These behavioral patterns indicate that infants are highly likely to be exposed to harmful substances present in products.

Guney et al. (2014) evaluated the concentration of heavy metals (As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sb, and Se) in 72 children's products that were randomly purchased from the US market. They found that the concentration of heavy metals in 32 products exceeded the established safety thresholds(European Council, 2009). Cui et al. (2015) evaluated the concentration of heavy metals (As, Cd, Cr, Ni, Pb, and Sb) in 45 children's products randomly purchased from the Chinese market and reported that the concentration of the heavy metals in 16 products exceeded the safety criteria (European Council, 2009). Numerous studies have demonstrated harmful substances at concentrations exceeding the safety thresholds in various consumer products, including products for children. Gao et al. (2018b) analyzed the content of heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb, and Sb) in 32 lip cosmetics (e.g., lip balms, lip glosses, lipsticks) and detected the presence of Cr, Cu, Mn, and Pb in three of those products. Turner (2019) analyzed the concentration of heavy metals in glass bottles using X-ray fluorescence spectrometry and found that the concentrations of Cd, Cr, and Pb in glass were 1100, 3000, and 1100 mg/kg, whereas those of Cd and Pb in the enamel of glass bottles were 100,000 and 20,000 mg/kg, respectively. Zhao et al. (2018) purchased 101 chopsticks from the Chinese market and analyzed their heavy metal concentrations. They validated that heavy metals were present in substantially high concentrations in the paint coating of chopsticks, which varied depending on the type of paint used (Cd: 0.002–120,000 mg/kg, Co: 0.004–2600 mg/kg, Cr: 2.2–8500 mg/kg, Ni: 0.1–150,000 mg/kg, Pb: 0.12–500,000 mg/kg). Since many studies have reported the presence of harmful substances, especially heavy metals, in various consumer products that come into oral contact, as well as products intended for children, the human health risks of such products must be reasonably assessed.

Bioaccessibility refers to the fraction of a substance that has the potential to be absorbed into the human body of the total amount of harmful substances present in the environmental medium. For assessing site-specific human health risks of harmful substances, relative bioavailability must be determined using animal models. However, the utilization of animals for toxicity studies has consistently been the subject of economic and ethical concerns (Cardoso et al., 2015; Xia et al., 2016); therefore, various in vitro methods that can replace the in vivo toxicity tests (i.e., methods with a good in vitro-in vivo correlation [IVIVC]) have been proposed. The following in vitro methods are widely recognized and used to assess the oral bioaccessibility of heavy metals in soils: the in vitro gastro-intestinal method (IVG) (Rodriguez et al., 1999), the Physiologically Based Extraction Test method (PBET) (Wragg et al., 2007), the OSWER 9285.7–77 of the United States Environmental Protection Agency (USEPA) (USEPA, 2007), the ISO 17924 of the International Organization for Standardization (ISO) (ISO, 2018), and the DIN 19738:2 of the Deutsches Institut für Normung e.V. (DIN) (DIN, 2004). To assess the in vitro bioaccessibility of harmful substances in children's products, researchers have employed the BS EN 71–3 of the British Standards Institution (BSI) (EN71–3, 2019), the EUR 19899 EN of the Joint Research Centre (JRC) of the European Commission (Simoneau and Rijk, 2001), and the RIVM report 320102004 of the Dutch National Institute for Public Health and the Environment (RIVM) (Oomen et al., 2005).

The RIVM presented the suck, suck-swallow, swallow-fasted, and swallow-fed models to assess the oral bioaccessibility of harmful substances in children's products that may pose risks owing to sucking behaviors (Oomen et al., 2005). Because the human body is exposed to harmful substances present in many consumer products, including children's toys, through intentional and/or unintentional sucking, an exposure assessment using artificial saliva must be performed. In particular, the suck model was subdivided into the "one-compartment model," which involves extraction solely by saliva, and the "three-compartment model," which takes into account the interactions of the harmful substances with gastric and intestinal fluids upon ingestion after being extracted by saliva. The one-compartment model assumes that 100% of the heavy metals extracted from products by saliva are absorbed into the body, whereas the three-compartment model assumes that some of the extracted heavy metals may form precipitates and/or complexes by reacting with gastric and intestinal fluids and that such precipitates and/or complexes are not absorbed into the body. According to the findings of the RIVM, the oral bioaccessibility of Pb in finger paint was 9.2–13.2% using the one-compartment model, whereas it was 4.5–6.3% using the three-compartment model (Oomen et al., 2005). Cationic metals, such as Pb, precipitate in the high pH conditions of the intestinal fluid (Brandon et al., 2006; Ljung et al., 2007). Since the absorption of substances in the human body mostly occurs in the intestines (Kiela and Ghishan, 2016), using the three-compartment model appears to be reasonable. However, test procedures for the three-compartment method are considerably complex, and a conservative assessment of oral bioaccessibility with the one-compartment model can be yielded (e.g., Pb in finger paint aforementioned). In most oral bioaccessibility test methods, the effect of sucking behaviors is therefore evaluated by extracting heavy metals using artificial saliva and subsequently subjecting the eluate to instrumental analysis (Cui et al., 2015; Guney and Zagury, 2014; Zhao et al., 2018).

This study compares the one-compartment model and the three-compartment model within the suck model, which is a method suitable for assessing the oral bioaccessibility of heavy metals in products associated with non-nutritive sucking habits. The RIVM only compared these models for determining the concentration of Pb (Oomen et al., 2005); therefore, herein, we determined whether the test methods can be applied to various heavy metals/metalloids found in consumer products and the differences between the methods. We assumed that the characteristics of the extraction of oxyanions, such as As and Se, by digestive fluids and their behavioral patterns in the digestive fluids were different from those of cationic metals (Basta et al., 2007; Li et al., 2017). The bioaccessibility assessment results obtained using the two models were compared to supplement the limitations of each method, and a more suitable test method with relative simplicity that can be used to assess the heavy metals that are highly likely to be absorbed into the body is presented.

2. Materials and methods

2.1. Certified reference materials (CRMs)

The bioaccessibility of heavy metals resulting from sucking behavior was assessed using 14 CRMs containing heavy metals. To quantitatively compare the oral bioaccessibility assessment methods, we used CRMs as homogeneous samples with certified element contents, rather than using actual consumer products, which were likely to cause errors owing to heterogeneities depending on the sampling point and method used. In particular, 12 target heavy metals (As, Ba, Cd, Cr, Cu, Ni, Pb, Sb, Se, Sn, Sr, and Zn) were selected in accordance with the EU Directive 2009/48/EC related to the safety of toys (European Commission, 2009), and the CRMs were selected so that at least one of them would contain each of the target heavy metals. The CRMs used were as follows. The following six plastic CRMs were used: CRM 113–01–013 (acrylonitrile butadiene styrene [ABS]; Korea Research Institute of Standards and Science [KRISS], Korea), JSM P 701–1 (polyethylene [PE]; JFE Techno-Research

Corporation [JFE-TEC], Japan), ERM EC680m (PE) and ERM EC681m (PE) (European reference materials [JRC Geel], Belgium), SRM 2861 (polyvinyl chloride [PVC]; National Institute of Standards and Technology [NIST], MD, USA), and JSAC 0602–3 (polyethylene terephthalate [PET]; The Japan society for analytical chemistry [JSAC], Japan). The following two paint CRMs were used: 110–05-patint-02 (Korea Testing and Research institute [KTR], Korea) and CRM 013–50 G (Sigma-Aldrich, MO, USA). The glass CRM used was BAM-S004 (Federal institute for materials research and testing [BAM], Germany). The following five metal CRMs were used: SRM 856a (aluminum; NIST), SRM 875 (cupro-nickel; NIST), SRM 899 (nickel-base; NIST), SRM 872 (phosphor bronze; NIST), and 102–02-SBSI5 (silicon; KTR). Table S1 shows the concentration of heavy metals in each of the CRMs.

2.2. Preparation of the artificial digestive fluids

To assess the oral bioaccessibility of heavy metals in the CRMs, we prepared artificial digestive fluids (saliva, gastric fluid, duodenal fluid, and bile fluid) by referring to ISO 17924 (ISO, 2018). This standard is based on in vivo validation and is known to result in a high IVIVC of As, Cd and Pb in mouse and swine (Li et al., 2015; Wragg et al., 2011) (Table S2). During the preparation of each artificial digestive fluid, inorganic and organic component solutions were prepared separately and mixed. A solid enzyme component was then added and the mixture was stirred.

2.3. Determination of bioaccessible metal concentrations resulting from non-nutritive sucking habits

Artificial saliva (21 mL) was added to 0.4 g of CRMs, and the mixture was stirred at 55 rpm in a water bath at 37 °C for 1 h (stirring time was as per the Korean NIER guidelines) to induce a reaction and extract the heavy metals from the CRMs. The mixture was then centrifuged at 4500 × *g* for 5 min, and 3 mL of the supernatant was collected for heavy metal concentration analysis. The concentrations determined in this manner were considered the bioaccessibility assessment results of the one-compartment model (Fig. 1). Meanwhile, 18 mL of the remaining supernatant was sequentially mixed with 12 mL of gastric fluid and allowed to react for 2 h. Moreover, the same volume of supernatant was mixed with 12 mL of duodenal fluid and 6 mL of bile fluid (and allowed to react for 2 h) to simulate a scenario where the swallowed saliva reacts with the gastric and intestinal fluids (three-compartment model). Centrifugation was then performed at 4500 × *g* for 5 min, and the supernatant was collected for heavy metal concentration analysis (Fig. 1).

2.4. Determination of the total concentration of metals extracted using artificial saliva

To determine the total concentration of the extracted heavy metals, we subjected the complexes formed between the heavy metals extracted from the CRMs and the artificial saliva components to decomposition through acid digestion. Briefly 1 mL of 67% HNO_3 was injected into 10 mL of the centrifugation supernatant obtained after employing the one-compartment model, and the mixture was subjected to decomposition for 20 min in a microwave (Mars 6, CEM Corporation, USA) at 1200 W and 180 °C ± 5 °C to prepare the samples for heavy metal concentration analysis.

2.5. Analysis of the metals/metalloids and data treatment

To analyze the concentration of the heavy metals in the solutions, we used an inductively coupled plasma mass spectrometer (ICP-MS, 7900, Agilent Technologies, USA). An ICP-MS equipped with a collision cell was used to minimize the mass interferences (He flow rate = 5 mL/min), and the analysis was conducted under the following conditions: radio frequency power: 1550 W; radio frequency matching: 2 V; and nebulizer gas (Ar) flow rate: 1.05 L/min. The limits of detection (LODs) were as follows: As: 0.010 μg/L; Ba: 0.009 μg/L; Cd: 0.01 μg/L; Cr: 0.006 μg/L; Cu 0.133 μg/L; Ni: 0.013 μg/L; Pb: 0.011 μg/L; Sb: 0.032 μg/L; Se: 0.050 μg/L; Sn: 0.067 μg/L; Sr: 0.005 μg/L; Zn: 0.059 μg/L. To correct

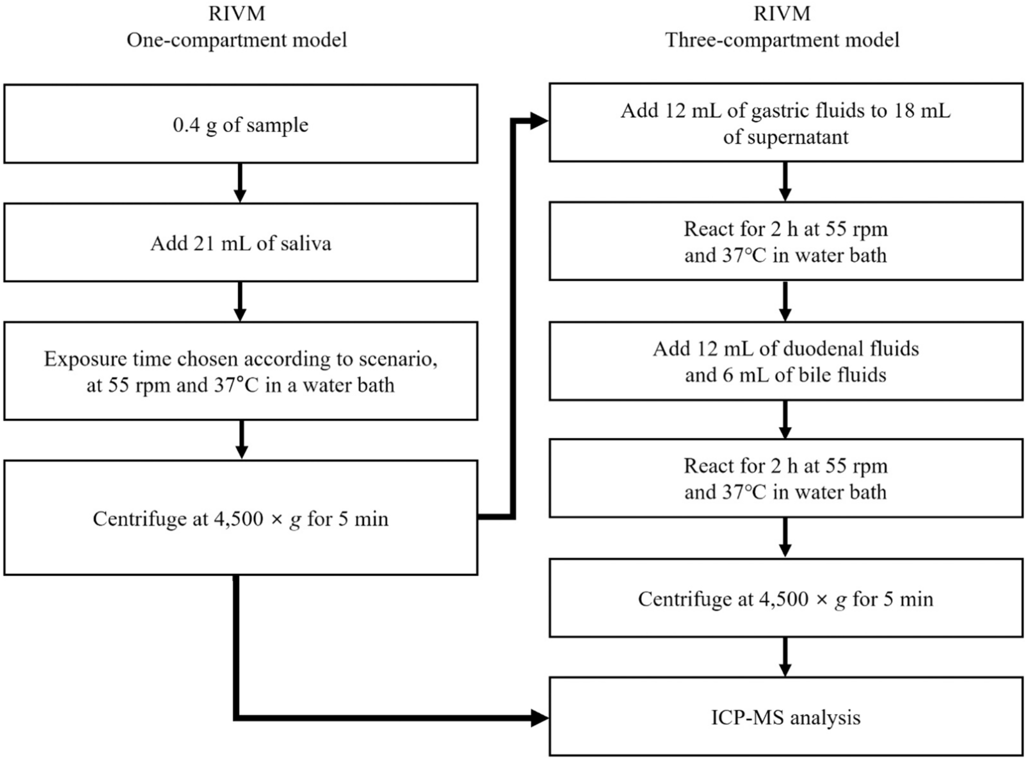

Fig. 1. Schematic diagrams of the one-compartment model and the three-compartment model of the RIVM suck model.

the matrix effect, the analytical signal of the blank (i.e., artificial digestive fluid without CRM) was subtracted from that of each sample. In addition, three samples were prepared and evaluated using each test method (i.e., based on the total concentration of heavy metals extracted using artificial saliva, the one-compartment model, and the three-compartment model) for each CRM. The evaluated samples were used to calculate the average and standard deviation to determine the bioaccessible concentration.

Bioaccessibility was calculated using Eq. (1).

$$Bioaccessibility(\%) = \frac{CRM_C}{CRM_T} \times 100 \tag{1}$$

where CRM_C is the heavy metal concentration measured in the centrifugation supernatant of artificial digestive fluids using ICP-MS (mg/kg), and CRM_T is the heavy metal content (certified value, mg/kg) of the CRM used in the experiment.

The ratio of the complexes formed between the extracted heavy metals and artificial saliva components was reported by Li et al. (2013). Eqs. (2) and (3) were used to calculate this ratio.

$$OC \quad complexes_R = 1 - \frac{OC_c}{ST_c} \times 100 \tag{2}$$

$$TC \quad complexes_R = 1 - \frac{TC_c}{ST_c} \times 100 \tag{3}$$

In Eq. (2), OC $complexes_R$ represents the ratio of complexes formed between extracted heavy metals and artificial saliva components, and OC_C represents the bioaccessible metal concentration (mg/kg) determined using the one-compartment model. In Eq. (3), TC $complexes_R$ represents the ratio of complexes formed between extracted heavy metals and artificial saliva components, TC_C is the bioaccessible metal concentration (mg/kg) determined using the three-compartment model, and ST_C is the total concentration of the metals extracted using artificial saliva bioaccessible metal concentration (mg/kg).

3. Results and discussion

3.1. Bioaccessible metal concentrations determined using the one-compartment model and the three-compartment model

Table 1 shows the bioaccessible concentrations of heavy metals in the 14 CRMs as determined using the one-compartment model and the three-compartment model, respectively. Higher heavy metal concentrations, except for those of Cu and Pb, were detected using the three-compartment model than using the one-compartment model. When Cu and Pb were excluded, the average concentration of the heavy metals determined using the one-compartment model was 4.81 ± 13.1 mg/kg, and that of those obtained using the three-compartment model was 7.89 ± 19.4 mg/kg. However, the average concentration of Cu and Pb determined using the one-compartment model was 58 ± 128 mg/kg and that determined using the three-compartment model was 8.51 ± 22.0 mg/kg.

While heavy metals were extracted using artificial saliva and instrumental analysis (ICP-MS) was performed through solid-liquid separation (centrifugation) in the one-compartment model, instrumental analysis was conducted after an additional step that involved the reaction of the separated supernatant and artificial digestive fluids (gastric and intestinal fluids) in the three-compartment model (Fig. 1). Therefore, it is estimated that a portion of the heavy metals extracted from the CRMs using artificial saliva was not measured during instrumental analysis; however, they may be measured after the reactions with the artificial gastric and intestinal fluids. In particular, a number of studies have reported that SCN^- in artificial saliva forms complexes with metals (Arvand et al., 2007; Sanna et al., 2002; Salgado-Salgado et al., 2016). According to RIVM, during the centrifugation process, large particles, such as samples, are separated into solid-liquid phases, whereas small particles, including metals, remain unseparated (Oomen et al., 2006). Laird et al. (2015) validated the differences in bioaccessible concentrations by separating metals and samples extracted through the digestive fluid under centrifugation conditions of 5000 × *g* and 12,000 × *g*. They determined that the centrifugation process did not affect bioaccessible concentrations. In addition, they validated that complexes formed as a result of interactions between gastric fluids and metal are less than 1000 kDa. According to RIVM, such complexes remain in the supernatant without undergoing effective separation during the centrifugation process and subsequently dissociate when exposed the low pH of the gastric fluid (Oomen et al., 2006). The bioaccessible concentrations determined using the one-compartment model were lower than those determined using the three-compartment model because such complexes exhibit a relatively low ionization efficiency in plasma of ICP-MS (D'Ilio et al., 2011).

Conversely, the bioaccessible concentrations of Cu and Pb determined using the one-compartment model were higher than those determined using the three-compartment model; therefore, Cu and Pb exhibited a behavior from that of the other heavy metals (Table 1). In other words, Cu and Pb extracted from the CRMs using artificial saliva formed various complexes in large quantities by reacting with artificial gastric and intestinal fluids. Moreover, such complexes separated during the centrifugation process of the three-compartment model or exhibited low ionization efficiency in plasma of ICP-MS, even when they were present in the supernatant and thus exhibited low bioaccessible concentrations. Among the various CRMs, SRM 872 exhibited the highest difference in Pb concentration. The bioaccessible concentration of Pb was determined to be 35.4 ± 2.81 mg/kg using the one-compartment model and 7.09 ± 3.42 mg/kg using the three-compartment model. The bioaccessible concentration of Cu was determined to be 83.9 ± 3.68 mg/kg using the one-compartment model, whereas it was determined to be 0.40 ± 0.11 mg/kg using the three-compartment model. Similarly, among the various CRMs, SRM 875 exhibited the highest difference in Cu concentration. The one-compartment model yielded a concentration of 392 ± 29.7 mg/kg, whereas the three-compartment model yielded a concentration of 67.9 ± 4.92 mg/kg. A similar pattern was also confirmed in a study by the RIVM on the presence of Pb in finger paint(Oomen et al., 2005). Gao et al. (2018a) assessed the intake bioaccessibility of particulate dust from urban sources. Although the sucking behavior was not evaluated, they reported that the concentration of Pb eluted by saliva was 0.30 ng/m^3, whereas that eluted by intestinal fluid was 0.17 ng/m^3. Li et al. (2020) and Li et al. (2013) reported that enzyme components in digestive fluids, such as bile, mucin and pepsin affect the bioaccessibility assessment results by forming sediments with heavy metals. The mixing of intestinal juice with gastric juice is known to raise the pH and enzyme concentration, leading to the formation of complexes between metals and components rich in carbonates and chelates in the intestine, thereby resulting in their stabilization or precipitation as insoluble substances (Turner and Radford, 2010). Moreover, sediments such as $Pb_5(PO_4)_3Cl$ may be formed under the high pH conditions of the intestinal fluid (Grøn and Andersen, 2003; Wragg et al., 2011). Phosphate, a digestive fluid component, may form complexes with Cu and Pb through ligand exchange reactions (Basta et al., 2007; Li et al., 2017).

3.2. Total concentrations of the metals extracted using artificial saliva

The formation of complexes between artificial saliva components and heavy metals was validated by measuring the total concentration of the extracted heavy metals through acid digestion using artificial saliva solutions (Fig. 2). The total concentration of the extracted heavy metals was found to be the highest for 10 heavy metals. This was followed by the concentration values determined using the three-compartment model and the one-compartment model, with the exception of Cu and Pb (Table 1). In particular, in the case of SRM 872, the concentration of Zn was determined to be 58 ± 0.1, 82 ± 8.3, and 509 ± 31 mg/kg using

Table 1
Heavy metal concentrations in CRMs evaluated using the one-compartment model, the three-compartment model, and based on the total concentration of heavy metals extracted using artificial saliva.

CRMs	Composition	Methods	Bioaccessible concentration (mg/kg)											
			As	Ba	Cd	Cr	Cu	Ni	Pb	Sb	Se	Sn	Sr	Zn
JSM P701	PE	OC[a]	< 0.0005[e]	-[d]	0.01 ± [f] 0.0005	0.79 ± 0.04	-	-	0.004 ± 0.001	-	-	-	-	-
		TC[b]	0.005 ± 0.002	-	0.02 ± 0.002	1.28 ± 0.18	-	-	< 0.0006	-	-	-	-	-
		ST[c]	0.01 ± 0.002	-	0.04 ± 0.003	2.69 ± 0.66	-	-	0.10 ± 0.01	-	-	-	-	-
CRM 113–01–013	ABS	OC	0.10 ± 0.003	-	< 0.0005	< 0.0003	-	< 0.0007	< 0.0006	< 0.002	-	-	-	-
		TC	0.23 ± 0.01	-	< 0.0005	< 0.0003	-	< 0.0007	< 0.0006	< 0.002	-	-	-	-
		ST	0.36 ± 0.04	-	< 0.0006	0.04 ± 0.03	-	0.02 ± 0.01	0.01 ± 0.002	0.01 ± 0.0005	-	-	-	-
JSAC 0602–3	PET	OC	-	-	0.01 ± 0.0001	0.01 ± 0.0004	-	-	< 0.0006	-	-	-	-	-
		TC	-	-	0.02 ± 0.001	0.01 ± 0.001	-	-	< 0.0006	-	-	-	-	-
		ST	-	-	0.05 ± 0.004	0.07 ± 0.04	-	-	0.03 ± 0	-	-	-	-	-
ERM EC680m	PE	OC	< 0.0005	-	< 0.0005	0.002 ± 0.001	-	-	< 0.0006	< 0.002	-	< 0.004	-	0.05 ± 0.02
		TC	< 0.0005	-	< 0.0005	< 0.0003	-	-	< 0.0006	0.005 ± 0.0004	-	< 0.004	-	< 0.003
		ST	0.003 ± 0.001	-	< 0.0006	0.02 ± 0.01	-	-	< 0.0006	0.01 ± 0.003	-	< 0.004	-	0.08 ± 0.002
ERM EC681m	PE	OC	0.004 ± 0.0002	-	< 0.0005	0.01 ± 0.001	-	-	< 0.0006	< 0.002	-	< 0.004	-	< 0.003
		TC	0.02 ± 0.01	-	< 0.0005	0.01 ± 0.003	-	-	< 0.0006	0.003 ± 0.001	-	< 0.004	-	0.65 ± 0.78
		ST	0.02 ± 0.001	-	< 0.0006	0.04 ± 0.003	-	-	< 0.0006	0.01 ± 0.004	-	< 0.004	-	< 0.003
SRM 2861	PVC	OC	0.01 ± 0.0002	1.53 ± 0.03	0.003 ± 0.0003	-	< 0.007	-	< 0.0006	0.07 ± 0.005	0.12 ± 0.01	10.4 ± 0.19	-	-
		TC	0.02 ± 0.0005	0.75 ± 0.28	0.007 ± 0.0002	-	< 0.007	-	< 0.0006	0.23 ± 0.01	0.14 ± 0.01	24.4 ± 0.86	-	-
		ST	0.03 ± 0.001	12.2 ± 0.10	0.02 ± 0.002	-	< 0.008	-	0.01 ± 0.003	0.35 ± 0.09	0.35 ± 0.01	47.9 ± 1.37	-	-
BRM S004	Glass	OC	-	-	-	0.01 ± 0.001	-	-	-	-	-	-	-	-
		TC	-	-	-	< 0.0003	-	-	-	-	-	-		-
		ST	-	-	-	0.06 ± 0.01	-	-	-	-	-	-	-	-
110–05-paint-02	Paint	OC	-	-	0.002 ± 0.001	-	-	-	1.43 ± 0.02	-	-	-	-	-
		TC	-	-	0.003 ± 0.0001	-	-	-	1.36 ± 0.02	-	-	-		-
		ST	-	-	0.85 ± 0.23	-	-	-	9.05 ± 0.74	-	-	-	-	-
CRM 013–50 G	Paint	OC	-	-	1.07 ± 0.05	2.74 ± 0.13	-	-	1.75 ± 0.06	-	-	-	-	-
		TC	-	-	2.04 ± 0.05	4.61 ± 0.06	-	-	0.31 ± 0.09	-	-	-		-
		ST	-	-	6.49 ± 0.64	14.6 ± 1.55	-	-	20.0 ± 2.31	-	-	-	-	-
102–02-SBSI5	Silicon bronze	OC	-	-	-	-	4.23 ± 0.20	0.68 ± 0.05	-	-	-	-	-	46.4 ± 6.66
		TC	-	-	-	-	0.12 ± 0.002	1.39 ± 0.15	-	-	-	-	-	68.8 ± 8.90
		ST	-	-	-	-	33.8 ± 1.95	2.60 ± 0.19	-	-	-	-	-	129 ± 6.62
SRM 856a	Aluminum	OC	-	-	-	0.002 ± 0.0004	< 0.007	< 0.0007	3.93 ± 0.24	-	-	7.19 ± 0.16	0.45 ± 0.01	1.47 ± 0.01
		TC	-	-	-	< 0.0003	< 0.007	0.005 ± 0.002	0.34 ± 0.04	-	-	14.9 ± 0.74	1.44 ± 0.02	2.18 ± 0.06
		ST	-	-	-	0.11 ± 0.09	0.14 ± 0.04	0.04 ± 0.01	35.4 ± 4.52	-	-	28.6 ± 1.24	2.48 ± 0.15	5.93 ± 0.20
SRM 872	Phosphor bronze	OC	-	-	-	-	83.9 ± 3.68	-	35.4 ± 2.81	-	-	2.27 ± 0.13	-	57.9 ± 0.11
		TC	-	-	-	-	0.40 ± 0.11	-	7.09 ± 3.42	-	-	3.64 ± 0.43	-	81.7 ± 8.31
		ST	-	-	-	-	126 ± 26.1	-	912 ± 42.2	-	-	28.9 ± 1.45	-	509 ± 30.8
SRM 875	Cupronickel	OC	-	-	0.04 ± 0.002	-	392 ± 29.7	20.0 ± 0.54	0.02 ± 0.01	-	0.01 ± 0.001	< 0.004	-	0.58 ± 0.08
		TC	-	-	0.09 ± 0.001	-	67.9 ± 4.92	36.5 ± 0.83	0.04 ± 0.002	-	0.03 ± 0.003	< 0.004	-	0.47 ± 0.12
		ST	-	-	0.59 ± 0.06	-	694 ± 94.3	206 ± 29.8	0.94 ± 0.14	-	0.05 ± 0.01	0.33 ± 0.12	-	6.62 ± 0.75
SRM 899	Nickel, alloy	OC	-	-	-	-	-	-	< 0.0006	-	0.02 ± 0.0004	-	-	-
		TC	-	-	-	-	-	-	0.02 ± 0.02	-	< 0.003	-	-	-
		ST	-	-	-	-	-	-	0.03 ± 0.01	-	0.07 ± 0.01	-	-	-

[a] OC: One-compartment model
[b] TC: Three-compartment model
[c] ST: Total concentration of metals extracted using artificial saliva
[d] -: Elements not contained in the CRM
[e] < LOD (mg/kg)
[f] ± : standard deviations (n = 3)

the one-compartment model, the three-compartment model, and based on the total concentration of heavy metal extracted using artificial saliva, respectively. In the case of SRM856a, the concentration of Cu was determined to be lower than the detection limit for the one-compartment model and the three-compartment model; however, it was 0.14 ± 0.04 mg/kg based on the total concentration analysis of the heavy metals extracted using artificial saliva (Table 1). In other words, we speculate that the difference in bioaccessible concentration between the total concentrations of metals extracted using artificial saliva and those determined using the one-compartment model is attributed to the formation of complexes between KSCN (potassium thiocyanate) and metal substances among the artificial saliva components. For example, SCN$^-$ is known to form various complexes with metals through interactions such as covalent and ionic bonding, thereby forming complexes such as $Cd(SCN)_2$, CuSCN, $Pb(SCN)_2$, $Se(SCN)_2$, $Sn(SCN)_2$, and $Zn(SCN)_2$ (Wechwithayakhlung et al., 2021). The complexes formed between the artificial saliva components and the extracted heavy metals could be included in the analysis because they were dissociated during the reactions with the artificial digestive fluids (gastric and intestinal fluids). This also means that 100% of the complexes formed between the artificial saliva components and heavy metals were not dissociated during the reactions with the gastric and intestinal fluids, or that some of the extracted heavy metals also formed complexes with the gastric and intestinal fluid components.

For Cu and Pb, the total concentration values obtained from acid digestion were the highest, followed by those obtained using the one-compartment model and those using the three-compartment model (Table 1). In the case of SRM 872, the Pb concentration was 35 ± 2.81, 7.09 ± 3.42, and 912 ± 20 mg/kg according to the one-compartment model, the three-compartment model, and based on the total concentration of heavy metal extracted using artificial saliva, respectively. In the case of SRM 875, the Cu concentration was 392 ± 29.7, 67.9 ± 4.92, and 694 ± 94.3 mg/kg using the one-compartment model, the three-compartment model, and based on the total concentration of heavy metals extracted using artificial saliva, respectively (Table 1). This means that the Cu and Pb extracted from CRMs using artificial saliva formed different complexes in large quantities through reactions with the artificial digestive fluids (gastric and intestinal fluids).

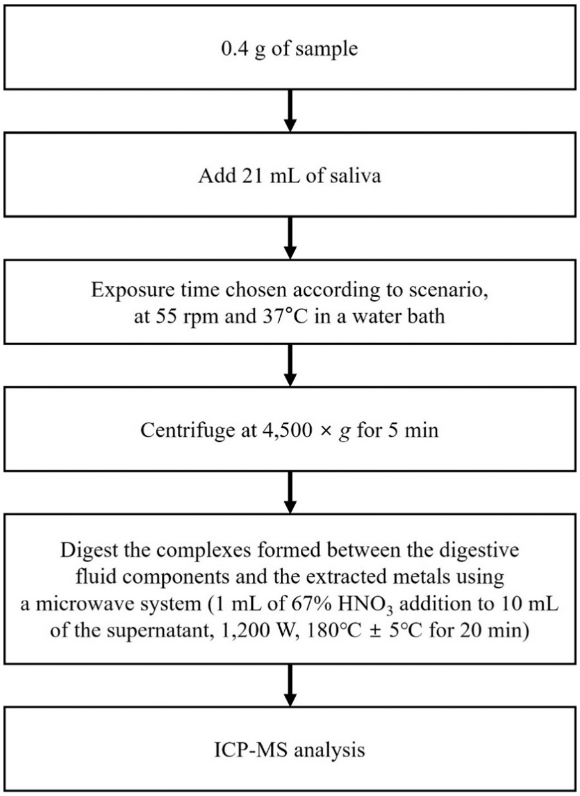

Fig. 2. Flow chart for determining the total metal/metalloid concentrations extracted from the consumer products using artificial saliva.

3.3. *Comparison among the calculated bioaccessibility values*

Table 2 shows the bioaccessibility values calculated based on the concentrations evaluated using the one-compartment model, the three-compartment model, and based on the total concentration of heavy metals extracted using artificial saliva. The average bioaccessibility value determined using the one- compartment model, three-compartment model, and based on total concentration of heavy metals extracted using artificial saliva were 0.19% ± 0.47%, 0.37% ± 0.92%, and 0.93% ± 2.45% respectively. However, in the case of Cd in CRM 013–50 G (paint), the bioaccessibility values determined using the one-compartment model, the three-compartment model, and based on the total concentration of heavy metal extracted using artificial saliva were 2.84%, 5.41%, and 17%, respectively (Table 2). Therefore, the bioaccessibility of heavy metals may notably vary depending on the total concentration of heavy metals present in a product and the composition of the product. For plastics, the average bioaccessibility was determined to be 0.13% ± 0.25%, 0.29% ± 0.54%, and 0.37% ± 0.87% using the one-compartment model, the three-compartment model, and based on the total concentration of heavy metals extracted using artificial saliva, respectively. For paints, the bioaccessibility was determined to be 0.74% ± 1.19%, 1.27% ± 2.33%, and 4.48% ± 7.01%, respectively. For metals, the bioaccessibility was determined to be 0.11% ± 0.18%, 0.20% ± 0.39%, and 0.77% ± 1.05%, respectively. These results indicate that metals and metalloids present in paints are more readily eluted compared to those present in other products. This finding indicates that the experimental assessment of bioaccessibility is essential for reasonably evaluating the human health risks associated with exposure to heavy metal-containing products.

3.4. *Proportion of complexes formed*

Table 3 shows the values obtained by dividing the heavy metal concentrations determined using the one-compartment model and the three-compartment model by the total concentration of heavy metals extracted using artificial saliva. Each value is expressed as a percentage and indirectly represents the amount of complexes that the heavy metals extracted from the CRMs formed with the saliva components and with the gastric/intestinal fluid components. The results of dividing the heavy metal concentrations determined using the one-compartment model by the total concentration of heavy metals extracted using artificial saliva ranged from 0.28% to 66.5%. Conversely, the results of dividing the heavy metal concentrations determined using the three-compartment model by the total concentration of heavy metals extracted using artificial saliva ranged from 0.33% to 105% (Table 3).

In the case of 110–05-paint-02, 99.7% of the total Cd extracted using artificial saliva seemed to have formed complexes with the saliva components since a ratio of 0.28% was obtained when comparing it to the total concentration. Moreover, this ratio did not notably vary, even after the reactions with the artificial gastric/intestinal fluids. In the case of ERM EC681m, 76.8% of the total quantity of as extracted using artificial saliva appears to have formed complexes with the saliva components (a ratio of 23.2% was obtained when comparing it to the total concentration). All of the complexes seem to have dissociated because a ratio of 100% was obtained when reacting with gastric and intestinal components. The ratio of Cr that participated in the formation of complexes with artificial saliva was found to be 81.2%; however, after the reactions with the artificial gastric/intestinal fluids, this ratio decreased to 68.4% in CRM 013–50 G. Nevertheless, in JSAC 0602–3, the ratio of Cr that was involved in the formation of complexes with artificial saliva was 84.3%;

Table 2

Bioaccessibility calculated based on the concentrations obtained using the one-compartment model and the three-compartment model, as well as the total concentration of heavy metals extracted using artificial saliva.

CRMs	Composition	Methods	Bioaccessibility (%)											
			As	Ba	Cd	Cr	Cu	Ni	Pb	Sb	Se	Sn	Sr	Zn
JSM P701	PE	OC[a]	< 0.0003[e]	-[d]	0.01	0.69	-	-	0.003	-	-	-	-	-
		TC[b]	0.003	-	0.02	1.12	-	-	< 0.001	-	-	-	-	-
		ST[c]	0.01	-	0.04	2.34	-	-	0.09	-	-	-	-	-
CRM 113–01–013	ABS	OC	0.07	-	< 0.004	< 0.0002	-	< 0.001	< 0.0004	< 0.001	-	-	-	-
		TC	0.17	-	< 0.004	< 0.0002	-	< 0.001	< 0.0004	< 0.001	-	-	-	-
		ST	0.27	-	< 0.004	0.03	-	0.01	0.01	0.01	-	-	-	-
JSAC 0602–3	PET	OC	-	-	0.02	0.01	-	-	< 0.001	-	-	-	-	-
		TC	-	-	0.03	0.01	-	-	< 0.001	-	-	-	-	-
		ST	-	-	0.09	0.06	-	-	0.03	-	-	-	-	-
ERM EC680m	PE	OC	< 0.01	-	< 0.003	0.02	-	-	< 0.01	< 0.02	-	< 0.02	-	0.03
		TC	< 0.01	-	< 0.003	< 0.003	-	-	< 0.01	0.05	-	< 0.02	-	< 0.002
		ST	0.07	-	< 0.003	0.20	-	-	< 0.01	0.07	-	< 0.02	-	0.04
ERM EC681m	PE	OC	0.03	-	< 0.0004	0.02	-	-	< 0.001	< 0.002	-	< 0.004	-	< 0.000004
		TC	0.12	-	< 0.0004	0.03	-	-	< 0.001	0.004	-	< 0.004	-	0.001
		ST	0.11	-	< 0.0004	0.10	-	-	< 0.001	0.01	-	< 0.004	-	< 0.00001
SRM 2861	PVC	OC	0.004	0.21	0.01	-	< 0.01	-	< 0.001	0.10	0.05	0.81	-	-
		TC	0.01	1.02	0.01	-	< 0.01	-	< 0.001	0.34	0.06	1.89	-	-
		ST	0.01	1.65	0.03	-	< 0.02	-	0.02	0.52	0.14	3.70	-	-
BRM S004	Glass	OC	-	-	-	0.002	-	-	-	-	-	-	-	-
		TC	-	-	-	< 0.0001	-	-	-	-	-	-	-	-
		ST	-	-	-	0.01	-	-	-	-	-	-	-	-
110–05-paint-02	Paint	OC	-	-	0.001	-	-	-	0.15	-	-	-	-	-
		TC	-	-	0.001	-	-	-	0.14	-	-	-	-	-
		ST	-	-	0.38	-	-	-	0.96	-	-	-	-	-
CRM 013–50 G	Paint	OC	-	-	2.84	0.44	-	-	0.27	-	-	-	-	-
		TC	-	-	5.41	0.75	-	-	0.05	-	-	-	-	-
		ST	-	-	17.2	2.36	-	-	3.12	-	-	-	-	-
102–02-SBSI5	Silicon bronze	OC	-	-	-	-	0.0005	0.03	-	-	-	-	-	0.07
		TC	-	-	-	-	0.00001	0.05	-	-	-	-	-	0.10
		ST	-	-	-	-	0.004	0.10	-	-	-	-	-	0.18
SRM 856a	Aluminum	OC	-	-	-	0.0004	< 0.00002	< 0.00002	0.36	-	-	0.72	0.25	0.02
		TC	-	-	-	< 0.0001	< 0.00002	0.0001	0.03	-	-	1.49	0.80	0.02
		ST	-	-	-	0.02	0.0004	0.001	3.22	-	-	2.86	1.38	0.06
SRM 872	Phosphor bronze	OC	-	-	-	-	0.01	-	0.09	-	-	0.01	-	0.14
		TC	-	-	-	-	0.00005	-	0.02	-	-	0.01	-	0.20
		ST	-	-	-	-	0.01	-	2.21	-	-	0.07	-	1.27
SRM 875	Cupronickel	OC	-	-	0.16	-	0.04	0.02	0.03	-	0.15	< 0.1	-	0.05
		TC	-	-	0.40	-	0.01	0.04	0.05	-	0.63	< 0.1	-	0.04
		ST	-	-	2.70	-	0.08	0.20	1.02	-	1.35	0.37	-	0.60
SRM 899	Nickel, alloy	OC	-	-	-	-	-	-	< 0.000001	-	0.00002	-	-	-
		TC	-	-	-	-	-	-	0.0001	-	< 0.000003	-	-	-
		ST	-	-	-	-	-	-	0.0001	-	0.0001	-	-	-

[a] OC: One-compartment model

[b] TC: Three-compartment model

[c] ST: Total concentration of metals extracted using artificial saliva

[d] -: Elements not contained in the CRM

[e] < LOD (mg/kg)

Table 3
Ratios of the heavy metal concentrations determined using the one-compartment model and the three-compartment model to the total concentration of heavy metals extracted using artificial saliva (i.e., 100[%] – the ratio of the formation of complexes between the components of artificial digestive fluids and the extracted heavy metals [%]).

CRMs	Composition	Methods	Ratio (%)											
			As	Ba	Cd	Cr	Cu	Ni	Pb	Sb	Se	Sn	Sr	Zn
JSM P701	PE	OC[a]/ST[c]	-[d]	-	21.3	39.5	-	-	3.65	-	-	-	-	-
		TC[b]/ST	37.2	-	46.7	47.7	-	-	-	-	-	-	-	-
CRM 113–01–013	ABS	OC/ST	26.8	-	-	-	-	-	-	-	-	-	-	-
		TC/ST	63.6	-	-	-	-	-	-	-	-	-	-	-
JSAC 0602–3	PET	OC/ST	-	-	16.0	15.7	-	-	-	-	-	-	-	-
		TC/ST	-	-	35.4	8.01	-	-	-	-	-	-	-	-
ERM EC680m	PE	OC/ST	-	-	-	9.20	-	-	-	-	-	-	-	64.0
		TC/ST	-	-	-	-	-	-	-	64.5	-	-	-	-
ERM EC681m	PE	OC/ST	23.2	-	-	17.9	-	-	-	-	-	-	-	-
		TC/ST	105	-	-	26.5	-	-	-	52.8	-	-	-	-
SRM 2861	PVC	OC/ST	27.6	12.5	16.0	-	-	-	-	19.0	33.7	21.8	-	-
		TC/ST	78.7	61.9	34.2	-	-	-	-	65.5	41.2	51.0	-	-
BRM S004	Glass	OC/ST	-	-	-	13.2	-	-	-	-	-	-	-	-
		TC/ST	-	-	-	-	-	-	-	-	-	-	-	-
110–05-paint-02	Paint	OC/ST	-	-	0.28	-	-	-	15.8	-	-	-	-	-
		TC/ST	-	-	0.33	-	-	-	15.0	-	-	-	-	-
CRM 013–50 G	Paint	OC/ST	-	-	16.5	18.8	-	-	8.75	-	-	-	-	-
		TC/ST	-	-	31.5	31.6	-	-	1.53	-	-	-	-	-
102–02-SBSI5	Silicon bronze	OC/ST	-	-	-	-	12.5	26.1	-	-	-	-	-	35.9
		TC/ST	-	-	-	-	0.35	53.3	-	-	-	-	-	53.2
SRM 856a	Aluminum	OC/ST	-	-	-	2.18	-	-	11.1	-	-	25.1	18.1	24.8
		TC/ST	-	-	-	-	-	10.9	0.96	-	-	51.9	58.1	36.7
SRM 872	Phosphor bronze	OC/ST	-	-	-	-	66.5	-	3.88	-	-	7.86	-	11.4
		TC/ST	-	-	-	-	0.32	-	0.78	-	-	12.6	-	16.0
SRM 875	Cupronickel	OC/ST	-	-	5.97	-	56.5	9.73	2.48	-	10.8	-	-	8.71
		TC/ST	-	-	14.7	-	9.63	17.7	4.66	-	46.5	-	-	7.16
SRM 899	Nickel, alloy	OC/ST	-	-	-	-	-	-	-	-	29.0	-	-	-
		TC/ST	-	-	-	-	-	-	71.2	-	-	-	-	-

[a] OC: One-compartment model
[b] TC: Three-compartment model
[c] ST: Total concentration of the metals extracted using artificial saliva
[d] -: Elements not contained in the CRM

nonetheless, after the reactions with the artificial gastric/intestinal fluids, this ratio increased to 92.0% (Table 3).

In summary, the ratio of the formation of complexes with the extracted metals and the digestive fluids components (i.e., artificial saliva, gastric/intestinal fluid) notably varied depending on the characteristics of the CRMs and the target heavy metals. Since the formation and dissociation of complexes can occur multiple times due to changes in the digestive environment (for example, through the influence of ingested food), the degree of heavy metal absorption may vary. Pelfrêne et al. (2020) and Rodrigues et al. (2018) reported that 0.65% HCl and 0.43 M HNO_3 could replace the bioaccessibility assessment using ISO 17924, which is known for its complexity and the excessive use of reagents. These studies are acknowledged to not accurately reflect potential metals that are absorbed into the human body, in relation to physiological factors, such as the human digestive process and the interactions between digestive fluid components and metal, which can be evaluated using ISO 17924. Therefore, the bioaccessibility evaluation was divided into stages and presented. The first stage involves a single extraction method, and the second stage is suggested to be evaluated using ISO 17924, which can simulate the composition and digestion steps in a manner similar to those occurring in the human body, thereby supplementing and validating the study. Additionally, measuring the total concentrations of heavy metals extracted from products using artificial saliva is favorable for a conservative assessment (Fig. 2). Regarding supplementation and validation of the findings, evaluation should be conducted using ISO 17924, taking into consideration the physiological aspects.

4. Conclusions

We compared the one-compartment model and the three-compartment model within the suck model of the RIVM, which is a method used for assessing the oral bioaccessibility of heavy metals in products associated with non-nutritive sucking habits. By comparing the two models, we validated that a considerable amount of complexes between saliva components and the extracted heavy metals are formed and that some of these complexes dissociate during reactions with the gastric/intestinal fluids. We also observed that in the cases of Cu and Pb, additional complexes are formed during the reactions with gastric/intestinal fluids. We measured the total concentrations of the extracted heavy metals through acid digestion using artificial saliva solutions and found that up to 99.7% of the heavy metals formed complexes, depending on the characteristics of the sample (e.g., composition) and the target element. This finding indicates that the current RIVM suck model notably underestimates the oral bioaccessibility of heavy metals in products associated with sucking habits. Considering the possibility that the digestive environment can change owing to ingested food and the inconsistency of the complex formation–dissociation characteristics, we believe that measuring the total concentrations of heavy metals extracted using artificial saliva is a conservative and simple method to assess the bioaccessibility of heavy metals in products associated with non-nutritive sucking habits.

Author contributions

Dong-Jun Baek: Writing – original draft, Investigation, Methodology. **Deok Hyun Moon:** Writing – original draft. **Seon-Woo Kwon:** Investigation. **Haeun Kim:** Investigation. **Sang-Gyu Yoon:** Investigation. **Ganesh T. Chavan:** Investigation. **Jung-Hwan Kwon:** Conceptualization, Funding acquisition. **Jinsung An:** Writing – review & editing, Conceptualization, Resources, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115270.

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