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TOXIC ELEMENTS IN TOYS AND CHILDREN'S LOW-COST JEWELRY:
OCCURRENCE, BIOACCESSIBILITY, AND RISKS FROM ORAL EXPOSURE

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OCCURRENCE, BIOACCESSIBILITY, AND RISKS FROM ORAL EXPOSURE

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RÉSUMÉ

La contamination des jouets et des bijoux de fantaisie destinés aux enfants par des éléments potentiellement toxiques est un problème courant. Les enfants peuvent être exposés aux jouets et aux bijoux qui peuvent contenir du Pb, du Cd ou d'autres métaux et métalloïdes toxiques par voie d'exposition orale. Le problème de contamination des bijoux et des jouets et la possibilité d'exposition des enfants à ces produits ont déjà été évalués dans une certaine mesure dans la littérature. En résumé, l'exposition devrait avoir lieu en raison du contact avec la bouche, qui est spécifique aux enfants. Plus précisément, les enfants pourraient être exposés aux éléments potentiellement toxiques dans les jouets et les bijoux pour enfants par (1) la mobilisation dans la salive suite au contact prolongé avec la bouche ou par (2) la mobilisation dans le système gastro-intestinal suite à l'ingestion des jouets ou des bijoux. Cependant, la bioaccessibilité orale des éléments potentiellement toxiques trouvés dans les jouets et les bijoux de fantaisie n'a pas encore été complètement évaluée, le potentiel d'exposition des enfants aux éléments toxiques dans les articles contaminés n'a pas été estimé et les risques n'ont pas encore été caractérisés.

Lors de cette thèse, une revue de la littérature a été premièrement réalisée pour déterminer et comprendre l'état des connaissances scientifiques, les différentes approches de sécurité, le contexte législatif et les besoins de recherche. Les législations des États-Unis, du Canada et de l'Union européenne (UE) qui contrôlent le contenu des métaux dans les jouets et bijoux ont été évaluées et comparées. Une revue de la littérature sur la contamination par les métaux dans les jouets et les bijoux de fantaisie, le contenu en métaux et leur biodisponibilité et l'évaluation de l'exposition a été effectuée. Après cela, afin d'évaluer l'ampleur du problème de contamination dans les jouets et les bijoux destinés aux enfants, la première étape du travail expérimental a été conduite. Les concentrations totales de l'As, du Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sb, et Se dans des jouets et des bijoux achetés sur le marché nord-américain (n = 72) ont été déterminées et, ensuite, comparées aux valeurs des limites réglementaires. En plus, la biodisponibilité orale des métaux a été estimée dans certains échantillons (n = 4) par des essais *in vitro* de bioaccessibilité et le potentiel de mobilisation des éléments a été démontré. À l'étape suivante, la mobilisation des éléments par la salive après le contact avec la bouche a été évaluée. Les jouets et bijoux contaminés (appartenant à l'une des catégories suivantes : jouets et bijoux métalliques (MJ),

jouets en plastique, jouets peints ou vernis, et jouets fragiles/flexibles; n = 32) ont été testés par l'étape de l'extraction par la salive des protocoles *in vitro* de bioaccessibilité DIN et RIVM. Après cela, afin d'évaluer la mobilisation des éléments dans le système gastro-intestinal suite à l'ingestion, la biodisponibilité de l'As, du Cd, Cu, Ni, Pb et du Sb a été évaluée (n = 24) par trois différents protocoles de bioaccessibilité *in vitro* (IVG, PBET et EN 71-3). Enfin, les risques sanitaires pour les enfants par la voie d'exposition orale ont été caractérisés en détail pour les jouets métalliques et les bijoux choisis (n = 16) en considérant trois scénarios d'exposition : ingestion de parties ou pièces provenant des jouets ou des bijoux, ingestion de matière grattée, mobilisation par la salive suite au contact avec la bouche. Par la suite, une approche globale comprenant l'établissement de limites bioaccessibles pour huit éléments prioritaires a été développée.

La revue de la littérature a révélé que bien que les législations américaines et canadiennes mettent l'accent sur la prévention de l'exposition au Pb (et dans une certaine mesure au Cd), d'autres éléments potentiellement toxiques dans les jouets et bijoux ne sont pas réglementés, sauf pour la peinture et le vernis. La législation de l'UE a été trouvée plus complète du point de vue des contaminants et des limites, et son approche scientifique est plus claire et avancée. Les limites actuelles et les procédures de test de jouets incluses dans les législations ne sont pas toujours basées sur le concept de biodisponibilité. Bien que le problème de contamination et les risques potentiels aient été suggérés dans une certaine mesure dans la littérature scientifique, la recherche sur la bioaccessibilité des éléments dans les jouets contaminés et les bijoux était insuffisante. En plus, aucune étude sur la caractérisation des risques n'a pu être trouvée. Finalement, il y a un besoin pour des études sur le développement des essais de bioaccessibilité *in vitro* validés avec des études *in vivo* pour les jouets et les bijoux, et les données de bioaccessibilité de divers métaux pour différentes matrices de jouets et de bijoux sont nécessaires.

La recherche sur les concentrations totales de métaux dans les jouets et bijoux de fantaisie pour les enfants achetés sur le marché nord-américain (n = 72) a révélé que, pour la catégorie MJ (n = 24), 20 articles avaient des concentrations totales qui dépassent les limites de concentrations de migration définies par la directive de sécurité des jouets de l'UE. Sept des dix-sept bijoux testés n'étaient pas conformes aux limites de concentrations totales présentées dans les règlements américains et canadiens. Ces échantillons comprenaient des articles en très haute teneur en Cd (37% [p/p]), Pb (65 %) et Cu (71%). Pour les jouets en plastique (n = 18), les jouets peints ou

vernis (n = 12) et les jouets fragiles/flexibles (n = 18), les concentrations totales étaient inférieures aux limites de migration de l'UE (sauf dans un échantillon pour chacune des catégories). Dans l'ensemble, les concentrations totales de l'As, du Cd, Cu, Ni, Pb, et du Sb étaient très élevés dans certains échantillons analysés. En outre, des essais de bioaccessibilité orale sur quatre articles ont montré qu'un bijou testé a fortement lessivé le Pb (quantité gastrique : 698 µg, intestinal : 705 µg) et le Cd (1,38 et 1,42 µg). Par conséquent, il était nécessaire de faire des essais additionnels de bioaccessibilité (salivaire et gastro-intestinale) pour les échantillons contaminés par des métaux.

La biodisponibilité des métaux des jouets et des bijoux de fantaisie dans la salive a été estimée par deux tests *in vitro* (compartiments d'extraction de salive des protocoles de bioaccessibilité DIN et RIVM, n = 32). Quatre métaux ont été mobilisés à partir de 16 MJ en quantités significatives (>1 µg pour le Cd et le Pb, >10 µg pour le Cu et le Ni). Ces concentrations bioaccessibles du Cd ont mené à des risques inacceptables pour les jeunes enfants entre 6 mois et 3 ans selon les résultats des deux protocoles. Les concentrations totales et bioaccessibles des métaux étaient différentes et pas toujours corrélées. Cela encourage l'utilisation de la bioaccessibilité au lieu des concentrations totales pour des évaluations de risques plus justes. De plus, la bioaccessibilité des métaux augmentait avec la durée de l'extraction (30 – 120 minutes).

Compte tenu de l'ingestion potentielle des jouets ou des bijoux, la biodisponibilité gastro-intestinale des éléments toxiques a été évaluée par trois protocoles de bioaccessibilité *in vitro* (IVG, PBET, EN 71-3; n = 24). Le Cd, le Cu, le Ni, et le Pb ont été mobilisés à partir de 19 MJ, et un ensemble de crayons. Les concentrations bioaccessibles du Cd, Ni, ou du Pb ont dépassé les limites de l'UE dans quatre à six MJ, selon le protocole. L'utilisation des essais de bioaccessibilité *in vitro* avec deux phases (gastrique et intestinale, ex. IVG ou PBET) est recommandée par rapport aux tests avec une phase (ex. la norme EN 71-3), parce que les essais avec deux phases sont plus conservateurs et représentent mieux la physiologie gastro-intestinale. Encore une fois, les concentrations des métaux bioaccessibles et totales étaient différentes et pas toujours corrélées, ce qui encourage l'utilisation de la bioaccessibilité pour une caractérisation des risques plus juste.

Finalement, la caractérisation des risques pour trois scénarios d'exposition (ingestion de pièces, ingestion de matériau gratté, mobilisation via la salive, n = 16) a montré que l'ingestion de pièces

ou de morceaux des jouets ou des bijoux a causé des niveaux de risque inacceptables (indice de risque [HI] >1) pour huit articles et ce pour le Cd, le Ni, et le Pb (HI aussi élevé que 75, 5,8 et 43, respectivement). L'ingestion de matériel gratté a indiqué un risque limité avec des valeurs de HI inférieures à 1 pour tous les échantillons (jusqu'à 0,29 pour le Cd, 0,16 pour le Pb). Le scénario de solubilisation dans la salive a mené à des valeurs de HI supérieures à 1 pour trois échantillons (deux pour le Cd, un pour le Ni). Les échantillons potentiellement dangereux identifiés par la caractérisation des risques étaient différents de ceux identifiés par les approches des États-Unis, du Canada et de l'UE. Une méthodologie globale a été développée pour pallier aux insuffisances des différentes approches de sécurité des jouets dues aux différences des définitions exactes d'un jouet et bijou, des méthodes d'essai de laboratoire, des scénarios d'exposition, et des éléments métalliques couverts. L'approche présentée dans cette thèse donne des définitions, des recommandations d'essai et des directives à appliquer pendant la caractérisation des risques ainsi que les limites bioaccessibles pour huit éléments prioritaires pour différents cas d'exposition.

La contamination des jouets et des bijoux par le Pb et le Cd, et dans une moindre mesure par le Cu, le Ni, l'As et le Sb, pose encore un problème important en Amérique du Nord, en particulier pour les jouets métalliques et les bijoux. Par conséquent, les réglementations américaine et canadienne actuelles qui reposent principalement sur la prévention de l'exposition au Pb et au Cd ne permettent pas toujours efficacement d'identifier les échantillons potentiellement dangereux. Il est montré dans la présente thèse que les éléments toxiques dans les jouets et les bijoux de fantaisie peuvent devenir bioaccessibles dans la salive suite au contact prolongé avec la bouche (surtout le Cd) ou dans le système gastro-intestinal suite à l'ingestion (surtout le Cd, le Ni, et le Pb). Les quantités bioaccessibles des métaux mobilisés par la salive ou les fluides gastro-intestinaux mènent à des valeurs de HI supérieures à 1 et entraînent donc un risque inacceptable pour les enfants en cas d'exposition. Le risque dû à l'exposition aux articles contaminés est plus important pour le scénario de l'ingestion de pièces, suivi par la solubilisation dans la salive après un contact prolongé. Comme les concentrations bioaccessibles et totales sont différentes et ne sont pas toujours bien corrélées, la bioaccessibilité devrait être utilisée pour identifier les objets contaminés et potentiellement dangereux, ainsi que pour caractériser l'exposition et évaluer les risques.

En ce qui concerne les éléments toxiques dans les jouets et les bijoux destinés aux enfants, des travaux de recherche supplémentaires sur la contamination par des éléments toxiques, la

bioaccessibilité, et l'exposition des enfants sont recommandés. Il est également nécessaire de développer un test de bioaccessibilité *in vitro* validé avec des études *in vivo*. Pour la caractérisation des risques, les paramètres d'exposition utilisés actuellement sont accompagnés de grandes incertitudes, donc, plus de recherche sur le temps de jeu des enfants, la fréquence et le temps de contact avec la bouche et les estimations des taux d'ingestion est nécessaire. La présence de Cr (VI) et Sn (org) devrait être recherchée dans les jouets et les bijoux, et des limites devraient être mises en œuvre si nécessaire. Enfin, le scénario d'exposition prolongée aux jouets métalliques et bijoux (le cas dans lequel un échantillon ingéré peut rester dans la voie gastro-intestinale pendant des jours ou des semaines) devrait être évalué et une caractérisation plus détaillée des risques qui l'accompagne est également recommandée.

ABSTRACT

Contamination of toys and low-cost jewelry with potentially toxic elements is a widespread problem. Children can be orally exposed to toys and children's jewelry that contain Pb, Cd, and other toxic metals and metalloids. Contamination problem in jewelry and toys, and possibility of children's exposure to these items have been previously investigated to some extent in the literature. Exposure may occur via oral pathway as a result of common child-specific behavior of object mouthing. The oral exposure may lead toxic elements to become bioavailable. More specifically, children can be exposed to potentially toxic elements in toys and children's jewelry (1) via saliva mobilization following mouthing, or (2) via mobilization in the gastro-intestinal tract following ingestion of toy or jewelry material. However, oral bioaccessibility of elements in toys and inexpensive jewelry has not yet been fully evaluated, and children's potential exposure has not been characterized.

In the present work, a literature review has been initially conducted in order to determine and clearly understand the state of scientific knowledge, different toy safety approaches, legislative background, and research needs. The U.S., Canadian, and European Union (EU) legislations regulating metals in toys and jewelry have been evaluated. A literature review on metals in toys and low-cost jewelry (content, bioavailability, children's exposure, and testing) has been performed. Then, the extent of contamination problem in toys and low-cost children's jewelry has been assessed by the first phase of experimental work. Total concentrations of As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sb, and Se in toys and jewelry (n = 72) bought on the North American market have been determined and compared to different regulatory limits, and, oral metal bioavailability in selected items (n = 4) has been estimated via bioaccessibility testing. In the next phase, selected toys and jewelry with contamination (categories: metallic toys and jewelry (MJ), plastic toys, toys with paint or coating, brittle/pliable toys; n=32) were tested using the saliva extraction (mouthing) compartment of the DIN and RIVM *in vitro* bioaccessibility protocols to assess mobilization of elements via saliva. Then, mobilization of elements in the gastro-intestinal tract following ingestion has been investigated by assessing As, Cd, Cu, Ni, Pb, and Sb gastro-intestinal bioavailability (n=24) via three different *in vitro* bioaccessibility protocols (IVG, PBET, and EN 71-3). Finally, health risk for children from oral exposure to contaminated

metallic toys and jewelry (n=16) has been characterized considering three scenarios: ingestion of parts or pieces, ingestion of scraped-off material, and saliva mobilization following mouthing. To deal with the shortcomings of current procedures on chemical safety of jewelry and toys, a comprehensive approach was developed including bioaccessible limits for eight priority elements.

The literature review has revealed that while the U.S. and Canadian legislations put emphasis on Pb (and to some extent Cd) exposure prevention, other toxic elements in toy and jewelry are not regulated except in paint or coating. The EU legislation is more comprehensive in terms of selected contaminants and limits, and its scientific approach is more advanced. Current limits and toy testing procedures mandated by legislations are not always based on bioavailability concept. Although the problem of toy and jewelry contamination and accompanying potential risks have been demonstrated to some extent in the scientific literature, research on the bioaccessibility of elements in contaminated toys and jewelry was very limited, and risk characterization studies were not available. Finally, *in vitro* bioaccessibility tests developed and validated for toys and jewelry, and metal bioaccessibility data for different toy and jewelry matrices were not available.

The investigation of total metal concentrations in toys and children's low-cost jewelry bought from the North American market (n=72) has revealed that for the category MJ (n = 24), 20 items had total concentrations exceeding migratable concentration limits defined in the EU Toy Safety Directive. Seven of 17 jewelry items did not comply with total concentration limits in U.S. and Canadian regulations. These samples included articles with very high Cd (37% [w/w]), Pb (65%), and Cu (71%) concentrations. For plastic toys (n = 18), toys with paint or coating (n = 12), and brittle or pliable toys (n = 18), total concentrations were below the EU migration limits (except in one toy for each category). Overall, total concentrations of As, Cd, Cu, Ni, Pb, and Sb were elevated in some of the analyzed samples. Testing for gastro-intestinal bioaccessibility on selected items (n=4) showed that a tested jewelry item strongly leached Pb (gastric: 698 µg, intestinal: 705 µg) and some Cd (1.38 and 1.42 µg). Therefore, it was found necessary to carry out saliva and gastro-intestinal bioaccessibility tests.

Considering the saliva mobilization of toxic elements following mouthing, elemental bioavailability in toys and low-cost jewelry has been estimated via two different *in vitro* tests (saliva extraction compartments of the DIN and RIVM bioaccessibility protocols, n = 32). Four

metals were mobilized to saliva from 16 MJ in significant quantities (>1 µg for highly toxic Cd and Pb, >10 µg for Cu and Ni). Bioaccessibility increased with extraction time. Bioaccessible concentrations of Cd caused unacceptable risk for young children between 6 mo- and 3 y-old. Total and bioaccessible concentrations were different and not always correlated, encouraging the use of bioaccessibility for more accurate hazard assessments.

Considering the ingestion of toy or jewelry material, gastro-intestinal bioavailability of toxic elements has been assessed via three *in vitro* bioaccessibility protocols (IVG, PBET, EN 71-3; n = 24). Cd, Cu, Ni, and Pb were mobilized from 19 MJ, and one crayon set. Bioaccessible Cd, Ni, and Pb concentrations exceeded EU migratable concentration limits in four to six MJ, depending on the protocol. Using two-phase (gastric and intestinal) protocols like IVG or PBET would be recommended over one-phase EN 71-3 since the former are more conservative and better represent gastro-intestinal physiology. Total and bioaccessible metal concentrations were also different and not always correlated, and the use of bioaccessibility in risk characterization is recommended.

Finally, risk characterization based on three exposure scenarios (whole ingestion of parts or pieces, ingestion of scraped-of material, saliva mobilization following mouthing; n = 16) showed that whole ingestion of parts or pieces caused unacceptable risk for eight items for Cd, Ni, and Pb (Hazard Index [HI]>1 and as high as 75, 5.8, and 43, respectively). Saliva mobilization scenario caused HI to exceed 1 in three samples (two for Cd, one for Ni), indicating a lower risk than ingestion of parts or pieces. Ingestion of scraped-off material lead to limited risk as HI was less than 1 for all samples (up to 0.287 for Cd, 0.156 for Pb). Potentially hazardous items identified via the risk characterization were different than the ones identified via the U.S., Canadian, and EU toy safety approaches. A comprehensive methodology has been developed to deal with the shortcomings of various approaches of toy safety and complexity due to different toy and jewelry definitions, test methods, exposure scenarios, and regulated elements. The presented approach provides definitions, testing recommendations, and guidelines for risk characterization, together with bioaccessible quantity limits for eight priority elements for different exposure scenarios.

Contamination of toys and jewelry by Pb and Cd, and to a lesser extent by Cu, Ni, As, and Sb, still poses an acute problem in North America. This is valid especially for the category of MJ. The U.S. and Canadian regulations, which mainly focus on Pb (and to a lesser extent Cd)

exposure prevention and also does not take contaminant bioavailability fully into account (limits for Pb and Cd for toys are based on total concentrations), may not be able to efficiently screen hazardous items in some cases. It has been shown in the present study that toxic elements can become bioaccessible in saliva following mouthing (especially Cd), or in gastro-intestinal system following ingestion (especially Cd, Ni, and Pb). Bioaccessible quantities of metals mobilized to saliva or gastro-intestinal fluids can cause HI to exceed 1, meaning that they may indicate unacceptable risk for children in case of exposure. The risk is the greatest for the scenario of whole ingestion of parts or pieces, which is followed by saliva mobilization. Since bioaccessible and total concentrations are not the same and are not always correlated, bioaccessibility should be preferably used for screening of contaminated items and during risk evaluations.

Regarding toxic elements in toy and low-cost or jewelry, further research on elemental contamination, bioaccessibility, and children's exposure is recommended. Developing an *in vitro* bioaccessibility test with *in vivo* validation is also needed. For risk characterization, exposure parameter estimations carry high amounts of uncertainty, and more research on play time for children, mouthing frequency and times, and toy material ingestion rate estimates is suggested. The presence of Cr(VI) and Sn(org) in toys and jewelry should be investigated, and additional limits should be implemented if necessary. Finally, research on prolonged exposure (the case in which ingested items may stay in gastro-intestinal tract for days or weeks) to MJ, and an accompanying more detailed risk characterization is also highly suggested.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

Al	aluminum
As	arsenic
ASTM	American Society for Testing and Materials
ATSDR	Agency for Toxic Substances and Disease Registry
B	boron
Ba	barium
BP	brittle or pliable toys
BSI	British Standards Institution
Cd	cadmium
CDC	Centers for Disease Control and Prevention
CDI	chemical daily intake
Cr	chromium
Cr(III)	trivalent chromium
Cr(VI)	hexavalent chromium
CMR	carcinogens, mutagens, and reproductive toxins
Co	cobalt
CPSA	Consumer Product Safety Act
CPSIA	Consumer Product Safety Improvement Act
Cu	copper
DIN	German Institute for Standardization
DL	detection limit
EU	European Union
Hg	mercury

HI	hazard index
IVG	*in vitro* gastro-intestinal protocol
MJ	metallic toys and jewelry
Mn	manganese
Ni	nickel
Pb	lead
PBET	physiologically based extraction test
PC	toys with paint or coating
PL	plastic toys
RfD	reference dose
RIVM	Dutch National Institute for Public Health and the Environment
RSD	relative standard deviation
Sb	antimony
Se	selenium
Sn	tin
Sn(org)	organic tin
Sr	strontium
TDI	tolerable daily intake
U.S.	United States
U.S.CPSC	Unites States Consumer Product Safety Commission
U.S.EPA	United States Environmental Protection Agency
Zn	zinc

LIST OF APPENDICES

PREFACE

Research activities realized for the completion of this dissertation have lead to five publications in journals with peer review, as well as to a number of conference presentations. The details of the publications and presentations are given below:

Peer-reviewed publications in scientific journals:

Guney, M. and Zagury, G. J. "Heavy Metals in Toys: Critical Review of U.S. and Canadian Regulations and of Scientific Literature", *Environmental Science & Technology*, 46, 4265-4274, 2012.

Guney, M. and Zagury, G. J. "Contamination by Ten Harmful Elements in Toys and Children's Jewelry Bought on the North American Market", *Environmental Science & Technology*, 47, 5921-5930, 2013.

Guney, M., Nguyen, A. and Zagury, G. J. "Estimating Children's Exposure to Toxic Elements in Contaminated Toys and Children's Jewelry via Saliva Mobilization", *Chemosphere,* submitted on September 17, 2013 (manuscript #: CHEM30359).

Guney, M. and Zagury, G. J. "Bioaccessibility of As, Cd, Cu, Ni, Pb, and Sb in Toys and Children's Jewelry", *Environmental Science & Technology*, accepted with minor revisions on November 10, 2013 (manuscript #: ES-2013-036122).

Guney, M. and Zagury, G. J. "Children's Exposure to Harmful Elements in Toys and Low-cost Jewelry: Characterizing Risks and Developing a Comprehensive Approach", *Science of the Total Environment*, submitted on October 7, 2013 (manuscript #: STOTEN-D-13-03258).

Conference presentations:

Guney, M., Zagury, G. J. "Concentrations élevées en Cd, Cr et Pb dans divers jouets et bijoux de fantaisie vendus au Québec : Mythe ou réalité? ", 15th Annual Symposium of Chapitre Saint-Laurent of SETAC, 26-27 May 2011, Montréal, QC, Canada, (oral presentation).

Guney, M., Zagury, G. J. "Biodisponibilité des métaux dans des jouets et des bijoux de fantaisie contaminés : Y a-t-il un danger pour nos enfants?", 16th Annual Symposium of Chapitre Saint-Laurent of SETAC, 7-8 June 2012, Québec, QC, Canada, (oral presentation).

Guney, M., Nguyen, A., Zagury, G. J. "Mobilisation des métaux par la salive via le contact avec la bouche des jouets et des bijoux de fantaisie contaminés : Y a-t-il un danger pour nos enfants?", 17th Annual Symposium of Chapitre Saint-Laurent of SETAC, 6-7 June 2013, Montréal, QC, Canada, (oral presentation).

Guney, M., Nguyen, A., Zagury, G. J. "Assessing mouthing bioavailability of Pb and Cd from low cost jewelry and metallic toys using synthetic saliva", 7th International Workshop on Chemical Bioavailability, 4-8 November 2013, Nottingham, U.K. (poster presentation).

Guney, M., Zagury, G. J. "Gastrointestinal bioavailability of Pb and Cd in inexpensive jewelry and metallic toys: Assessment via three *in vitro* tests", 7th International Workshop on Chemical Bioavailability, 4-8 November 2013, Nottingham, U.K. (poster presentation).

Other:

Guney, M. and Zagury, G. J. "Letter to Editor: Toxic chemicals in toys and children's products", *Environmental Science & Technology*, 45, 3819-3819, 2011 (letter to the editor).

INTRODUCTION

Background and problem definition

Children are more sensitive to contaminant exposure than adults due to their developmental status, and they have the potential to be exposed to higher levels of contaminants since they eat, drink, and breathe relatively more due to their lower bodyweight. Exposure to potentially toxic elements above toxicity thresholds has been shown to cause several serious, well documented toxic effects. As children are already exposed to certain levels of toxic elements via water, food, and soil, their additional exposure via contaminated toys and jewelry may pose additional risk and therefore must be investigated in detail.

Presence of toxic elements in toys and jewelry and related regulations

Toys and jewelry may contain high levels of potentially toxic elements due to several factors: use of metals as stabilizers in plastics, application of paint containing metal pigments, and use of contaminated recycled plastics or toxic metals in production. The extent of contamination of toys and low-cost jewelry seems to be large in North America considering numerous product recalls comprising millions of items within the last decade. Lack of regulations for certain contaminants combined with the problems in enforcement of current regulations is the main reason for the presence of contaminated toys and jewelry in the market. The focus of the U.S. and Canadian legislations regulating chemical safety of toys and children's jewelry is on Pb exposure prevention. While limits for Cd were recently introduced for jewelry, other potentially toxic elements are only regulated in paints and coatings. Both for the U.S. and Canadian regulations, the critical problem in legislations seems ignoring other potentially toxic elements which may be present in toys and jewelry while concentrating mainly on Pb exposure prevention, contrary to the new Directive on toy safety of the EU which includes migratable concentration limits for 19 metals defined for three material categories.

Elemental bioavailability and children's exposure to contaminated toys and jewelry

Oral bioavailability is the fraction of a contaminant reaching the systemic circulation from the gastrointestinal tract after ingestion, and oral bioaccessibility is the fraction of the substance that becomes soluble in the gastrointestinal tract and is thus available for absorption. Bioaccessibility can be used as an estimation of bioavailability and, when available, validated *in vitro* bioaccessibility tests might be preferred over *in vivo* bioavailability tests for their cost advantages and ethical considerations regarding *in vivo* testing. Especially for children younger than 6-y old, object mouthing is an important behavior where mouthing frequency and times vary according to specific age category. Due to prevalent mouthing behavior of young children, potentially toxic elements in contaminated toys and inexpensive children's jewelry may become bioavailable (1) via saliva mobilization following mouthing, (2) via mobilization in gastro-intestinal tract following ingestion of scrapings (i.e. scraped coatings, fibers or textile, or broken or brittle sections), or, (3) via mobilization in gastro-intestinal tract following ingestion of parts or pieces (i.e. jewelry parts or detachable toy pieces). Following exposure, there is a possibility that high amounts of contaminants may become bioaccessible and reach the systemic circulation. This may cause damage to various target organs depending on the exact chemical content of the toy, physiological and behavioral exposure parameters, and the level of bioavailability of the chemical of concern.

State of knowledge and research needs

The problem of contamination with potentially toxic elements in toys and jewelry has been demonstrated to some extent by the previous research, and the level of contamination was shown to reach very high levels especially in contaminated jewelry (an in-depth detailed discussion on this problem with references provided in the literature review presented in *Chapter 2*). However, research on the bioavailability estimation of potentially toxic elements in toys and jewelry is limited. Furthermore, although exposure potential of children via oral pathway to contaminated toys and inexpensive jewelry has been shown, risk characterization studies could not be found in the literature. Finally, the shortcomings of current toy safety approaches should be addressed in

detail and enhancements should be suggested, which could help better screen contaminated items, improve test procedures, more competently characterize risks, and more effectively protect children's health.

Plan of dissertation

The main purpose of this study is to determine the potential risk for young children in the case of oral exposure to toxic elements found in contaminated toys and children's low-cost jewelry. In this context, presence and bioaccessibility of various toys and jewelry have been investigated, and risks are characterized via deterministic approach.

In this dissertation, objectives, research approach, and hypotheses are first given in detail in the following chapter (*Chapter 1*). Then, a detailed literature review is presented on the contamination problem of toys and jewelry (*Chapter 2*). In this chapter, the extent of contamination in toys and jewelry, legal aspects, children's possible exposure to metals, and testing practices for toys and jewelry are investigated and discussed in detail. The investigation of the problem of contamination in toys and jewelry from the North American market consists of the first experimental phase of the present work, and findings of this phase are discussed (*Chapter 3*). Later, metals bioavailability in contaminated items have been assessed via bioaccessibility testing for the cases of saliva mobilization following mouthing of toys and jewelry (presented in *Chapter 4*), and ingestion of toy or jewelry material (presented in *Chapter 5*). Finally, a detailed risk characterization has been done considering different toys and jewelry, scenarios, and child age categories; and a comprehensive approach regarding chemical safety of toys for children has been developed (*Chapter 6*). A brief general discussion (*Chapter 7*), and conclusions and recommendations are presented at the end.

CHAPTER 1   OBJECTIVES, RESEARCH APPROACH, AND HYPOTHESES

1.1 Objectives and research approach

The principal objective of the present thesis is:

- To determine whether there is significant potential risk for young children in the case of oral exposure to toxic elements found in contaminated toys and children's low-cost jewelry.

The following specific objectives are presented and findings are discussed:

Objective 1

To evaluate current state of scientific knowledge on toxic elements in toys and children's low-cost jewelry: legislation, contamination, possibility of exposure, and bioaccessibility; and address research needs.

An in-depth literature review has been performed and data synthesis has been done. The outcome of this objective has lead to the publication of the following article, which is presented in *Chapter 2*:

> **Article #1:** Guney, M. and Zagury, G. J. "Heavy Metals in Toys: Critical Review of U.S. and Canadian Regulations and of Scientific Literature", *Environmental Science & Technology*, 46, 4265-4274, 2012.

Objective 2

To determine the current extent of contamination problem in toys and children's inexpensive jewelry sold on the North American market.

A laboratory study has been conducted to determine the extent of contamination in 72 toys and jewelry with ten harmful elements. The outcome of this objective resulted in the publication of the following article, which is presented in *Chapter 3*:

Article #2: Guney, M. and Zagury, G. J. "Contamination by Ten Harmful Elements in Toys and Children's Jewelry Bought on the North American Market", *Environmental Science & Technology*, 47, 5921-5930, 2013.

Objective 3

To assess the mobilization potential of toxic elements found in contaminated toys and low-cost jewelry via saliva following mouthing.

Saliva bioavailability of ten elements in 32 samples has been assessed via two different *in vitro* bioaccessibility protocols. The results from this laboratory study have lead to the submission of the following article, which has been presented in *Chapter 4*:

Article #3: Guney, M., Nguyen, A. and Zagury, G. J. "Estimating Children's Exposure to Toxic Elements in Contaminated Toys and Children's Jewelry via Saliva Mobilization", *Chemosphere,* submitted on September 17, 2013 (manuscript #: CHEM30359).

Objective 4

To assess the mobilization potential of toxic elements found in contaminated toys and low-cost jewelry in gastrointestinal tract via gastrointestinal fluids following ingestion.

Gastro-intestinal bioavailability of six selected metals in 24 samples has been assessed via three different *in vitro* bioaccessibility protocols. The results from this laboratory study resulted in the publication of the following article, which is presented in *Chapter 5*:

Article #4: Guney, M. and Zagury, G. J. "Bioaccessibility of As, Cd, Cu, Ni, Pb, and Sb in Toys and Children's Jewelry", *Environmental Science & Technology*, accepted with minor revisions on November 10, 2013 (manuscript #: ES-2013-036122).

Objective 5

To characterize risk for children following oral exposure to contaminated toys and inexpensive jewelry by considering contaminant bioavailability and different exposure scenarios.

The risk for children from oral exposure to contaminated toys and jewelry has been characterized in detail by considering three different exposure scenarios, and by using the experimental data from the present work and selected exposure parameters taken from the literature.

Objective 6

To develop a comprehensive methodology to assess safety of toys and children's jewelry for children in case of oral exposure which includes different scenarios, recommendations for testing and risk characterization, and bioaccessible limits for priority elements.

To overcome the shortcomings of current approaches and to reduce complexity due to various definitions, test methods, and risk evaluation guidelines presented in these approaches, a comprehensive methodology has been developed and presented.

The results from the conducted work regarding objectives 5 and 6 have contributed to the submission of the last article within the body of the present work, which is presented in *Chapter 6*:

Article #5: Guney, M. and Zagury, G. J. "Children's Exposure to Harmful Elements in Toys and Low-cost Jewelry: Characterizing Risks and Developing a Comprehensive Approach", *Science of the Total Environment*, submitted on October 7, 2013 (manuscript #: STOTEN-D-13-03258).

1.2 Hypotheses

Based on the context mentioned on the *Introduction* section, the following research hypotheses (in the form of null hypothesis) are formulated:

1. Extent of contamination of toys and jewelry in North America: Contamination of toys and children's low-cost jewelry sold on the North American market with toxic elements is not an ongoing widespread problem. While contaminated articles may be encountered from time to time, the extent of contamination is not significant. The null hypothesis will be refuted if a significant percentage of articles in a sample subcategory (>10% of items) contains one or more toxic elements above the limits presented in current regulations.

2. Saliva bioaccessibility: Toxic elements which may be found in toys and children's inexpensive jewelry are not mobilized into saliva following prolonged mouth contact. The null hypothesis will be refuted if saliva bioaccessibility tests simulating exposure via

mouthing yield detectable concentrations of one or more toxic elements in collected saliva samples.

3. Gastro-intestinal bioaccessibility: Toxic elements found in toys and children's low-cost jewelry are not solubilized into gastro-intestinal fluids following ingestion. The null hypothesis will be refuted if gastrointestinal bioaccessibility tests that simulate exposure via ingestion result in detectable concentrations of one or more toxic elements in gastric and intestinal fluids taken at the end of gastric and intestinal phases.

4. Risk for children: Exposure to toxic metals in toys and children's jewelry via saliva mobilization following mouthing or via mobilization in gastro-intestinal tract following ingestion does not cause unacceptable risk to children. The null hypothesis will be refuted if risk characterization conducted by using experimental results and selected exposure parameters yields hazard index values exceeding 1 for at least one or more elements.

CHAPTER 2   PUBLICATION #1 – LITERATURE REVIEW:

HEAVY METALS IN TOYS AND LOW-COST JEWELRY: CRITICAL REVIEW OF U.S. AND CANADIAN LEGISLATIONS AND RECOMMENDATIONS FOR TESTING

This chapter presents a literature review on heavy metals in toys and low-cost jewelry. It provides an extensive assessment on elemental contamination problem in various categories of children's toys and jewelry, children's possibility of exposure to these items, and testing methods for metals in potentially contaminated items. It also presents a critical evaluation and comparison of legal aspects and toy safety approaches from the U.S., Canada, and EU. Finally, by addressing specific information gaps and research and testing needs on elemental contamination in toys and low-cost jewelry, it provides basis for the justification of latter work conducted for and presented in this dissertation. This article has been published in the journal *Environmental Science and Technology* (volume: 46, page: 4265-4274, 2012)*, which ranks #1 in total citations in the Environmental Engineering and Environmental Sciences categories and has an Impact Factor of 5.257 according to the 2012 Journal Citation Reports from Thomson Reuters.

HEAVY METALS IN TOYS AND LOW-COST JEWELRY: CRITICAL REVIEW OF U.S. AND CANADIAN LEGISLATIONS AND RECOMMENDATIONS FOR TESTING

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ABSTRACT

High metal contamination in toys and low-cost jewelry is a widespread problem, and metals can become bioavailable, especially via oral pathway due to common child-specific behaviors of mouthing and pica. In this review, the U.S., Canadian, and European Union (EU)

legislations on metals in toys and jewelry are evaluated. A literature review on content, bioavailability, children's exposure, and testing of metals in toys and low-cost jewelry is provided. A list of priority metals is presented, and research needs and legislative recommendations are addressed. While the U.S. and Canadian legislations put emphasis on lead exposure prevention, other toxic elements like arsenic and cadmium in toy materials are not regulated except in paint and coatings. The EU legislation is more comprehensive in terms of contaminants and scientific approach. Current toy testing procedures do not fully consider metal bioavailability. In vitro bioaccessibility tests developed and validated for toys and corresponding metal bioaccessibility data in different toy matrices are lacking. The U.S. and Canadian legislations should put more emphasis on metal bioavailability and on other metals in addition to lead. A two-step management approach with mandatory testing of toys for total metal concentrations followed by voluntary bioaccessibility testing could be implemented.

2.1 Introduction

Human exposure to heavy metals is an important current problem. According to a recent report on human exposure to chemicals, urine analyses of Americans show proof of recent and cumulative exposures to various elements including arsenic (As), cadmium (Cd), and lead (Pb) (CDC, 2009). Children are highly sensitive to exposure to toxic substances due to their physiological and developmental properties and are already exposed to certain levels of metals via air, water, and soil (Trejo-Acevedo et al., 2009). In addition to background exposure, further exposure from other sources may increase the carcinogenic and non-carcinogenic risks. Specifically, mouthing or ingestion of contaminated toys and low-cost jewelry or dermal contact with those items may create additional risk.

Toys may contain high levels of toxic metals. Use of metals as stabilizers in plastics during manufacturing, application of paint containing metal pigments to toys and jewelry, and use of contaminated recycled plastics or metals in toy production are the main reasons for the metal contamination. Millions of toys have been recalled in the past few years because of chemical safety hazards (Morrisson, 2009), which included Mattel's recalls of 2 million toys for violating

lead paint standard (Mattel & Fisher-Price, 2007) and recall of 12 million glasses intended for children use by McDonald's due to the Cd detected in surface coating (U.S.CPSC, 2010a).

Oral bioavailability is the fraction of a contaminant reaching the systemic circulation from the gastrointestinal tract after ingestion, whereas oral bioaccessibility is the fraction of the substance that becomes soluble in the gastrointestinal tract and is thus available for absorption (Ruby et al., 1999). Bioaccessibility can be used as an estimation of bioavailability and, when available, in vitro bioaccessibility tests are preferred over in vivo bioavailability tests for their cost advantages and ethical considerations regarding in vivo testing. Metals may be released from toy and low-cost jewelry matrices by different pathways such as the following: via saliva during mouthing, via sweat during dermal contact, or via gastric fluids after partial ingestion (i.e., scraped coatings, fibers or textile, or broken sections) or whole ingestion (jewelry or detachable pieces). Hence, high amounts of metals can become bioavailable by reaching the systemic circulation and then potentially damage various target organs depending on the chemical content, physiological and behavioral exposure parameters, and bioavailability of the chemical of concern. For example, there is no safe level of Pb for children and, exposure to even very low concentrations are hazardous, adversely affecting IQ (Canfield et al., 2003). For other metals, potential risk from additional pathways is important because background exposure may be already high (As) or allowable exposure levels are low (Cd).

In the past, children's exposure to Pb via ingestion of various items has resulted in several cases with serious acute or chronic adverse effects, including death (CDC, 2004, 2006; Dargan, Evans, House, & Jones, 2000; Esernio-Jenssen, Donatelli-Guagenti, & Mofenson, 1996; Fergusson, Malecky, & Simpson, 1997; McKinney, 2000; Merritt, 2005; Mowad, Haddad, & Gemmel, 1998; VanArsdale, Leiker, Kohn, Merritt, & Horowitz, 2004). The ingested items were various, including necklaces, charms, costume jewelry, lead shots, snooker chalk, fishing sinkers, and clothing accessory. In general, the main focus on metal poisoning from objects including toys is on whole ingestion of Pb-containing items because Pb-contaminated items or paint are widely present, toxic effects of Pb are serious, and consequences of these incidents are severe. However, there are other metals posing serious toxic effects after uptake of low concentrations with high probability of exposure due to their possible presence in toys and low-cost jewelry. Moreover, in addition to whole ingestion and acute toxicity cases, chronic toxicity by metals can be observed following repeated exposure.

The present article includes a discussion of legal framework in the U.S. and Canada. The EU legal framework is also discussed since it contains a more thorough coverage of children's health issues. Moreover, some of the tests included in the current U.S. and Canadian regulations are based on EU documents. The current focus of North American legislations is on mechanical safety of toys and mainly Pb exposure prevention, while there are regulatory gaps in the area of chemical safety (Becker, Edwards, & Massey, 2010; Guney & Zagury, 2011).

This article aims to provide a critical literature review on metal content, bioavailability, and children's exposure to metals in toys and low-cost jewelry which are categorized here as plastic toys, toys with paint or coating, brittle or pliable toys, and metallic toys and jewelry. Phthalates and other organic contaminants of concern that can be present in toys are not addressed in this review due to space limitations. The article provides a list of priority metals specific to toys selected based on their likeliness of presence in toys, their potential bioavailability, and severity of their toxicological profile. Special emphasis is put on bioavailability and bioaccessibility concepts because they can provide a more accurate estimation of risk. The bioavailability concept is now well accepted in the field of soil metal pollution, and a number of in vitro tests validated against in vivo studies have been developed to measure metal bioaccessibility (more specifically As, Pb, and Cd) in contaminated soils (Juhasz, Weber, Smith, Naidu, Rees, et al., 2009; Rodriguez, Basta, Casteel, & Pace, 1999; Ruby et al., 1999; Schroder et al., 2004; U.S.EPA, 2008a). The concept of bioaccessibility, which can be used as an estimation of bioavailability, is useful to scientists and risk assessors to make a better estimation of risk following exposure to metal contaminated sites. Hence, current test methods and importance of bioavailability estimation via bioaccessibility testing for toys and low-cost jewelry are discussed. Future research needs and recommendations on legislative and scientific issues are also given.

2.2 Review of Legal Frameworks

2.2.1 United States

The Consumer Product Safety Act (CPSA) of 1972 and its amended 2008 version, the Consumer Product Safety Improvement Act (CPSIA) by Consumer Safety Product Commission (CPSC) of the U.S., include legal requirements for consumer products. Section 101 (a) of the updated

CPSIA in the U.S. has set the allowable amount of Pb in children's metal jewelry to 300 mg.kg^{-1} as of August 2009. Also, the maximum allowable amount of Pb in paint and surface coating has been limited to 90 mg.kg^{-1} in Section 101 (f) of CPSIA by August 2009. The CPSC has also stated that it may decide to decrease 300 mg.kg^{-1} limit to 100 mg.kg^{-1} by August 2011 (U.S.CPSC, 2012). As of August 14, 2011, all toys and children's products containing Pb in excess of 100 mg.kg^{-1} became banned hazardous substances. Section 106 (Mandatory Toy Safety Standards) of the CPSIA (U.S.CPSC, 2009) requires toys to satisfy the safety criteria set by ASTM standard F963-08 (ASTM International, 2008). The part of this standard on metals in toys, which is based on a section of the EU's former toy safety standard of migratable metals in toys EN 71-3 (BSI, 2006), sets migratable limits for 8 elements in paint and similar surface coating materials (60 mg.kg^{-1} for Sb, 25 mg.kg^{-1} for As, 1000 mg.kg^{-1} for Ba, 75 mg.kg^{-1} for Cd, 60 mg.kg^{-1} for Cr, 90 mg.kg^{-1} for Pb, 60 mg.kg^{-1} for Hg, and 500 mg.kg^{-1} for Se). These limits are exactly the same as the ones defined in EN 71-3, and the proposed test method for determination of migration limits is also taken from EN 71-3. This test is a 2 h migration test which more or less simulates human gastric conditions (the extraction of metals from a toy sample is performed in 0.07 M HCl at 37 ∘C). Although lower metal migration limits for modeling clay are defined in EN 71-3, these values are not present in ASTM F963-08 as it only includes limits for paint and coating. Recently published (November 2011) ASTM standard F2923-11 (ASTM International, 2011) on children's jewelry limits the presence of total Pb to 100 mg.kg^{-1} in jewelry and defines migration limits for Sb, As, Ba, Cd, Cr, Hg, and Se (having the same values as in ASTM 963-08) from paint and surface coating of children's jewelry. Moreover, a limit for total Cd concentration has been introduced as 300 mg.kg^{-1}. Any jewelry exceeding this value is required to go under a migration test (EN 71-3 for plastic jewelry and CPSC-CH-E1004-11 (U.S.CPSC, 2011) for metallic jewelry) where total migratable limit is stated as 200 μg.

Toy safety standards in the U.S. use an approach mainly based on Pb exposure prevention and on total metal content (except leaching from paints and coatings of toys and jewelry, and Cd in jewelry). Main toys and toy materials of concern are jewelry items, applied paint, and coating. Limits on total concentration of metals other than Pb in toys are not defined in the U.S. regulations, except for Cd in jewelry. Bioavailability of metals in toy materials is not properly considered in regulations.

2.2.2 Canada

Laws H-3 (Hazardous Products Act) and c.931 (Hazardous Products (Toys) Regulations) set the fundamental requirements for toy safety in Canada. H-3 limits the total Pb in surface coating materials to 600 mg.kg^{-1} and Hg to 10 mg.kg^{-1} (Government of Canada, 2005a). Coating on furniture and other articles for children, and coating on pencils and artists' brushes cannot contain more than 600 mg.kg^{-1} of Pb (Articles 2 and 18). Jewelry primarily appealing to children younger than 15 years old cannot contain more than 600 mg.kg^{-1} of total Pb and 90 mg.kg^{-1} of migratable Pb (Government of Canada, 2005b). Finally, total Pb limit in toys for children younger than 3 years old was recently reduced from 600 to 90 mg.kg^{-1} (Government of Canada, 2010). The law does not apply to certain items, provided that Pb is necessary to produce an essential characteristic of the part, no alternative part containing less Pb is available, and the part does not release more than 90 mg.kg^{-1} of Pb when tested in accordance to standard EN 71-3.

Similar to toy safety standards in the U.S., standards in Canada use an approach mainly based on Pb exposure prevention and focus on total Pb content with the main concern being on jewelry, paint, and coating. Limits on metal content other than Pb are not defined in regulations, except for Sb, As, Cd, Se, and Ba in surface coatings as 1000 mg.kg^{-1} dissolved in 5% HCl at 20 °C (limits for As, Cd, and Sb are very high compared to ASTM). For jewelry, the laboratory protocol proposed by Health Canada (2008) to test the 90 mg.kg^{-1} limit of migratable Pb is taken from the EU's EN 71-3. Migration and bioavailability of metals in consumer products are considered only for jewelry and coatings.

For both the U.S. and Canadian regulations, the critical problem in legislations seems ignoring other toxic metals which can be present in toys while mainly concentrating on Pb exposure prevention. On the contrary, the new Directive on toy safety in the EU (European Council, 2009) includes migratable concentration limits for 19 metals. Moreover, metal speciation is also considered, and separate values for highly toxic Cr(VI) and organic Sn are included in the European toy safety Directive. Recent recalls of large number of jewelry items due to their high Cd content in the U.S. and Canada (CBC, 2010; Health Canada, 2010a, 2010b; U.S.CPSC, 2010b, 2010c, 2010d) and studies showing contamination in paint and coating of toys (Kawamura, Kawasaki, Mine, Mutsuga, & Tanamoto, 2006; Kawamura, Mutsuga, Yamauchi, Ueda, & Tanamoto, 2009) and plastic toys (Kumar & Pastore, 2007) support the need to include

limits for other metals in the U.S. and Canadian regulations for other toy materials, in addition to paint and coating. Currently, the U.S. has made changes limiting the presence of total and migratable Cd in jewelry, and the Canadian government is in preparation to establish a new limit of 130 mg.kg^{-1} based on total concentration of Cd. Health Canada also made a call to industry to voluntarily stop use of Cd in jewelry manufacturing (2010c).

The concept of contaminant bioavailability is not properly included in the test methods proposed by the regulations. Therefore, regulations on total Pb concentration (100 mg.kg^{-1} for the U.S. and 90 mg.kg^{-1} for Canada) might be too strict in some cases. Another important concern is that in the regulations of both countries, there is no test developed to assess long-term migration of metals from ingested jewelry. A long-term migration test which has been developed by CPSC (U.S.CPSC, 2005a) was found in the literature (simulation of long-term leaching via a 6 h extraction test in an HCl solution). The scientific basis of this test is questionable since ingested jewelry items can stay in the gastrointestinal tract much longer than 6 h, in some cases many days before effects of metal poisoning are detected (VanArsdale et al., 2004). Moreover, to the authors' knowledge, there is no practical application of this test, and no regulatory article mandates its use. Ingestion of leaded jewelry by children caused tragic cases resulting in serious injuries and death in the past; and, a proper test which measures leaching potential and sets migration limits is therefore needed.

Published studies on toxic substances in toys (Becker et al., 2010; Weidenhamer & Clement, 2007a; Yost & Weidenhamer, 2008a, 2008b), recalled consumer products data including cases comprising millions of toys (Mattel & Fisher-Price, 2007; Morrisson, 2009; U.S.CPSC, 2007, 2010a, 2010b, 2010c, 2010d), and incidents reported in scientific literature (Merritt, 2005; VanArsdale et al., 2004) and national media (Ecology Center, 2008; Lynett, 2008; Pentland, 2008) from Canada and the U.S. show that there is also a lack of enforcement of the current regulations for both countries.

2.2.3 European Union

Toy safety in the EU was formerly regulated by the Council Directive 88/378/EEC (European Council, 1988). It contained migration limits for 8 elements (Sb, As, Ba, Cd, total Cr, Pb, Hg, and Se) from toys, and testing for migration of metals was performed according to the standard EN 71-3 (BSI, 2006). This standard had two separate migration limits: for modeling clay and for all

other toy materials. Contrary to the U.S. and Canadian regulations, the migration limits and specific test methods were defined not only for paint and coating but also separately set for polymeric toy materials, paper and paperboard, textiles, glass, ceramic and metallic material (specifically for wholly ingestible items like jewelry), and for other materials of any kind. EN 71-3 proposes a test method which mimics human gastric conditions. All the limits are based on ingesting 8 mg of toy material, and correction factors are applied to the results. Both of these points raised criticisms as an ingested amount would be deemed as too low, and the basis for the correction of results is unclear (European Commission, 2004).

The new EU Directive 2009/48/EC on the safety of toys (European Council, 2009) sets migration limits for 19 elements (Al, Sb, As, Ba, B, Cd, Cr(III), Cr(VI), Co, Cu, Pb, Mn, Hg, Ni, Se, Sr, Sn (total), Sn (organic), and Zn) for three different toy categories. The section regulating metal content in toy materials has been prepared after evaluating different studies done by government agencies and consultants, and the limits have been selected after evaluating the data from the toy safety report of Dutch National Institute for Public Health and Environment (RIVM) (van Engelen et al., 2006). The scientific basis underlying the selection of limit values and details of this report are discussed in detail in the *Review of Scientific Literature* section of this article. Very shortly, limit values are based on the assumptions of a percentage of tolerable daily intake for each metal for children, calculated based on a certain mass of ingested toy material. The highest concentration limits are defined for 'scraped-off toy material', followed by 'liquid or sticky toy material' and 'dry, brittle, powder-like or pliable toy material'. These limit values do not apply to toys or components of toys which does not pose any hazard due to sucking, licking, swallowing, or prolonged contact (i.e., not accessible).

Another important element of the EU Directive 2009/48/EC is the prohibition of carcinogens, mutagens, and reproductive toxins (CMRs) in toys. According to the Directive, the CMRs of category 1A, 1B, and 2 under the regulation EC 1272/2008 (European Council, 2008) cannot be used in toys. This part of the Directive could lead manufacturers to find safer alternatives to toxic substances during product design and ensure the production of safer toys for children.

At first glance, it can be said that the new EU regulation provides a more thorough coverage on children's health issues regarding metal exposure. First of all, it includes a higher number of contaminants than the U.S. and Canadian regulations. Some elements included in the Directive

(i.e., Al and Sr) may not necessarily be considered because their toxicity is of little concern due to either toxic effects, concentrations at which they are toxic, or their limited presence in toys. Second, speciations of Cr(VI) and organic Sn are considered in the legislation, although in practice there are difficulties in measuring Cr(VI) and organic Sn in consumer products, and, therefore, implementing these limits can be difficult. Third, three material categories are defined which may help for a better risk evaluation and prevention. However, a wide variety of all other toy materials except 'liquid or sticky toy material' and 'dry, brittle, powder-like or pliable toy material' has to fall into the category of 'scraped-off toy material'. It may not be proper to evaluate all the remaining toy materials in this category. Metal concentration limits on 'scraped-off toy material' category can be high since they are calculated on the assumption of ingesting only 8 mg toy material. This may not represent the most of the real life cases since (1) ingested amounts from scraping different toys (i.e., a plastic toy, a painted toy and a metallic toy) would differ from one to the other, (2) ingested amounts can easily exceed 8 mg, and (3) cumulative exposure from different toys is possible (Rastogi & Pritzl, 1996).

Finally, there is no specific test mandated on ingested contaminated jewelry in the new EU regulation to the authors' knowledge. In the new regulation, the bioavailability concept is taken into account. Current studies from governmental organizations on toy contamination and recall data show that practical application of the EU regulations on toy market is fairly successful.

2.3 Review of Scientific Literature

2.3.1 Metals contamination and bioaccessibility in toys

There are a limited number of studies in the literature on toy metal contamination and exposure. These studies generally focused on Pb since it is a high priority potential neurotoxin having well documented and irreversible effects on young children such as (1) a decrease in IQ (Canfield et al., 2003; Lanphear, Dietrich, Auinger, & Cox, 2000), (2) an antisocial and criminal behavior (Dietrich, Ris, Succop, Berger, & Bornschein, 2001), and (3) an increased risk of diagnosis with attention deficit hyperactivity disorder (Braun, Kahn, Froehlich, Auinger, & Lanphear, 2006). These studies have been reviewed and categorized as follows:

2.3.1.1 Plastic toys

Metals including Pb, Cd, and Zn are used as stabilizers (Kumar & Pastore, 2007), coloring agents, catalysts, or filling materials (Soares, Saiki, & Wiebeck, 2005) in PVC, and it was shown that these metals can leach from plastics (Kumar & Pastore, 2007). Lithner et al. (2009) reported that leaching from several plastic items caused chronic toxicity to fish, which included objects manufactured specifically for children (a bath tub toy, an inflatable bathing ring made of PVC, and a polyurethane children's handbag). They suggested that the toxicity in PVC was potentially caused by the metal-containing (Pb, Sn, Cd, Ba/Zn) stabilizers or plasticizers (mainly phthalates). The Danish EPA (Borling, Engelund, & Sørensen, 2006) tested a limited number of toys made of soft plastic foam (n = 8) by using the method stated in EN 71-3 and detected low metal concentrations, being below allowed limits. They also found that organic tin compounds and phthalates concentrations were above regulatory limits on some items.

Although direct release of metals from plastics may be expected to be low due to the complex and strong polymer structure of these toys, scenarios of repeated exposure to contaminated plastic toys or exposure to contaminated paint on the plastic toys can be important concerns for children's health.

2.3.1.2 Toys with paint or coating

Paint and coating can contain heavy metals. For example, leaded paint is cheaper than the nonleaded version (Barboza, 2007) and is sometimes used to reduce manufacturing costs. Kawamura et al. (2006) measured contents and migration of 8 elements in baby toys (mainly PVC) and paint according to EN 71-3. They found that Ba, Cd, Cr, and Pb were present in several samples, but leaching of metals was lower than the specified limits. They later reported that Cd and Pb leach from paint films under acidic conditions (Kawamura et al., 2009). Dutch Food and Consumer Product Safety Authority (Bouma & Reus, 2004) has made a market surveillance study and investigated toys for their chemical contents. In wooden toys (n = 64), Pb and Cd migration from paint layers were measured according to the EN 71-3. They detected Pb in 9 of the 64 samples, and in 2 samples the limit was exceeded, whereas Cd was not detected in any sample.

In general, paint can contain various pigments for coloration, of which, some may include metals. Children's exposure to painted toys can be dangerous if the paint is ingested after scraping, or when metals migrate from paint through saliva via mouthing or through sweat via dermal contact.

2.3.1.3   Metallic toys and jewelry

Contamination of children's jewelry items with heavy metals, especially by Pb and Cd is a widespread and acute problem. One of the main sources of Pb in jewelry items is thought to be the use of recycled electronic wastes and battery lead as a source material (Weidenhamer & Clement, 2007b, 2007c). Leaded electronic waste is exported from the U.S. to several Asian countries where solder is recovered and circuit boards are stripped of parts in small workshops. Weidenhamer and Clement found a composition of Pb, Sn, and Cu in some jewelry items, which were similar to the composition of solders in circuit boards. They also found that Pb−Sb composition was very similar to composition of battery Pb standard reference material. After the regulatory focus on Pb in children's products, some manufacturers turned to Cd as a substitute since Cd prices significantly dropped due to the decreased demand (Becker et al., 2010). Weidenhamer and Clement (2007a) detected widespread Pb contamination of imported low-cost jewelry in the U.S. Specifically, 42.6% of 139 jewelry items tested were found heavily leaded with an average concentration of 44% (w/w) Pb. Further testing of 10 items exceeding 30% Pb showed leaching of Pb greater than 175 μg over 6 h. They also noted a great variability of Pb concentration between individual samples of the same kind of jewelry. Yost and Weidenhamer (2008a) tested leaded jewelry and found 50 pieces exceeded 175 μg accessible Pb limit. Eighteen of these items leached 3000 μg of Pb or more. Yost and Weidenhamer also investigated the Pb content in plastic jewelry (2008b). Nine out of 100 items tested were Pb-polluted, with accessible Pb per bead being under 50 μg. Coatings obtained by scraping had Pb content far exceeding 600 mg.kg^{-1}. Similarly, Maas et al. (Maas, Patch, Pandolfo, Druhan, & Gandy, 2005) found that 169 of 285 collected items of children's and adult's jewelry contained Pb higher than 3%. Approximately 40% of the items had at least 50% Pb. Surface Pb transfer from laboratory wiping or handling (20 s of contact time) of these items yielded a transfer between 10 and 90 μg Pb to the wipe for 16 samples.

For metallic toys and jewelry, the ingestion of Pb-contaminated items is an important exposure pathway for children. Recent recalls of jewelry items due to the Cd content suggest that Cd

toxicity after whole ingestion can be another major issue, although there are no studies found in the literature on Cd contamination in toys and its leaching potential. While the primary concern for inexpensive jewelry is the exposure through ingestion of pieces, for other metallic toys, leaching during contact via sweat or during mouthing via saliva can also be important.

2.3.1.4 Brittle or pliable toys

Brittle and pliable toys can be ingested repeatedly in higher amounts than plastic toys or paint and coating on toys. Rastogi and Pritzl (1996) investigated the migration of 8 metals stated in EN 71-3 from crayons and water colors (n = 48). Migration of all target elements, except Cr and Se, occurred from both crayons and water colors. Mainly As, Ba, Hg, and Pb leached. Metal leaching from crayons and water colors were found below the limits stated in EN 71-3 (except for Ba for one sample and Pb for one sample), but authors noted that children may ingest much more material than the amount assumed in the EN 71-3 (8 mg). They also reported that migration limits may be far exceeded in real life cases. They recommended addition of migration limits for other elements and differentiation of migration limit for Cr(III) and Cr(VI) due to their different toxic potentials. In their studies on Pb bioaccessibility from chalk and paint, Oomen et al. (2004) stated that amount of toy matrix should be considered (solid to solution ratio) when determining the bioaccessibility and using results for exposure assessment. Low solid to fluid ratios better represent single events of excess ingestion, where high values represent hand-to-mouth behavior.

Contaminated brittle or pliable toys like chalk, crayons, or carton puzzles can be mouthed for long times. Due to their structural properties, large amounts of these materials can be directly ingested by children. Some fraction of brittle or pliable toys can also stick to hands, resulting in ingestion after mouthing of hands or uptake via dermal exposure.

2.3.1.5 Other toys and products

Rastogi (1992) measured Cd, Cr, Hg, and Pb residues in finger paints and makeup paints following total digestion. No Cd or Hg has been detected, but Cr and Pb were found in 19% and 28% of samples, respectively. Two samples had less than 10 mg.kg^{-1} of Cr, and 3 samples had less than 10 mg.kg^{-1} of Pb. Inexpensive, seasonal, and holiday products are items where quality of product and possibility of contamination can be underestimated due to their limited time of use. Weidenhamer (2009) investigated Pb contamination in inexpensive seasonal and holiday

products and found 20 out of 95 items having Pb concentrations higher than 60 mg.kg^{-1}. He reported high variability of Pb content of samples due to difficulties of sampling (done via scraping) and observed high variability between different samples from the same kind of item due to variation in different production lots. He concluded that mouthable items (i.e., fake teeth, sippers, and pens) could be especially of high concern.

2.3.2 Children's exposure to metals in toys

Children are already exposed to Pb and other metals from different sources (Glorennec et al., 2007; Yost et al., 2004). Infant, child, and adolescent exposures to environmental toxicants are different from those of adults because of the behavioral and physiological differences (Moya, Bearer, & Etzel, 2004). On a body weight basis, children breathe more air, drink more water, and consume more of certain foods than adults. They also often put their hands, toys, and other objects in their mouths during normal exploration of their environment. Children with pica behavior (the habitual eating of nonfood objects) are at even greater risk.

The main consideration in exposure of children to toy contaminants is the oral pathway, followed by dermal and inhalatory pathways. The category and properties of toy become important when considering the exposure pathways (Table 2.1).

Table 2.1 Exposure probabilities for children to elements in toys and jewelry considering various pathways

Category	*Oral exposure*				*Dermal exposure*	*Inhalatory exposure*
	Mouthing of toy	*Mouthing of hand residue*	*Ingestion (partial)*	*Ingestion (whole)*	*Dermal uptake*	*Inhalatory uptake*
Plastic toys	**High**	**High**	**High**	Low	**High**	Low
Painted or coated toys	**High**	**High**	**High**	Low	**High**	Low
Metallic toys and jewellery	Low	**High**	Low	**High**	**High**	Low
Brittle or pliable toys	**High**	**High**	**High**	Low	**High**	**High**

An important parameter for estimating children's exposure to metals in toys is mouthing frequency and time. Recommended values for mouthing frequencies suggested by the U.S.EPA (2008b) are between 1 and 24 contacts.hr^{-1}, where maximum value (24) belongs to 6−12 month-old babies, then the value decreases as the child gets older (10 contacts.hr^{-1} for 3−6 years). After the age of 6, mouthing is not a primary concern. Mean and 95th percentile durations for mouthing times were also suggested by the U.S.EPA for ages between 3 months and 3 years, changing between 8 and 13 min.hr^{-1}. The U.S.EPA stated that confidence in those recommended values may be low because magnitude of bias in original studies is unknown, representativeness of data to all the U.S. is low, data collection periods of base studies were short, and characterization of variability in population and description of uncertainties were unaddressed in those studies. Tulve et al. (2002) stated that toy mouthing showed large variability depending on age; for example, median contacts per hour in children younger than 24 months was 39 (95% C.I.: 31−48) and in children older than 24 months was 9 (95% C.I.: 7−12) for Washington State children (n = 186). Juberg et al. (2001) reported estimated daily mean mouthing times of New York State children for plastic toys as 17 min (n = 107) for children 0−18 months-old, which then decreased to 2 min for children 18−36 months old. Smith and Norris (2003) state that estimated average mouthing time is the longest between 6 and 9 months (39 min.d^{-1}) and decreases to 11 min.d^{-1} at 3 years old. The weighted average of the values from 1 month to the end of 3 yr is 15 min. A meta-analysis revealed that there was no statistically significant difference in hand-to-mouth frequency between genders or across studies (Xue et al., 2007). The authors of this study also stated that there is greater uncertainty and the need for additional data for the age groups of <3 months, 3−6 months, and 3−6 yrs.

Availability, variability, and uncertainty in other exposure parameters of children to contaminated toys, as well as in mouthing times and frequencies, are a concern (Bremmer & van Veen, 2002). For example, there are no data on the amount of ingested toy material although this information is highly needed for different materials and age categories. Estimations for other exposure parameters specific to exposure from toys such as body surface areas, leaching rates, and play time are scarcely available in the literature, and many parameters still have to be roughly estimated.

2.3.3 Bioaccessibility of metals

Evaluation of toy safety based on total metal concentrations is generally the main approach used in risk management since it is simple to understand and apply, and total metal content can be assessed easily and quickly. However, safety studies based on this approach may fail to sufficiently consider the differences in types of toys, pathways, and exposure scenarios and may lead to over-estimation of risk since bioaccessibility of contaminants is not taken into account. In assessing the risk via exposure, use of bioaccessibility data, rather than total concentrations of contaminant, provides a more realistic estimation of exposure. For this reason, the use of bioaccessibility concept is well established in the area of soil contamination, and in vitro tests that reproduce the human physiology have been developed and validated against in vivo studies for contaminated soils (Juhasz, Weber, Smith, Naidu, Marschner, et al., 2009; Rodriguez et al., 1999; Schroder et al., 2004; U.S.EPA, 2008a).

Former EU toy safety regulation EN 71-3 considers bioavailability of metals via a test which simulates gastric conditions and sets migration limits for 8 metals from different toy materials. Moreover, some tests required by the U.S. and Canadian authorities are also based on EN 71-3 as previously discussed. However, there are no in vivo studies performed to determine bioaccessibility of metals on toy materials. To the authors' knowledge, there is only one in vitro consumer product test for testing bioaccessibility of contaminants in toys (Brandon et al., 2006). This test is a modified version of an in vitro soil bioaccessibility test (Oomen, Rompelberg, et al., 2003) and is not validated against any in vivo studies for Pb or any other metals. This test is a three compartment model with mouthing, gastric, and intestinal phases, which aims to determine bioaccessibility of contaminants in toys by scenarios of suck, suck-swallow, swallow-fasted, and swallow-fed. The RIVM model is very detailed in terms of compartments, equipment, and chemicals required to mimic human physiology. For this reason, practical application of this test seems more difficult than other bioaccessibility tests designed for soils and impractical and costly compared to total metal content determination or HCl leaching tests (i.e., EN 71-3).

Apart from the consumer in vitro digestion model, RIVM has conducted further work on availability and exposure of contaminants in toys (van Engelen et al., 2006). The adequate approach to exposure of metals in toys by the authors considers mouthing, ingestion, dermal uptake, and inhalation as possible mechanisms. A risk based methodology was developed by the

authors using the requirement that children's exposure to elements should not exceed a certain percentage of their tolerable daily intakes (TDI). Under the assumption that ingestion is the primary exposure pathway and children ingest 400 mg of brittle or pliable toys, 100 mg of liquid or sticky toys or 8 mg of scraped-off toy material per day, alternative metal concentration limits corresponding to 5%, 10%, and 20% of TDIs for 18 metals have been proposed for the case of ingestion. The selected values were later used in the new toy safety regulation by the European Council (2009).

Although the new approach proposed by RIVM is more thorough than the approach in former regulations, limiting intake to a certain value of TDI has its drawbacks. For example, a TDI value would not be defined for carcinogenic substances like As or for the non-carcinogenic metal Pb since the effects of these elements are significant even at low uptake values. Also, correctness of TDI values is open to discussion for some metals, as most references are based on intake from food and water due to lack of studies in the scientific literature. Finally, limiting intake of metals for children, for example, to 10% of TDI can be an oversimplification and unnecessary in some cases, if the uptake of the metal from other sources is already very low or nonexistent (In such cases, higher percentages of TDI can be safely selected). It is recommended to select TDI values and corresponding percentages for limits based on the elemental toxicity and level of exposure from other sources. Also more research is needed in estimating the amount of ingested toy material similar to the detailed studies in the field of soil ingestion, as the values selected by RIVM are based on sole assumptions. Especially, the selected value for the ingestion of scraped-off toy material (8 mg/day) can be easily exceeded in certain cases. A very important critical point would be the case of pica behavior in children, and it must be definitely considered in exposure assessment of children to metals, as it is a common behavior in children.

The uptake of metals by mouthing and toy material ingestion is well covered in RIVM's report (van Engelen et al., 2006) and the new EU regulation of toy safety (European Council, 2009). Less importance is given to metal uptake via dermal contact or inhalation. It is not clear if the ingested material is taken following direct ingestion, during mouthing of toy or mouthing of hands containing residue from toys. These ingestion pathways should be considered separately since some pathways can be dominant for certain types of toys, and ingested amounts can be quite different for each pathway. Also mouthing of toys can cause additional exposure to metals even when toy ingestion does not occur, and based on the mouthing frequency and time data

from literature, it can be considered as an important pathway for metal exposure. RIVM suggests that dermal bioavailability should be taken into account only in the cases of Cr(VI) and Hg and does not consider neither dermal nor inhalatory exposure in detail. Oral pathway is definitely dominant in metal exposure from toys, but these additional pathways can be also important for specific toys or metals, hence additional literature research and a more detailed study of these pathways are needed.

Unfortunately, there are no scientific studies in the literature on metal bioaccessibility in toys other than some research done in The Netherlands. In their specific report on assessing Pb bioaccessibility in toy matrices, Oomen et al. (Oomen, van Twillert, Hofhuis, Rompelberg, & Versantvoort, 2003) applied three in vitro digestion models for Pb from 4 toy matrices (chalk for exterior use, chalk, finger-paint, paint flakes). The bioaccessibility in the intestinal compartment was lowest for the suck model (between <0.2% and 5.4%), higher for the suck-swallow model (between 0.1% and 23%), and the highest for the swallow model (between 1.6% and 51%). The bioaccessibility in the mouth compartment of the suck model was low (between <0.2 and 8.4%). These results may mean that risk assessment based on migration of Pb from toys under acid conditions is likely to overestimate the oral bioavailability, as the bioaccessibility of Pb in the small intestinal compartment (where absorption of the contaminant takes place) was 2- to 50-fold lower than the bioaccessibility of Pb in the stomach (van Engelen et al., 2006). While risk assessment based on metal migration under acidic conditions may overestimate bioavailability (and consequently, the risk) for the cases of mouthing or partial ingestion of toy items, risk can also be underestimated for the case of whole ingestion of toys (i.e., contaminated jewelry). This is because the exposure duration to ingested items (sometimes weeks) is much more than typical test durations (generally from 1 to 6 h).

Under these conditions, development and validation of more in vitro bioaccessibility tests specific to toys is recommended, considering mouthing, partial ingestion, and whole ingestion of toy items. Since there are already various published in vitro bioaccessibility tests which successfully predict metal oral bioavailability in soils when compared to in vivo tests (i.e., for As and Pb as stated by Juhasz et al. (Juhasz, Weber, Smith, Naidu, Marschner, et al., 2009; Juhasz, Weber, Smith, Naidu, Rees, et al., 2009)), these tests could be used as starting points. Because there are many kinds of toy materials, in vivo studies, in vitro test validation, and reference material development must first be done for priority toy materials (i.e., for metallic

toys and jewelry). Lacking bioaccessibility data for various toy materials should be generated by the use of appropriately developed and validated in vitro tests.

2.3.4 Testing of toys for metals

There are various tests used by different countries to investigate metals in toys. Although the main focus in the U.S. and Canada is on Pb content of toy materials and jewelry items, migration limits of 7 other elements (Sb, As, Ba, Cd, Cr, Hg, and Se, same metals in EN 71-3) are determined in surface coating materials and for Cd in jewelry in the U.S., and migration limits are set for 5 metals (Sb, As, Ba, Cd, Se) in Canada. In the EU, on the other hand, the toys are tested for the migratable concentrations of 19 metals and metalloids. When considering EN 71-3, it can be said that the elements to analyze (Sb, As, Ba, Cd, Cr, Hg, Pb, and Se) are properly selected. As, Cd, Pb, and Hg are highly toxic, especially Pb and Cd are found in various toy and jewelry samples, so these metals should be the priority contaminants in toys. Ba is used in paint and plastics manufacturing. Cr is widely used in manufacturing; however, only the hexavalent state seems important to consider in toy exposure as Cr(III) toxicity is only dangerous in high doses. Sb is used in batteries and in some alloys and may end up in metallic toys and jewelry. Like Ba, Se is a constituent of paint and plastics. In addition to the 8 elements provided by EN 71-3, for the cases of mouthing and partial ingestion of toy material (i.e., scraped-off material), testing of toys for Mn is recommended, since it has various uses including paint, and for total Sn, as it can be a possible indicator of the presence of highly toxic organic Sn. For the case of whole ingestion of metallic toys and jewelry, testing of Cu and Ni is recommended due to their possible presence in metallic items.

Speciation can be defined as the distribution of an element among defined chemical species in a system (UNEP - WHO, 2006). Metal speciation is important when considering exposure because it may strongly affect bioavailability. Sn exists in the oxidation states of 0, (II), and (IV) in inorganic tin compounds and as organotin compounds of tetravalent tin which is more toxic. The mobility and toxicity of Cr(VI) is high compared to Cr(III). For those reasons, testing of toys for Cr(VI) and organic Sn is recommended if total Cr and Sn measurements in toys give detectable concentrations.

In summary, testing of toys and inexpensive jewelry is suggested for the following elements:

1. As, Ba, Cd, Cr(total), Hg, Mn, Pb, Sb, Se, Sn(total); for the cases of mouthing and partial ingestion.

2. As, Ba, Cd, Cr(total), Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn(total); for the case of whole ingestion of metallic toys and jewelry.

For both cases, if Cr and Sn are present, further testing for Cr(VI) and organic Sn is recommended.

Finally, establishing extensive and rapid testing of toys for metals in the U.S. and Canada is important since numerous recalls of hundreds of thousands of various toys show that there are problems with toy contamination and proper enforcement of regulations. Rapid screening of toys for metals can be achieved by different methods. Portable X-ray fluorescence devices are capable of giving a close estimate of metal content in toys and toy materials in minutes (Kalnicky & Singhvi, 2001). Another analytical technique which can be used for direct analysis of toys for metals is laser induced ICP; however, high initial costs and operating expenses are important concerns for this technology. To test Pb presence in toys, there are commercially available Pb test kits on the market, but Health Canada does not recommend the use of these kits. Former experience shows that leaded jewelry is exceptionally heavy to its size, damp gray in color, and easily leaves gray trails when rubbed on white paper. Therefore, suspicious jewelry items can be quickly identified by using this technique for further testing. At last, toys made of PVC are of special concern because in addition to toxic metals they may contain organic contaminants and phthalates might be present. To quickly and economically screen the presence of PVC in plastic toys, a copper wire heated in Bunsen burner is put on plastic material to have a melted sample and is reheated in flame (Kumar & Pastore, 2007). A blue-green flame persisting for a few seconds indicates the presence of a halogen other than fluoride (possibly Cl in PVC items).

Development and use of a two-step approach for testing of metals in toys, toy materials, and inexpensive jewelry is suggested:

- Total metal determination (Step 1): Quick screening methods mentioned above can be used to identify potentially contaminated items. Total metal concentrations are then measured in potentially contaminated items, and results are compared to the maximum allowable limits, which can be defined by considering oral (mouthing and ingestion), dermal, and inhalatory pathways for a sufficient number of categories of toy materials (i.e., plastic toys, toys

with paint and coating, brittle or pliable toys, and metallic toys and jewelry) and by assuming 100% bioaccessibility of metals. More research on the estimation of important exposure parameters such as, mouthing times, play times, ingested amounts of toy materials, and dermal absorption is needed for proper determination of these limits. Furthermore, the percentages of TDI values used as limits must be selected for each metal by considering multiple exposures from different sources. Doing so will ensure that the combined exposure from all sources including toys will not exceed the TDI for each metal and, therefore, will not pose an unacceptable risk to children's health.

- Determination of bioavailable amount (Step 2): Assessing risk based on total metal concentrations by assuming 100% bioavailability may lead to an overestimation of risk. Hence, for toys having concentrations of metals above maximum allowable limits, to estimate bioavailability, manufacturers or importers can get voluntary bioaccessibility tests done at their own expense by third party testers. For this, in vitro bioaccessibility tests need to be developed and, if possible, validated against in vivo studies, considering the wide variety of toy materials present on the market. After the development of in vitro bioaccessibility tests, these can be used to determine the bioaccessible amount of metals in toy material, which is required to be less than the maximum allowable limits defined in step 1.

2.4 Conclusions and Recommendations

Presence of metal-contaminated toys and jewelry in the market is a widespread problem in the U.S. and Canada. The toxic metals in toys may come from poor manufacturing practices or use of contaminated materials in toy production. Child specific behaviors such as mouthing and ingestion put children at risk of being exposed to contaminants in toys. The U.S. and Canadian regulations put special emphasis on Pb exposure prevention, while other toxic metals which can be present in toy materials are not fully considered in the regulations, except for surface coating materials and Cd in jewelry. The EU has a more thorough coverage of toy safety by having a larger list of contaminants, diverse toy material categories and limits, and via a more efficient implementation of regulations. Contaminant bioavailability is incompletely considered in the U.S. and Canadian regulations and tests, but is taken into account in the EU regulations.

Adverse effects of metals and metalloids on children's health are well documented in the literature, and health consequences of exposure are quite severe for some of these elements. Total metal content in toys and low-cost jewelry has been scarcely studied, and the few published studies suggest that contamination and migration can be important problems. However, there is more research needed on child specific exposure pathways, determination of primary exposure parameters, development of in vitro bioaccessibility tests (with validation against in vivo bioavailability studies), derivation of bioaccessibility data for different toy materials, and assessing the quantitative risk for children.

Not all toy test methods consider contaminant bioavailability. Risk evaluation through total metal content determination might overestimate the risk, although this kind of testing is practical as it is quick and easy to apply. Other toy test methods based on migration under acidic conditions may overestimate the risk in the cases of mouthing and partial ingestion of toy materials or underestimate it in the case of whole ingestion of toy items. To the authors' knowledge, there is no consumer product in vitro bioaccessibility test for toys validated against in vivo studies for metals. Toy and jewelry testing is recommended for As, Ba, Cd, Cr(total), Hg, Mn, Pb, Sb, Se, Sn(total) for the cases of mouthing and partial ingestion and As, Ba, Cd, Cr(total), Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn(total) for the case of whole ingestion of metallic toys and jewelry. If Cr and/or Sn are present, further testing is appropriate for Cr(VI) and organic Sn due to their specific toxicological profiles.

Use of a two-step approach for testing of metals in toys and toy materials is suggested. First, by using quick screening methods and total metal determination, toys could be evaluated based on the limits separately defined for mouthable toys, toy material having potential of being partially ingested, and toys with potential of whole ingestion. Second, if total concentrations of certain metals are above the proposed limits, the manufacturer can voluntarily test the toy for the bioavailable amount of these contaminants by using in vitro bioaccessibility tests developed specifically for toys, to ensure that the bioavailable amounts are below the limit values.

CHAPTER 3   PUBLICATION #2 – CONTAMINATION BY TEN HARMFUL ELEMENTS IN TOYS AND CHILDREN'S JEWELRY BOUGHT ON THE NORTH AMERICAN MARKET

This chapter presents the results from the first experimental phase of the present dissertation. The main purpose of the work presented here is to determine the extent of contamination problem in a large sample set of toys and low-cost jewelry bought from the North American market and categorized into different groups. Oral contaminant bioavailability has also been estimated by subjecting selected samples to bioaccessibility testing. In this chapter, particularly problematic samples and categories have been identified, and the mobilization potential of metals from contaminated items has been demonstrated. The work presented in this chapter has been published in the journal *Environmental Science and Technology* (volume: 47, page: 5921-5930, 2013) which ranks #1 in total citations in the Environmental Engineering and Environmental Sciences categories and has an impact Factor of 5.257 according to the 2012 Journal Citation Reports from Thomson Reuters.

CONTAMINATION BY TEN HARMFUL ELEMENTS IN TOYS AND CHILDREN'S JEWELRY BOUGHT ON THE NORTH AMERICAN MARKET

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ABSTRACT

Toys and children's jewelry may contain metals to which children can be orally exposed. The objectives of this research were (1) to determine total concentrations (TC's) of As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sb, and Se in toys and jewelry (n = 72) bought on the North American market and compare TC's to regulatory limits, and (2) to estimate oral metal bioavailability in selected items (n = 4) via bioaccessibility testing. For metallic toys and children's jewelry (n =

24) 20 items had TC's exceeding migratable concentration limits (European Union). Seven of seventeen jewelry items did not comply with TC limits in U.S. and Canadian regulations. Samples included articles with very high Cd (37% [w/w]), Pb (65%), and Cu (71%) concentrations. For plastic toys (n = 18), toys with paint or coating (n = 12), and brittle or pliable toys (n = 18), TC's were below the EU migration limits (except in one toy for each category). Bioaccessibility tests showed that a tested jewelry item strongly leached Pb (gastric: 698 μg, intestinal: 705 μg) and some Cd (1.38 and 1.42 μg). Especially in metallic toys and jewelry, contamination by Pb and Cd, and to a lesser extent by Cu, Ni, As, and Sb, still poses an acute problem in North America.

3.1 Introduction

Children are particularly sensitive to metal exposure due to their physiological and developmental properties, and they are already exposed to certain levels of metals via multiple pathways, i.e., food, air, water, and soil (Glorennec et al., 2007; Sexton et al., 2005; Trejo-Acevedo et al., 2009; Yost et al., 2004). Especially for children younger than 6 years old, mouthing of objects is an important behavior where mouthing frequency and times vary according to age category (Juberg et al., 2001; Smith & Norris, 2003; Tulve et al., 2002; U.S.EPA, 2008b). Release of metals from contaminated toys or children's jewelry via mouthing or ingestion of contaminated matrix is possible; therefore, this exposure pathway may add to the present carcinogenic and non-carcinogenic risks for children caused by elemental exposure.

Toys and jewelry may contain high levels of metals due to several factors, i.e., use of metals as stabilizers in plastics, application of paint containing metal pigments, and use of contaminated recycled plastics or toxic metals in production. Lack of regulations for certain contaminants and problems in enforcement of regulations are the main reasons for the presence of contaminated toys and jewelry in the market (Becker et al., 2010; Guney & Zagury, 2011, 2012). In 2007 and 2008 alone, more than 100 recalls of children's products were issued in North America, including the ones comprising millions of items that have been made in the past decade in North America because of chemical safety hazards (Mattel & Fisher-Price, 2007; Morrisson, 2009; U.S.CPSC, 2010a). Also, in the past, children's exposure to Pb via ingestion of low-cost jewelry resulted in

cases of serious acute or chronic adverse effects, including death (CDC, 2004, 2006; VanArsdale et al., 2004). The sources and toxic effects of various contaminants in toys and children's jewelry have been previously discussed in detail, and the presence and leaching potential of metals were reported in the recent reviews of Becker et al. (2010) and Guney and Zagury (2011, 2012).

Metals may be released from toy and jewelry matrices in gastric and intestinal fluids following partial ingestion (i.e., scraped coatings, fibers, textile, broken sections) or whole ingestion (small jewelry items, detachable pieces). Hence, significant amounts of metals may become bioavailable and harm various organs once having reached systemic circulation. Oral bioavailability is the fraction of a contaminant reaching the systemic circulation from the gastrointestinal tract after ingestion, and oral bioaccessibility is the fraction of the substance that becomes soluble in the gastrointestinal tract and is thus available for absorption (Ruby et al., 1999). Bioaccessibility can be used as an estimation of bioavailability and, when available, validated in vitro bioaccessibility tests might be preferred over in vivo bioavailability tests for their cost advantages and ethical considerations regarding in vivo testing.

A limited number of studies have investigated the bioaccessibility of metals in toys, concentrating mainly on jewelry and solely on Pb or Cd bioaccessibility. Yost and Weidenhamer (2008b) used a test proposed by U.S. CPSC to determine accessible Pb in contaminated plastic jewelry (test conducted in 0.07 M HCl without digestive enzymes or an intestinal phase). Total accessible Pb measured in 9 items was between 7.5 and 1290 μg per item. Weidenhamer et al. (2011) recently investigated bioaccessibility of Cd in contaminated jewelry using saline and diluted HCl solutions (representing mouthing and ingestion, respectively). They found that Cd can leach to saline and, to a larger extent, to HCl solution. Brandon et al. (2006) investigated bioaccessibility of different contaminants in consumer products by using a detailed physiologically based in vitro digestion model. This study included a few toy samples (one finger paint and one chalk spiked with Pb, and one chalk contaminated by Pb), and only Pb bioaccessibility was investigated (reported as <1% for spiked chalk, 0.1−0.3% for contaminated chalk).

A systematic study investigating contamination in toys and children's jewelry by various harmful elements in a large set of items belonging to different categories is missing in the literature. It was found important to assess metal contamination in toys and children's jewelry in order to

identify potentially problematic toy groups and metals when the following points are considered: children's significant exposure to metals from other sources, toy and jewelry recall data in North America due to chemical hazards comprising millions of items, documented incidences and significant potential of exposure to contaminated items, and the presence and possible leaching potential of metals in toys and children's jewelry. The objectives of this study are as follows:

(1) To determine the extent of elemental contamination in various toy groups and children's jewelry (n = 72) in terms of total concentrations (As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sb, Se)

(2) To estimate bioavailability of elements by assessing gastrointestinal leaching potential in selected items (n = 4) using a physiologically based bioaccessibility test (IVG protocol)

3.2 Experimental Section

3.2.1 Selection of toys and children's jewelry

Articles analyzed in this study were grouped into four categories: metallic toys and jewelry (MJ), plastic toys (PL), toys with paint or coating (PC), and brittle or pliable toys (BP). MJ represents a category that can have very high concentrations of one or more metals, including articles with or without paint or coating. It has already been shown that low-cost jewelry can contain high amounts of Pb and Cd (Désy, 2012; Weidenhamer & Clement, 2007a; Weidenhamer et al., 2011; Yost & Weidenhamer, 2008b) and leaching potential of these metals was demonstrated (Weidenhamer et al., 2011; Yost & Weidenhamer, 2008b). Cd is used as a cheap substitute for Pb by manufacturers (Becker et al., 2010). Pb, Sn, or Cu may come from leaded electronic waste which can be recycled for use in jewelry production (Weidenhamer & Clement, 2007c). The PL category comprises a wide variety of toys including teethers and rattles, and toys in this category may include metals used as stabilizers during plastics production (Kumar & Pastore, 2007). Paints and coatings applied to toys in the PC category can also contain heavy metals. For example, leaded paint is cheaper than the nonleaded version (Barboza, 2007), therefore its use in manufacturing can be preferred by the toy industry. Finally, BP includes toys like play dough, carton puzzle, and chalk where the probability of ingestion and ingested amounts can be higher than in the other categories.

Ten of the 72 toys selected for this study were exact or similar items from a list of previously screened toys for their metal content via XRF analysis (Ecology Center, 2008) (samples PC10, PC11, PC12, MJ21, and PL05) or similar items to jewelry recalled by Health Canada (Health Canada, 2010d) (samples MJ14−18). The rest of the items were previously unscreened. All items were bought from the North American market: from dollar stores, toy shops, low-cost jewelry stores, retail chains, and on the Internet.

3.2.2 Determination of total metal content

For each sample, a part representative of a section of the toy or children's jewelry which may be subject to exposure was sampled and acid digested. One composite sample was prepared for toys when found necessary by crushing and mixing parts with different colors from the same toy. Additional subsamples (different parts or sections) were analyzed for MJ24 (2 subsamples) and PL05 (7 subsamples). In particular, for the MJ category, the entire item was tested for 10 samples (MJ01, 02, 03, 04, 05, 06, 11, 12, 13, and 30) while an intact part of an item (i.e., pendant from a jewelry) was used for the remaining 14 articles (see Table 3.1 for sample details). In summary, a total of 24 toys and children's jewelry from MJ, 18 toys from PL, 12 toys from PC, and 18 toys from BP have been analyzed for their elemental content (detailed descriptions of items given in Tables 3.1−3.4).

The digestions were performed according to the Standard Method 3030 (via HNO_3 digestion on a hot plate, followed by optional additional digestion via $HClO_4$ or $HClO_4$ /HF when necessary) (Clesceri, Greenberg, & Eaton, 1999). All analyses were made in duplicate (with the exception of samples PC01−19, BP27, and BP28 due to the limited amount of toy material available). Digestates and procedure blanks were filtered (0.45 μm), diluted to 50 mL, and preserved at 4 °C until analysis. As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sb, and Se concentrations in the digestates were measured by ICP-OES (method detection limits (in $\mu g \cdot L^{-1}$); As: 19.5, Ba: 0.12, Cd: 1.2, Cr: 1.8, Cu: 2.4, Mn: 0.24, Ni: 5.7, Pb: 16.6, Sb: 15.6, and Se: 34.3). The concentrations of elements in toy/jewelry material were expressed in $mg.kg^{-1}$.

3.2.3 Determination of bioaccessibility

Selected samples (MJ14, PL05/1, BP04, and BP11, n = 4), based on total metal content and toy/jewelry category, were submitted to the IVG bioaccessibility protocol (Rodriguez et al.,

1999). The IVG protocol is a physiologically based test that mimics gastrointestinal conditions at 37°C with the presence of NaCl, pepsin, bile, and pancreatin under controlled pH (with HCl). It has been widely used in the assessment of metal bioaccessibility in soils (Juhasz et al., 2010; Juhasz, Weber, & Smith, 2011; Welfringer & Zagury, 2009; Yu, Du, Luo, Huang, & Jing, 2012) and validated against in vivo studies to assess As, Cd, and Pb bioaccessibility for some soils (Rodriguez et al., 1999; Schroder et al., 2004; U.S.EPA, 2000). Bioaccessibility tests were made in triplicate and two procedure blanks were processed. The gastric phase of IVG was conducted for 1 h at a pH of 1.8 followed by the intestinal phase for 1 h at a pH of 5.5. A sample amount around 1 g was tested. Ten milliliters of sample was taken from each triplicate and from the procedure blank at the end of gastric and intestinal phases. After centrifugation (5000×g) and filtration (0.45 μm), 1 mL of HNO_3 was added to the samples. The samples were then preserved at 4°C until analysis. The concentrations of As, Ba, Cd, Cu, Pb, and Sb (elements with significant concentrations in selected items) were measured by ICP-OES. From liquid concentrations, migrated amounts of elements (in μg, = elemental concentration × volume of gastric or intestinal liquid) and migratable concentrations of metals in toy/jewelry material (mg.kg^{-1}, = migrated amount / sample mass) were calculated (Table 3.5).

3.2.4 Quality assurance, quality control

To assess accuracy of the digestion protocol, one standard reference material (SRM54d, tin-base bearing metal) was analyzed in duplicate. The reference values (%w/w) were 7.04 for Sb, 3.62 for Cu, 0.62 for Pb, and 0.002 for Ni. The results obtained were consistent with the certified values (%w/w, 8.98 for Sb, 3.50 for Cu, 0.65 for Pb, and 0.004 for Ni; within 20% of certified values for Sb, Cu, and Pb). In order to assess the reproducibility of the IVG protocol, As bioaccessibility in one standard reference material (SRM2710, Montana highly elevated trace element concentration soil) was determined in triplicate. Intestinal bioaccessibility of As was 29.2% (relative standard deviation [RSD]: 4.9%) and this was comparable to the value reported by Pouschat and Zagury (25.2%) (2006).

A procedure blank was also processed and analyzed for each set of experiments (10 digestions and 2 IVG tests). Total metal concentrations in blanks were always below or very close to method detection limits. Also, RSD's of duplicates for each analyzed sample were calculated (Tables 3.1−3.4). In total, 69.0% of all RSD values calculated were equal to or below 25%

(the fraction of the RSD values smaller than 25%: 81.7% for BP, 70.0% for MJ, and 54.2% for PL). Although the RSD values were generally small for the majority of the analyses, results from duplicates showed greater difference in some cases (especially in samples with lower metal concentrations). This is attributed to (1) sample heterogeneity during sampling (samples had to be prepared from different sections of the same toy/jewelry, or similar sections of two or more identical toys/jewelry items were used) and (2) variability of chemical composition of different items of the same type. It has been reported before that individual samples of the same type of jewelry can vary greatly in terms of Pb concentrations (i.e., individual samples tested by different laboratories showed analyses of 99.1%, 67.0%, and 0.07% Pb [w/w] for the same type of bracelet sold in the market), indicating opportunistic use of source materials for jewelry (Weidenhamer & Clement, 2007a) resulting in varying concentrations between different production batches.

Table 3.1 Concentrations of metals and metalloids (average [mg.kg^{-1}] ± RSD [%]) in metallic toys and children's jewelry

#	Item	Description	As	Ba	Cd	Cr	Cu	Mn	Ni	Pb	Sb	Se
		EU migration limit for scraped toy material (mg.kg^{-1})	47	56000	23	460	7700	15000	930	160	560	460
1	MJ01	Pewter ornament for scrapbooking	<4.51	0.16 ± 90	9.58 ± 0.7	≤2.22	**2.68E+04** ± 20	2.26 ± 25	**2.50E+03** ± 24	102 ± 16	139 ± 49	≤9.25
2	MJ02	Smiley face brads for scrapbooking	10.1 ± 2.9	0.24 ± 18	**180** ± 5.0	205 ± 0.5	142 ± 3.1	3.55E+03 ± 0.6	265 ± 7.7	**325** ± 8.6	211 ± 9.6	<6.58
3	MJ03	Pewter embellishments for scrapbooking	**431** ± 36	<0.01	**187** ± 4.0	1.85 ± 21	**1.25E+04** ± 7.3	1.33 ± 6.0	**5.05E+03** ± 6.3	**4.36E+05** ± 3.9	**1.02E+03** ± 58	16.7 ± 22
4	MJ05	Pewter embellishments for scrapbooking	≤1.59	0.22 ± 81	9.45 ± 26	133 ± 138	**1.30E+04** ± 1.6	280 ± 75	**2.28E+03** ± 52	157 ± 3.9	25.0 ± 6.1	≤2.60
5	MJ06	Pewter embellishments for scrapbooking	1.57 ± 0.3	≤0.01	15.2 ± 21	3.47 ± 5.4	**1.61E+04** ± 12	147 ± 0.0	**3.23E+03** ± 2.6	119 ± 7.1	17.5 ± 1.4	<1.48
6	MJ07	Decorative metal beads for craft	3.56 ± 12	0.12 ± 43	1.40 ± 5.8	12.1 ± 22	**5.68E+05** ± 1.7	2.05 ± 73	**9.20E+03** ± 4.3	**163** ± 10	28.8 ± 5.0	<5.09
7	MJ08	Bracelet with metal beads	43.5 ± 0.9	0.17 ± 65	**88.2** ± 0.3	173 ± 0.5	1.34E+03 ± 19	4.32E+03 ± 0.5	**1.40E+04** ± 1.0	86.1 ± 46	53.0 ± 0.0	<6.86
8	MJ09	Decorative metal beads for craft	≤4.75	0.46 ± 74	**263** ± 3.7	5.91 ± 3.4	**7.10E+05** ± 21	13.9 ± 0.1	**1.10E+04** ± 28	**469** ± 3.5	27.6 ± 6.8	≤8.26
9	MJ10	Bracelet with metal beads	<2.86	<0.02	8.00 ± 84	7.33 ± 24	**5.75E+05** ± 0.2	1.37 ± 15	**8.24E+03** ± 6.7	106 ± 13	23.2 ± 27	<5.04
10	MJ12	Decorative pins for scrapbooking	20.7 ± 4.3	1.33 ± 27	**73.8** ± 4.9	265 ± 6.0	1.73E+03 ± 11	3.67E+03 ± 5.3	**4.38E+03** ± 2.7	31.8 ± 38	39.3 ± 16	<6.56
11	MJ13	Decorative pins for scrapbooking	**56.8** ± 46	0.72 ± 2.2	**76.0** ± 1.4	211 ± 58	**3.16E+04** ± 15	3.15E+03 ± 6.3	**5.29E+03** ± 36	≤5.82	38.8 ± 33	<6.50
12	MJ14	Jewelry piece - metal rose	**62.4** ± 0.2	<0.03	**203** ± 3.8	0.76 ± 41	4.35E+03 ± 0.5	1.35 ± 106	5.94 ± 25	**6.53E+05** ± 11	272 ± 33	≤14.55
13	MJ15	Jewelry piece - metal key	<4.76	<0.03	**2.59E+05** ± 14	≤0.52	**1.36E+05** ± 18	1.68 ± 6.5	**1.03E+03** ± 69	**1.08E+03** ± 7.0	60.7 ± 1.4	≤8.53
14	MJ16	Metal earring	<4.41	<0.03	**1.66E+05** ± 13	2.75 9± 4	**9.50E+04** ± 7.7	5.02 ± 66	1.87 ± 23	37.0 ± 16	35.7 ± 16	<7.75
15	MJ17	Jewelry metal pendant	<5.25	≤0.09	12.7 ± 70	≤0.48	**1.58E+04** ± 5.9	1.25 ± 5.0	≤1.74	88.5 ± 0.4	38.6 ± 17	12.0 ± 1.1
16	MJ18	Jewelry metal pendant	≤7.95	0.98 ± 30	**3.67E+05** ± 18	10.8 ± 0.4	**6.86E+04** ± 85	1.19 ± 41	17.7 ± 62	100.0 ± 1.2	32.7 ± 43	50.6 ± 2.0
17	MJ19	Bracelet beads	5.16 ± 2.1	212 ± 11	8.62 ± 41	7.87 ± 5.4	**1.53E+05** ± 1.7	2.61 ± 22	24.1 ± 13	10.7 ± 23	173 ± 21	≤3.18
18	MJ20	Bracelet charm	≤5.36	<0.03	**57.1** ± 74	1.44 ± 20	**9.79E+04** ± 29	1.62 ± 22	≤1.42	<3.61	40.6 ± 21	≤10.09
19	MJ21	Plastic wristband bracelet with painted car figure on it	0.86 ± 3.0	8.26 ± 28	5.98 ± 118	0.90 ± 90	8.18 ± 22	0.14 ± 53	1.67 ± 25	<0.71	≤0.80	<1.47
20	MJ22	Bracelet - green beads	<0.65	0.07 ± 21	<0.04	0.09 ± 23	3.85 ± 9.2	≤0.01	<0.19	<0.55	≤0.59	<1.15
21	MJ24/1	Bracelet - multicolour beads	<0.99	301 ± 6.7	0.45 ± 53	≤0.12	2.37 ± 71	≤0.02	<0.29	<0.84	<0.79	<1.74
	MJ24/2	Bracelet - metal chain	**85.2** ± 4.7	≤0.57	**82.5** ± 3.3	307 ± 1.9	**2.82E+04** ± 3.2	3.72E+03 ± 1.9	184 ± 2.5	<3.33	48.2 ± 18	<6.87
22	MJ25	Necklace - silver plastic	15.7 ± 15	780 ± 9.1	17.5 ± 2.8	16.2 ± 17	298 ± 39	19.7 ± 7.9	59.3 ± 47	85.2 ± 3.9	**631** ± 40	<6.59
23	MJ26	Plastic hairclip	<4.23	40.6 ± 40	<0.26	1.48 ± 17	16.0 ± 73	1.20 ± 49	<1.23	<3.60	12.9 ± 8.6	<7.44
24	MJ30	Plastic hair accessory (beads)	<2.23	12.8 ± 100	<0.13	0.84 ± 41	0.66 ± 79	0.39 ± 44	<0.65	<1.90	<1.78	<3.92

(**Value**): Total metal concentration exceeding EU migration limit

<(Value): Concentrations analyzed in both duplicates are below method detection limit (DL). Average of DL's is given.

≤(Value): Concentration analyzed in one of the duplicates is below DL and the other is above DL. Average of two measurements is given.

Table 3.2 Concentrations of metals and metalloids (average [mg.kg^{-1}] ± RSD [%]) in plastic toys

#	Item	Description	As	Ba	Cd	Cr	Cu	Mn	Ni	Pb	Sb	Se
		EU migration limit for scraped toy material (mg.kg^{-1})	47	56000	23	460	7700	15000	930	160	560	460
1	PL01	Composite sample from plastic cord bracelet set	<3.27	0.30 ±72	<0.20	1.28 ±3.9	4.67 ±1.3	2.16 ±30	<0.95	<2.78	<2.61	<5.75
2	PL02	Eraser set shaped as food	24.9 ±33	8.93 ±57	0.23 ±23	0.67 ±22	10.8 ±32	13.4 ±4.2	1.37 ±2.6	76.5 ±5.2	182 ±1.0	<1.35
3	PL05/1	Jewelry design set - very small glass beads	**81.2** ±22	375 ±50	**771** ±69	4.50 ±6.7	19.2 ±75	13.7 ±27	2.99 ±35	≤8.03	18.1 ±39	156 ±49
	PL05/2	Jewelry design set - small glass beads	**207** ±67	347 ±5.5	**351** ±19	329 ±45	338 ±6.9	4.17E+03 ±75	22.3 ±57	24.2 ±92	39.8 ±69	≤5.96
	PL05/3	Jewelry design set - small plastic beads	<2.20	47.0 ±7.4	<0.13	0.75 ±13	2.43 ±107	0.40 ±31	<0.64	<1.87	<1.76	<3.87
	PL05/4	Jewelry design set - medium plastic beads	<1.31	56.6 ±56	<0.08	0.37 ±18	≤3.57	0.22 ±38	<0.38	<1.11	<1.05	<2.30
	PL05/5	Jewelry design set - plastic cords	<1.04	161 ±24	<0.06	0.18 ±46	0.57 ±30	≤0.08	<0.30	<0.88	1.56 ±51	<1.83
	PL05/6	Jewelry design set - beads with letters	<4.25	<0.03	<0.26	0.69 ±9.6	≤1.16	<0.05	<1.24	<3.62	<3.40	<7.47
	PL05/7	Jewelry design set - other	<1.21	122 ±7.4	<0.07	0.23 ±5.3	<0.15	0.07 ±82	≤0.38	<1.03	<0.97	<2.13
4	PL06	Rubber duck (painted section)	<1.32	0.11 ±50	<0.08	0.21 ±48	<0.16	<0.02	<0.38	<1.12	2.92 ±28	<2.32
5	PL09	Plastic toy cars set	<2.41	≤0.06	<0.15	0.43 ±13	≤0.44	≤0.05	<0.70	<2.06	<1.93	<4.25
6	PL10	Balloon	<2.30	1.21 ±9.8	<0.14	0.68 ±23	5.68 ±33	2.30 ±7.2	1.00 ±18	<1.96	<1.84	<4.05
7	PL11	Teether	<3.95	1.07 ±8.6	<0.24	0.62 ±18	<0.48	<0.05	<1.15	<3.36	<3.16	<6.95
8	PL13	Plastic horn	<5.17	<0.03	<0.31	0.88 ±3.2	<0.63	<0.06	<1.51	<4.40	10.3 ±32	<9.09
9	PL14	Animal figurine	<2.86	3.59 ±14	<0.17	4.65 ±2.6	<0.35	<0.04	<0.83	≤3.28	≤3.55	<5.02
10	PL15	Farm figurine	<4.63	1.27 ±13	<0.28	1.47 ±7.1	<0.56	≤0.21	≤2.25	<3.94	<3.70	<8.14
11	PL16	Tea set	<2.81	<0.02	<0.17	1.32 ±89	105 ±17	≤0.05	≤0.98	<2.39	<2.25	<4.94
12	PL19	Food set	<3.25	2.37 ±27	<0.20	3.97 ±123	<0.40	0.81 ±16	<0.95	<2.77	<2.60	<5.72
13	PL20	Cooking set	<4.61	6.35 ±118	<0.28	20.8 ±81	<0.56	0.16 ±12	≤1.63	<3.92	<3.69	<8.11
14	PL21	Food set	<2.83	≤0.04	<0.17	3.47 ±20	<0.34	2.88 ±12	<0.83	<2.41	<2.27	<4.98
15	PL22	Teether	<3.91	24.5 ±18	<0.24	2.88 ±63	11.8 ±0.6	0.08 ±10	<1.14	<3.33	49.7 ±17	<6.88
16	PL23	Cooking set	<4.74	11.2 ±1.8	<0.29	1.14 ±20	≤2.44	1.12 ±6.1	<1.38	<4.04	≤7.37	<8.34
17	PL24	Rattle toy	<5.57	≤0.11	<0.34	1.95 ±25	≤1.56	0.21 ±76	<1.62	<4.74	<4.45	<9.79
18	PL25	Rattle toy	<5.19	≤0.20	<0.31	≤0.69	<0.63	<0.06	<1.51	<4.42	<4.16	<9.14

(**Value**): Total metal concentration exceeding EU migration limit
<(Value): Concentrations analyzed in both duplicates are below method detection limit (DL). Average of DL's is given.
≤(Value): Concentration analyzed in one of the duplicates is below DL and the other is above DL. Average of two measurements is given.

Table 3.3 Concentrations of metals and metalloids (average [mg.kg^{-1}] ± RSD [%]) in toys with paint or coating

#	Item	Description	As	Ba	Cd	Cr	Cu	Mn	Ni	Pb	Sb	Se
		EU migration limit for scraped toy material (mg.kg^{-1})	47	56000	23	460	7700	15000	930	160	560	460
1	PC01	Yellow colored metallic paint on metal toy bus	<67.2	<0.42	<4.07	16.5 ± -*	<8.17	<0.83	<19.6	<57.2	<53.8	<118.3
2	PC05	Carmine red colored metallic paint on metal toy car	<67.7	3.28 ± -*	<4.10	<6.22	<8.23	3.46 ± -*	<19.7	<57.6	370 ± -*	<119.1
3	PC07	Chartreuse colored metallic paint on metal toy car	<51.6	<0.32	<3.12	6.03 ± -*	129 ± -*	<0.63	<15.0	<43.9	<41.3	<90.7
4	PC09	Blue colored metallic paint on metal toy car	<66.3	<0.41	<4.01	<6.09	2139 ± -*	<0.82	<19.3	<56.5	<53.1	<116.7
5	PC10	Blue and white colored metallic paint on metal toy car	<51.1	9.54 ± -*	<3.09	<4.69	624 ± -*	<0.63	<14.9	<43.5	<40.8	<89.8
6	PC11	Red colored metallic paint on metal toy car	<61.7	<0.39	<3.73	<5.66	<7.50	<0.76	<18.0	<52.5	131 ± -*	<108.5
7	PC12	Carmine red colored metallic paint on metal toy car	<70.7	<0.44	<4.28	**968** ± -*	<8.59	10.1 ± -*	<20.6	<60.1	<56.5	<124.3
8	PC13	Khaki colored metallic paint on metal toy car	<75.0	<0.47	<4.54	<6.88	<9.12	<0.92	<21.9	<63.9	<60.0	<131.9
9	PC15	Dark grey colored metallic paint on metal toy car	<72.8	<0.46	<4.40	<6.68	21.7 ± -*	<0.90	<21.2	<61.9	<58.2	<128.0
10	PC17	Light grey colored metallic paint on metal toy car	<75.0	<0.47	<4.54	<6.88	<9.12	<0.92	<21.9	<63.9	<60.0	<131.9
11	PC18	Grey colored metallic paint on metal toy car	<36.4	3.83 ± -*	<2.20	<3.34	32.9 ± -*	1.15 ± -*	<10.6	<31.0	<29.1	<64.0
12	PC19	Maroon colored metallic paint on metal toy car	<56.4	1.69 ± -*	<3.41	212 ± -*	<6.85	8.49 ± -*	<16.4	<48.0	<45.1	<99.1

(**Value**): Total metal concentration exceeding EU migration limit

<(Value): Concentrations analyzed in both duplicates are below method detection limit (DL). Average of DL's is given.

≤(Value): Concentration analyzed in one of the duplicates is below DL and the other is above DL. Average of two measurements is given.

*: RSD not calculated since only one sample was analyzed due to limited amount of sample available

Table 3.4 Concentrations of metals and metalloids (average [mg.kg^{-1}] ± RSD [%]) in brittle or pliable toys

#	Item	Description	As	Ba	Cd	Cr	Cu	Mn	Ni	Pb	Sb	Se
		EU migration limit for brittle or pliable toy material (mg.kg^{-1})	3.8	4500	1.9	37.5	622.5	1200	75	13.5	45	37.5
1	BP01	Green apple-scented play dough	<0.84	0.40 ±14	<0.05	0.23 ±17	0.96 ±8.8	3.69 ±0.9	<0.25	<0.72	≤1.15	<1.49
2	BP02	Play dough set (composite sample)	<1.09	43.8 ±30	<0.07	0.27 ±14	20.9 ±40	1.71 ±5.3	≤0.38	<0.92	2.28 ±23	<1.91
3	BP03	Infant educational carton puzzle	<1.64	28.6 ±12	<0.10	2.55 ±6.5	35.9 ±12	26.1 ±2.3	1.48 ±30	<1.40	<1.31	<2.88
4	BP04	Crayon set (tip and coating composite sample)	<1.58	2210 ±49	0.37 ±24	12.6 ±3.4	54.5 ±17	48.7 ±7.1	11.4 ±1.7	<1.35	<1.27	<2.78
5	BP05	Oil crayon set (composite sample)	<1.53	319 ±10	<0.09	3.36 ±37	216 ±57	31.4 ±0.0	3.30 ±15	<1.30	<1.22	<2.69
6	BP09	Outdoor chalk set (composite sample)	<0.86	12.0 ±7.9	0.07 ±19	1.18 ±4.5	1.14 ±21	10.8 ±6.5	1.09 ±14	<0.73	<0.69	≤2.13
7	BP11	Infant educational carton puzzle (composite sample)	<1.61	2.38 ±2.8	<0.10	0.83 ±5.9	1.22 ±14	4.53 ±9.1	0.74 ±20	<1.37	<1.28	<2.82
8	BP12	Puffy fishball	<1.18	1.82 ±10	<0.07	0.81 ±12	8.44 ±89	0.54 ±26	0.95 ±10	<1.00	<0.94	<2.07
9	BP15	Chalk set (composite sample)	≤0.63	54.0 ±28	0.11 ±0.2	1.34 ±5.4	41.7 ±6.4	78.9 ±4.8	1.29 ±4.4	<0.52	<0.49	<1.07
10	BP16	Color paint set (composite sample))	<1.48	171 ±39	0.25 ±7.8	1.39 ±16	67.8 ±57	39.9 ±1.5	1.06 ±23	<1.26	3.38 ±16	<2.61
11	BP18	Crayon set (tip and coating composite sample)	<1.46	1971 ±1.9	**2.81** ±10	32.2 ±4.9	301 ±15	32.2 ±4.1	25.1 ±0.4	<1.24	<1.17	<2.57
12	BP19	Crayon set (tip and coating composite sample)	<1.54	3205 ±14	<0.09	5.78 ±0.3	0.38 ±48	17.8 ±0.9	2.14 ±2.0	≤2.47	<1.23	<2.71
13	BP20	Auto construction carton model kit (composite sample)	<1.51	57.6 ±19	<0.09	3.56 ±1.0	21.7 ±4.3	41.0 ±2.2	1.66 ±8.6	<1.28	<1.21	<2.65
14	BP21	Infant educational carton puzzle (composite sample)	<1.32	11.8 ±8.9	<0.08	0.68 ±40	1.60 ±27	14.9 ±7.4	≤0.46	<1.12	≤1.23	<2.32
15	BP27	Sticker set (composite sample)	<1.58	2.86 ±-*	<0.10	2.04 ±-*	19.3 ±-*	4.03 ±-*	0.69 ±-*	<1.35	<1.27	<2.79
16	BP28	Sticker set (composite sample)	<1.73	28.7 ±-*	<0.10	7.98 ±-*	59.4 ±-*	0.42 ±-*	0.60 ±-*	<1.47	<1.38	<3.05
17	BP29	Modeling clay set (composite sample)	≤0.61	690 ±2.2	0.13 ±21	1.69 ±2.8	12.3 ±24	107 ±16	1.16 ±10	<0.51	≤0.64	<1.06
18	BP30	Pacifier	<4.52	<0.03	<0.27	0.83 ±15	≤1.01	1.51 ±52	≤1.95	<3.85	<3.62	<7.96

(**Value**): Total metal concentration exceeding EU migration limit

<(Value): Concentrations analyzed in both duplicates are below method detection limit (DL). Average of DL's is given.

≤(Value): Concentration analyzed in one of the duplicates is below DL and the other is above DL. Average of two measurements is given.

*: RSD not calculated since only one sample was analyzed due to limited amount of sample available

3.3 Results and Discussion

3.3.1 Comparison with EU Directive

Total metal concentration results (Tables 3.1−3.4) were compared to limits stated in the EU Toy Safety Directive (European Council, 2009). The limits defined in this directive were mainly used in the discussion, because the EU Toy Safety Directive provides a more comprehensive approach to the chemical safety of toys than North American legislations due to its sound scientific basis, the number of selected contaminants in the legislation, and different limits defined for toy material categories (Guney & Zagury, 2012). It is assumed that when total metal concentration exceeds the migration limit for a metal, this item can be potentially dangerous for children and further migration analysis is needed to check if migration of metals exceeds the proposed limits. In summary, 20 of 24 MJ, 1 of 18 PL, 1 of 12 PC, and 1 of 18 BP samples had at least one total metal concentration exceeding the EU migration limits.

3.3.2 Comparison with the U.S. and Canadian limits

The results were also compared with North American limits. Unlike the new EU legislation that imposes limits on 17 elements, the U.S. and Canadian limits only restrict Pb and Cd in jewelry, and Pb in toys (ASTM International, 2011; European Council, 2009; Government of Canada, 2010; Health Canada, 2011; U.S.CPSC, 2012). Also, the E.U. limits are based on migratable (bioaccessible) concentrations for three toy categories whereas the U.S. and Canadian limits are based on total metal concentrations (except for paints and coatings where migratable concentrations for 8 metals are defined). It was found that the U.S. total Pb concentration limit (100 mg.kg^{-1}) in children's products (U.S.CPSC, 2012) (applicable to all 72 toys and jewelry) has been violated by 11 of 24 MJ samples. No other samples from PL, PC, or BP category violated Pb limit. Considering the U.S. limits for total concentrations of Pb (100 mg.kg^{-1}) and Cd (300 mg.kg^{-1}) (ASTM International, 2011) in children's jewelry (applicable to 17 items: MJ07−10, MJ14−22, MJ24−26, and MJ30), it was found that 7 of 17 items did not comply with those limits (MJ07, MJ09, MJ10, and MJ14 for Pb content; MJ16 for Cd content; MJ15 and MJ18 for Pb and Cd content).

Similarly, Canadian limit of Pb total concentration (90 mg.kg^{-1}, applicable to all 72 toys and jewelry) for toys for children less than three years of age, as well as other products whose normal

pattern of use involves the product being brought into contact with the user's mouth (Government of Canada, 2010) has been exceeded in 11 of 24 MJ samples. No other samples from PL, PC, or BP category violated this limit. Finally, considering Canadian limit of Cd total concentration in jewelry (Health Canada, 2011) (130 mg.kg^{-1}, applicable to 17 jewelry items given above) combined with the Canadian limit for Pb mentioned above, it was found that 7 out of 17 jewelry items did not comply with these limits (MJ07 and MJ10 for Pb content; MJ16 for Cd content; MJ09, MJ14, MJ15, and MJ18 for Pb and Cd content).

3.3.3 Metal content of metallic toys and children's jewelry

The majority of samples in the MJ category contain high concentrations of metals (Table 3.1). These 20 of the 24 MJ samples (having at least one total metal concentration exceeding EU migration limits) can be dangerous for children if metals are mobilized following mouthing and/or ingestion of these items. Cu was abundant in most samples, followed by Ni, Cd, Pb As, Sb, and Cr. In particular, two samples (MJ03 and MJ14) had extremely high Pb concentrations (44% and 65%, respectively). Six samples exceeded the EU migration limit of 160 mg.kg^{-1} for Pb in scraped toy material. Also, 12 samples had high Cd concentrations (up to 37% w/w) exceeding the migratable limit of 23 mg.kg^{-1}. Total concentrations of Pb, Cd, Cu, and Ni exceeded the EU limits up to several thousand times in different articles.

It should be noted that three of the Pb-contaminated items were jewelry items (MJ14, MJ15, and MJ18) similar to those recalled by Health Canada due to high Pb concentrations (Health Canada, 2010d). Two other similar items (MJ16 and MJ17) were not contaminated by Pb. Also, the sample MJ21 (already screened by XRF and reported as contaminated (Ecology Center, 2008)) was found complying with all regulations.

Our results clearly show that Pb and Cd, being the primary concern of children's jewelry regulations in North America, are still present in various jewelry and metallic toys. Also, other potentially toxic metals (As, Cu, Ni, and Sb) regulated neither in the U.S. nor in Canada are present in metallic toys and jewelry. In fact, almost any metallic toy or children's jewelry that is made of metal contains large quantities of metals (19 of the 20 items in MJ having at least one total metal concentration exceeding the EU migration limits were made partially or fully of metal). Nevertheless, jewelry items made of plastic or other nonmetallic materials had a

much lower metal content (MJ21, MJ22, MJ25, MJ26, and MJ30). Among these items, only one was found contaminated (MJ25, with a Sb concentration of 631 mg.kg^{-1}).

The extent of Pb contamination in metallic toys and jewelry was lower than that reported by Weidenhamer and Clement (2007a) (42.6% of 139 low-cost jewelry items found heavily leaded, average concentration 44%) and by Maas et al. (Maas et al., 2005) more than five years ago (children's and adults' jewelry, 169 of 285 items containing greater than 3% Pb), but higher than that reported by Yost and Weidenhamer (2008b) (plastic jewelry, 9 out of 100 items contaminated) and Cox and Green (Cox & Green, 2010) (about 4% of jewelry not complying with Californian Pb standard). Especially for metallic jewelry, it seems that the use of Pb or Pb-contaminated material as source material in production is decreasing. However, given the facts that there is evidence suggesting the use of recycled electronic wastes and battery lead as a source material (Weidenhamer & Clement, 2007b, 2007c) as well as manufacturers' tendency to turn to Cd as a substitute to Pb (Becker et al., 2010; Désy, 2012) an important fraction of low-cost jewelry available to consumers still poses a threat to children. Continuous chronic exposure to metallic toys and children's jewelry is a concern since children can scrape and ingest small amounts of toy material or applied paint. Detachable or broken parts of highly contaminated jewelry can be ingested during one exposure event leading to further dangerous consequences, especially if the ingested item stays in the gastrointestinal tract for a long time. Ingestion of some jewelry may be possible considering their high appeal to children due to their assorted colors and/or brightness. Therefore, young children with the potential of mouthing behavior should be kept away from metallic toys with small parts or inexpensive jewelry at all times.

3.3.4 Metal content of plastic toys

The level of contamination in the PL category was generally low (Table 3.2). Only 1 of 18 samples analyzed (PL05, a jewelry design set mainly made of plastic beads and cords, but also including glass beads) contained 2 subsamples which were highly contaminated with As and Cd (total metal concentrations exceeded the EU migratable limits). Cr, Mn, and Se were also present in considerable concentrations. These subsamples were very small ($\sim$1−2 mm in diameter, PL05/1) with small glass beads ($\sim$3 mm in diameter, PL05/2) probably colored with contaminated paints. In case of ingestion, As and Cd in these beads could be potentially

mobilized and pose a threat to children's health. It should be noted that item PL05 was one of the toys that has already been screened by XRF and found contaminated (Ecology Center, 2008).

Other items in this category had low metal concentrations, in most cases below detection limits. Generally, articles with a higher probability of mouthing (i.e., food-shaped erasers, plastic toy food sets, or cooking sets) did not contain high levels of metals with the exception of PL02 (eraser set shaped as food) which contained elevated concentrations of As, Pb, and Sb (24.9, 76.5, and 182 mg.kg^{-1}, below the E.U. migration limits). This toy can also be categorized as a brittle/pliable toy. If categorized as such, total Pb and Sb concentrations in PL02 would exceed the EU migration limits for this category. Finally, Sb was present in elevated concentrations in a teether (PL22, 49.7 mg.kg^{-1}, below the EU migration limits).

Compared to MJ, exposure to plastic toys seems to pose a lower threat for children due to the lower concentrations of metals and the lower probability of metals release due to the matrix of plastic toys. Although classified as plastic toys, potentially problematic samples (PL05/1, PL05/2, and PL02) were not made of PVC (commonly used in toy manufacturing). The overall level of contamination in our study samples was found lower than that reported by Kumar and Pastore (Kumar & Pastore, 2007) (plastic toys in Indian market, contamination by Pb and Cd reported in certain items) and comparable to that reported by Danish EPA (Borling et al., 2006) (soft foam plastic products, total metal concentrations not measured, very low metal migration reported).

In general for PL, small painted plastic pieces that can be easily ingested can be problematic. Also, some specific toys with high probability of mouthing and ingestion of scraped material like imitation plastic food or plastic kitchenware toys can be dangerous. This being said, the main reported problems associated with plastic toys are related to injury risk due to mechanical hazards and exposure to organic substances like phthalates (U.S.PIRG, 2012).

3.3.5 Metal content of toys with paint or coating

Only 1 out of 12 PC samples (Table 3.3) contained 1 total metal concentration exceeding the EU migration limits (Cr in PC12, 1 of the 3 items included in this study was similar to those already screened by XRF (Ecology Center, 2008)). Cr, Cu, and Sb were also detected in certain samples. The contamination levels were similar to those reported by Kawamura et al. (2006) in a local

Japanese journal (low levels of Ba, Cd, Cr, and Pb present in some paint samples). A sampling problem was experienced with this toy category. Paint samples were taken via scraping, and despite great care and effort, the amount obtained was generally very low. While it is fairly easy to take a sample of 1 g of metallic or plastic sample, the amount of paint sample that can be properly obtained via scraping is generally in the order of 10 mg. Therefore, it was not possible to perform these analyses in duplicate. This difficulty also caused high detection limits (when concentrations were converted to $mg.kg^{-1}$ of toy material) for As and detection limits slightly exceeded the migration limit value for As for 11 of 12 toys.

The use of Pb or Pb-contaminated material as a source material seems to have decreased in the PC category as well as in metallic jewelry as stated before. None of the 12 tested items had Pb content exceeding 100 $mg.kg^{-1}$. Weidenhamer (2009) reported that 12 of 95 seasonal toys with paint purchased in the US during 2007−2008 had more than 600 $mg.kg^{-1}$ of Pb. Although the present study has a smaller sample size, the findings support the conclusion that recent regulations have had a positive impact on Pb content of toys with paint or coating. Considering the low concentrations of metals found in this toy category and difficulty of scraping paint by teeth, it can be said that exposure to these toys poses a lower threat to children compared to MJ.

3.3.6 Metal content of brittle or pliable toys

Stricter migratable metal concentration limits are defined in the EU regulation for dry, brittle, powder-like, or pliable toy materials (BP) than for the scraped toy material (MJ, PL, and PC). This is due to the fact that BP represents a special toy category where ingestion of larger amounts of toy material is possible in case of exposure. Results (Table 3.4) show that only 1 out of 18 BP samples exceeded migration limits (BP18 for Cd). Ba was also present in elevated concentrations in crayons (less than the migration limit for BP04, BP18, and BP19). The levels of contamination in samples were similar to those reported by Rastogi and Pritzl (1996) (no or low level metal migration in 92 of 94 samples of crayons and water colors). Although concentrations of metals were generally very low in this category, BP toys might still be dangerous for children considering the elevated probability of ingesting very large amounts of toy material in specific cases (i.e., children with pica behavior).

3.3.7 Bioaccessibility of metals in selected samples

Four samples (one jewelry and three toys; MJ14, PL05/1, BP04, and BP11) were tested to estimate bioavailability of As, Ba, Cd, Cu, Pb, and Sb by using the IVG bioaccessibility protocol (Table 3.5). MJ14 was selected due to its very high Pb content and high concentrations of Cd and As. PL05 was selected due to its high As and Cd content, whereas BP04 was selected mainly due to its elevated Ba content. BP11 had low levels of all metals for total content and was selected in order to see whether a high proportion of the metal content was bioaccessible. Tests for MJ14 and PL05 were made on intact items, while composite samples were prepared for BP04 and BP11 by crushing and mixing parts with different colors from the same article.

For MJ14 (having 65.3% Pb w/w), Pb was found in gastric and intestinal extracts (dissolved amounts: 698 and 705 μg, respectively). These values correspond to bioaccessible concentrations of 642 and 647 mg.kg^{-1} in jewelry material. These concentrations are above the EU migratable limit for Pb of 160 mg.kg^{-1} specifically defined for scraped toy material. However, it should be noted that the IVG protocol is neither mandated nor specifically recommended to test metal migration according to the EU. These dissolved concentrations also far exceed the CPSC recommendation of children not ingesting more than 175 μg of accessible lead in a short period of time (U.S.CPSC, 2005b). Also, considering that there is no safe level for Pb exposure, considering that exposure to even very low concentrations of Pb is hazardous (Canfield et al., 2003), and considering the serious consequences of previous exposure events (CDC, 2004, 2006; Merritt, 2005; VanArsdale et al., 2004), it can be concluded that ingestion of a jewelry item such as MJ14 may pose a serious threat to children. The dissolved concentrations of Pb from MJ14 were found comparable to the findings of Yost and Weidenhamer (Yost & Weidenhamer, 2008b). A simple test was used by these researchers (U.S. CPSC method, a 6 h extraction at 37 °C in 0.07 M HCl) and more than 175 μg of Pb leached from the majority of highly contaminated jewelry subjected to leaching test.

Table 3.5 Gastric (G) and intestinal (I) bioaccessibility of metals and metalloids in selected samples

	As	Ba	Cd	Cu	Pb	Sb
	Bioaccessibility (µg dissolved)					
MJ14-G	<2.96	ND	**1.38**	≤0.86	**698**	<2.37
MJ14-I	<3.01	ND	**1.42**	<0.37	**705**	<2.41
PL05/1-G	<2.97	≤0.89	<0.18	≤6.47	ND	<2.37
PL05/1-I	<3.01	**32.5**	<0.18	<0.37	ND	<2.41
BP04-G	ND	0.019	0.179	≤0.74	ND	ND
BP04-I	ND	<0.02	<0.18	≤5.49	ND	ND
BP11-G	ND	≤7.14	ND	≤2.53	ND	ND
BP11-I	ND	≤25.4	ND	≤1.59	ND	ND
	Bioaccessible concentration (mg.kg^{-1})					
MJ14-G	<2.78	ND	**1.31**	≤0.73	**642**	<2.23
MJ14-I	<2.83	ND	**1.34**	<0.34	**647**	<2.26
PL05/1-G	<2.86	≤0.86	<0.17	≤6.20	ND	<2.29
PL05/1-I	<2.91	**31.5**	<0.18	<0.35	ND	<2.32
BP04-G	ND	<0.02	<0.15	≤0.61	ND	ND
BP04-I	ND	<0.02	<0.15	≤4.44	ND	ND
BP11-G	ND	≤6.42	ND	≤2.22	ND	ND
BP11-I	ND	≤22.8	ND	≤1.44	ND	ND

(**Value**): Value above method detection limit (DL)
<(Value): Values for all triplicates are below DL. Average of DL's is given.
≤(Value): Values in one triplicate or two triplicates below DL and the rest above DL. Average of three measurements given.
ND: Bioaccessibility not determined since total metal concentration is below DL

For MJ14 with a total Cd content of 203 mg.kg^{-1}, dissolved Cd values in gastric and intestinal fluids were 1.38 and 1.42 µg in gastric and intestinal phases, respectively. These values correspond to bioaccessible concentrations of 1.32 and 1.34 mg.kg^{-1} in jewelry material, which are less than the EU migratable limit for Cd of 23 mg.kg$_{-1}$ defined for scraped toy material. 1.38 µg at the end of 1 h gastric phase also corresponds to 33.12 µg for 24 h assuming that jewelry stays in the stomach and that HCl-extractable Cd is linearly correlated to residence time, 20 although the linearity assumption should be verified for the IVG protocol by experiments using longer testing times. This calculated value is lower than the maximum allowable acute exposure for a young child stated as a questionable 200 µg.d^{-1} by CPSC (U.S.CPSC, 2010e). The leaching of Cd from the same jewelry was lower than most of the 24 h accessibility values found by Weidenhamer et al. (2011); however, the total Cd in MJ14 (203 mg.kg^{-1}) was much lower than the concentrations found in that work (mean: 35.9%) and in some of our samples. For the other three toy samples, dissolved elemental concentrations and calculated migratable concentrations were below detection limits with the exception of Ba for PL05/1 for the intestinal phase.

Overall, the most problematic category was found to be metallic toys and jewelry due to not only very high concentrations of metals (Pb and Cd, followed by Cu and Ni, and to a lesser extent As and Sb), but also leaching potential of Pb and Cd in the gastrointestinal system. The categories of plastic toys, toys with paint or coating, and brittle or pliable toys were found less problematic. It is recommended that total concentration limits are immediately imposed for highly toxic elements such as Pb, Cd, and As not only in children's jewelry, but also in toys. In the meantime, since total metal content may not be an appropriate indicator of bioavailable metal, further research is recommended to determine oral bioaccessibility of metals (mainly Pb and Cd, followed by Cu, Ni, As, and Sb) in metallic toys and jewelry.

CHAPTER 4   PUBLICATION #3 – ESTIMATING CHILDREN'S EXPOSURE TO TOXIC ELEMENTS IN CONTAMINATED TOYS AND CHILDREN'S JEWELRY VIA SALIVA MOBILIZATION

Children's oral exposure to toxic elements in contaminated toys and children's inexpensive jewelry can occur via saliva mobilization following contact of these items with mouth, or via mobilization in gastrointestinal tract following ingestion of toy or jewelry material. The literature review in *Chapter 2* on toxic elements in toys and children's inexpensive jewelry addressed research needs, and *Chapter 3* identified contaminated samples and problematic categories from a large set of toys and children's low-cost jewelry bought on the North American market. This part of the present dissertation evaluates the mobilization potential of toxic elements via saliva in selected contaminated articles in case of mouthing. This has been done via *in vitro* laboratory testing, and risk for children has been characterized. The work presented in this chapter has been submitted to the journal *Chemosphere* (on September 17, 2013; manuscript #: CHEM30359) which is one of the leading journals in the category of Environmental Science with an Impact Factor of 3.137 as of 2012.

ESTIMATING CHILDREN'S EXPOSURE TO TOXIC ELEMENTS IN CONTAMINATED TOYS AND CHILDREN'S JEWELRY VIA SALIVA MOBILIZATION

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ABSTRACT

Children's potential for exposure to potentially toxic elements in contaminated jewelry and toys via mouth contact has not yet been fully evaluated. Various toys and jewelry (metallic toys and jewelry [MJ], plastic toys, toys with paint or coating, and brittle/pliable toys; n=32) were tested

using the saliva extraction (mouthing) compartment of the DIN and RIVM bioaccessibility protocols to assess As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sb, and Se mobilization via saliva. Total concentrations of As, Cd, Cu, Ni, Pb, and Sb were found elevated in analyzed samples. Four metals were mobilized to saliva from 16 MJ in significant quantities (>1 μg for highly toxic Cd and Pb, >10 μg for Cu and Ni). Bioaccessible concentrations and hazard index values for Cd exceeded limit values, for young children between 6 mo- and 3 y-old and according to both protocols. Total and bioaccessible metal concentrations were different and not always correlated, encouraging the use of bioaccessibility for more accurate hazard assessments. Bioaccessibility increased with increasing extraction time. Overall, the risk from exposure to toxic elements via mouthing was high only for Cd and for MJ. Further research on children's exposure to toxic elements following ingestion of toy or jewelry material is recommended.

4.1 Introduction

Children, highly sensitive to toxic substance exposure due to their physiological and developmental properties, are already exposed to certain levels of contaminants (CDC, 2013; Trejo-Acevedo et al., 2009). Recent reviews have shown that toys and children's jewelry may contain potentially toxic elements, that the scale of contaminated toy and jewelry problem is large, and that children can be exposed to metals in contaminated items (Becker et al., 2010; Guney & Zagury, 2011, 2012). Hundreds of recalls concerning various children's items have been made in North America within the last decade due to chemical safety hazards (Mattel & Fisher-Price, 2007; Morrisson, 2009; U.S.CPSC, 2010a). Highly toxic lead (Pb) and cadmium (Cd), as well as copper (Cu), nickel (Ni), arsenic (As), and antimony (Sb) can pose hazard to children due to their presence in children's toys and low-cost jewelry (Becker et al., 2010; Guney & Zagury, 2013a).

The main consideration in the exposure of children to contaminants in toys and jewelry is the oral pathway (Guney & Zagury, 2012), especially via the ingestion of scraped material or loose parts. However, exposure to contaminants via saliva mobilization during mouth contact via sucking and chewing can be also important, including cases where ingestion is not an issue (material is very hard to scrape i.e. a hard plastic toy, small parts are not loose therefore can't be ingested, parts

are large enough to prevent it from swallowing, or child only sucks a toy/jewelry piece rather than scraping the material off). Oral bioavailability is the fraction of a contaminant reaching the systemic circulation from the gastrointestinal tract, and oral bioaccessibility is the fraction of the substance that becomes soluble and is thus available for absorption (Ruby et al., 1999). *In vitro* oral bioaccessibility tests have been developed and used to assess bioavailability of elements (mainly As, Cd and Pb) in contaminated soils and in consumer goods, including tests with a mouthing compartment like the protocol of German Institute for Standardization (DIN) and consumer product *in vitro* digestion model (RIVM) (Brandon et al., 2006; DIN, 2004). Infants, toddlers, and young children tend to exhibit mouthing behavior especially up to 3 y-old (Tulve et al., 2002; Xue et al., 2007) and this can significantly add to toxic elemental exposure from contaminated toys and jewelry. Recommended values for mouthing frequencies given by the U.S. Environmental Protection Agency (U.S.EPA, 2008b) are high and vary where maximum mean value of 24 contacts.hr^{-1} belongs to 6−12 mo-old infants, then the value decreases to 20 for 1-2 yr-old toddlers, and to 10 for 2-3 yr-old toddlers. 95th percentile duration for mouthing times for these age groups are suggested by the U.S.EPA as 26, 22 and 16 min.hr^{-1}, respectively. According to Farmakakis et al. (2007), toys were the most frequent cause of medical emergency situations due to the aspiration or ingestion of inedible foreign bodies in Greek children, followed by coins and jewelry. Therefore, it can be hypothesized that mouthing behavior is significant for young children especially up to the age of 3, and toxic elements from contaminated toys and jewelry can become bioaccessible in saliva following mouth contact.

Although the problem of contamination in children's toys and jewelry has been demonstrated (Becker et al., 2010; Cox & Green, 2010; Greenway & Gerstenberger, 2010; Guney & Zagury, 2013a; Weidenhamer & Clement, 2007a; Yost & Weidenhamer, 2008b), studies considering mouthing are limited in terms of their number, sample variety, and elements tested. Brandon et al. (2006) tested finger paint (2 spiked samples) and chalk (2 spiked samples, 1 real-life case) to determine Pb bioaccessibility by using mouthing compartment of the RIVM protocol (reported bioaccessibility up to 13.2% for finger paint and <1% for chalk samples). Weidenhamer et al. (2011) tested bioaccessibility of Cd in Cd-contaminated jewelry via saliva extraction by using the U.S. Consumer Product Safety Commission (CPSC) method (n=34) and reported bioaccessible amounts up to 2189 µg following a 6-h extraction, suggesting the mobilization potential of Cd in

contaminated jewelry via saliva. Metals other than Pb and Cd, or different types of toys were not tested in these studies.

There is a strong need for data on the mobilization potential of different metals and metalloids in children's toys and low-cost jewelry via saliva. The objective of this study is to characterize the potential for children's exposure to As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sb, and Se via mobilization in saliva from various toys and jewelry (n=32) using two mouthing bioaccessibility tests.

4.2 Material and Methods

4.2.1 Total metal content determination

Samples (metallic toys and jewelry (MJ), plastic toys (PL), toys with paint or coating (PC), and brittle or pliable toys (BP)) were bought from the North American market: from dollar stores, toy shops, low-cost jewelry stores, retailer chains and on the internet. Seventy two toys and jewelry (24 MJ, 18 PL, 12 PC, and 18 BP) were first screened for their content of As, barium (Ba), Cd, chromium (Cr), Cu, manganese (Mn), Ni, Pb, Sb, and selenium (Se) via total digestion with HNO3, HCLO4, and HF followed by ICP-OES analysis (see S2.1 of *Annex* for details) (Guney & Zagury, 2013a). Thirty two toy and jewelry samples (20 MJ, 3 PL, 5 PC, and 4 BP) had at least one elevated metal concentration (in mg.kg^{-1}, >25% of the migration limit from the new European toy safety directive (European Council, 2009)). These samples were submitted to further analysis in the present study (See Table 4.1 for sample descriptions and results) to determine metal oral bioaccessibility via mobilization in saliva.

4.2.2 Determination of oral bioaccessibility via saliva

Since there is no Canadian method to determine mouthing bioaccessibility of metals in saliva for children's items, selected toy and jewelry samples (n=32) were tested by using slightly modified versions of the saliva extraction compartments of the DIN method 19738 (DIN, 2004) and the RIVM consumer product *in vitro* digestion model (Brandon et al., 2006). These tests were preferred over the U.S. saline extraction method to simulate mouthing (ASTM International, 2011) because it is not physiologically based, and selected test parameters raise questions (a very long extraction time of 6 h, no mechanical agitation to simulate sucking or chewing, no inorganic

or organic saliva compounds except NaCl). The DIN and RIVM tests were selected because they are physiologically based *in vitro* methods using mechanical agitation and synthetic saliva at 37 °C with the presence of various inorganic and organic chemical compounds (See S2.2 of *Annex* for experimental details). The bioaccessible quantities of selected elements (Cd, Cu, Ni, and Pb, in µg = saliva concentration in mg.L^{-1} × sample volume in mL) following 30, 60, and 120 minutes of extraction were calculated (Table S1.1 of *Annex*); and the bioaccessible concentrations in toy/jewelry material (in mg.kg^{-1} = bioaccessible quantity in µg ÷ sample weight in g) are presented in Table 4.2.

4.2.3 Risk characterization

Chemical daily intake (CDI, mg.kg^{-1}) for exposure to elements via saliva was calculated (Table 4.3) according to the following formula:

$$CDI = (BAcc \times ED) \div BW \quad (1)$$

Where BAcc (µg per 30 min of exposure) is the bioaccessible quantity of an element in saliva after 30 min of exposure, ED is the exposure duration (30 min.d^{-1}), and BW is the body weight (9.2 kg for 6−12 mo-old infants, 11.4 kg for 1-2 yr-old toddlers, and 13.8 kg for 2-3 yr-old toddlers (U.S.EPA, 2008b)). Alternatively, by using exposure durations specific to different age categories and theoretical sample-specific migration rates (Table S1.2, *Annex*) calculated from the experimental bioaccessibility data, CDI was also calculated according to the following formula:

$$CDI = (k_{mig} \times ED) \div BW \quad (2)$$

Where k_{mig} is the rate of migration (µg.min^{-1}), and ED is the age-specific exposure duration (min.d^{-1}) assuming 1 h.d^{-1} of play time and based on recommended 95th percentile mouthing times of 26 min.h^{-1} for 6−12 month-old infants, 22 min.h^{-1} for 1-2 yr-old toddlers, and 16 min.h^{-1} for 2-3 yr-old toddlers by the U.S.EPA (2008). A hazard index (HI, no unit) for oral exposure to elements via ingestion was calculated according to the following formula:

$$HI = CDI \div RfD \quad (3)$$

Where RfD is the reference dose (RfD for Cd: 0.5 µg.kg^{-1}.d^{-1} (U.S.EPA, 1994), suggested limit value for Pb exposure from toys: 3.6 µg.kg^{-1}.d^{-1} (van Engelen et al., 2006), minimal risk level for Cu:0.01 mg.kg^{-1}.d^{-1} (ATSDR, 2011), RfD for Ni: 0.02 mg.kg^{-1}.d^{-1} (U.S.EPA, 1996a)).

4.2.4 Quality assurance/quality control

In order to assess the accuracy of the digestion method, one standard reference material (SRM54d, tin-base bearing metal) was analyzed in duplicate and results were consistent with certified values (see S2.3 of *Annex* for details). The accuracy of the saliva extraction protocols could not be verified because a standard reference material certified for the DIN or the RIVM saliva extraction protocol is not available. However, SRM54d was tested by both protocols. This reference material is mentioned in Health Canada's protocol C-08 (Determination of migratable lead in consumer products) to be used as control sample (Health Canada, 2008), and therefore considered suitable for the present study. All digestion and mouthing experiments were conducted in duplicates. Total metal concentrations in blanks were always below or very close to the method detection limits. In order to evaluate analysis variability, the relative standard deviations (RSD) of duplicates for each analyzed sample were also calculated (see Table 4.1 for total digestions, the RSD values for the mouthing protocols were calculated but not reported). The RSD values were generally satisfactory for the majority of the analyses (see S2.3 of *Annex* for details).

Table 4.1 Concentrations of elements (average [mg.kg^{-1}] ± RSD [%]) in tested toys and jewelry

Item	Description	As	Ba	Cd	Cr	Cu	Mn	Ni	Pb	Sb	Se
MJ01	Pewter ornament for scrapbooking	<4.51	0.16 ± 90	[a]9.58 ± 1	≤2.22	[b]2.68E+04 ± 20	2.26 ± 25	2.50E+03 ± 24	102 ± 16	139 ± 49	≤9.25
MJ02	Smiley face brads for scrapbooking	10.1 ± 3	0.24 ± 18	180 ± 5	205 ± 0.5	142 ± 3	3.55E+03 ± 0.6	265 ± 8	325 ± 9	211 ± 10	<6.58
MJ03	Pewter embellishments for scrapbooking	431 ± 36	[c]<0.01	187 ± 4	1.85 ± 21	1.25E+04 ± 7	1.33 ± 6	5.05E+03 ± 6	4.36E+05 ± 4	1.02E+03 ± 58	16.7 ± 22
MJ05	Pewter embellishments for scrapbooking	[d]≤1.59	0.22 ± 81	9.45 ± 26	133 ± 138	1.30E+04 ± 2	280 ± 75	2.28E+03 ± 52	157 ± 4	25 ± 6	≤2.60
MJ06	Pewter embellishments for scrapbooking	1.57 ± 0	≤0.01	15.2 ± 21	3.47 ± 5.4	1.61E+04 ± 12	147 ± 0	3.23E+03 ± 3	119 ± 7	17.5 ± 1	<1.48
MJ07	Decorative metal beads for craft	3.56 ± 12	0.12 ± 43	1.40 ± 6	12.1 ± 22	5.68E+05 ± 2	2.05 ± 73	9.20E+03 ± 4	163 ± 10	28.8 ± 5	<5.09
MJ08	Bracelet with metal beads	43.5 ± 1	0.17 ± 65	88.2 ± 0	173 ± 0.5	1.34E+03 ± 19	4.32E+03 ± 0.5	1.40E+04 ± 1	86.1 ± 46	53 ± 0	<6.86
MJ09	Decorative metal beads for craft	≤4.75	0.46 ± 74	263 ± 4	5.91 ± 3.4	7.10E+05 ± 21	13.9 ± 0.1	1.10E+04 ± 28	469 ± 4	27.6 ± 7	≤8.26
MJ10	Bracelet with metal beads	<2.86	<0.02	8.00 ± 84	7.33 ± 24	5.75E+05 ± 0	1.37 ± 15	8.24E+03 ± 7	106 ± 13	23.2 ± 27	<5.04
MJ12	Decorative pins for scrapbooking	20.7 ± 4	1.33 ± 27	73.8 ± 5	265 ± 6	1.73E+03 ± 11	3.67E+03 ± 5.3	4.38E+03 ± 3	31.8 ± 38	39.3 ± 16	<6.56
MJ13	Decorative pins for scrapbooking	56.8 ± 46	0.72 ± 2	76.0 ± 1	211 ± 58	3.16E+04 ± 15	3.15E+03 ± 6.3	5.29E+03 ± 36	≤5.82	38.8 ± 33	<6.50
MJ14	Jewelry piece - metal rose	62.4 ± 0	<0.03	203 ± 4	0.76 ± 41	4.35E+03 ± 1	1.35 ± 106	5.94 ± 25	6.53E+05 ± 11	272 ± 33	≤14.55
MJ15	Jewelry piece - metal key	<4.76	<0.03	2.59E+05 ± 14	≤0.52	1.36E+05 ± 18	1.68 ± 6.5	1.03E+03 ± 69	1.08E+03 ± 7	60.7 ± 1	≤8.53
MJ16	Metal earring	<4.41	<0.03	1.66E+05 ± 13	2.75 ± 94	9.50E+04 ± 8	5.02 ± 66	1.87 ± 23	37 ± 16	35.7 ± 16	<7.75
MJ17	Jewelry metal pendant	<5.25	≤0.09	12.7 ± 70	≤0.48	1.58E+04 ± 6	1.25 ± 5	≤1.74	88.5 ± 0	38.6 ± 17	12 ± 1
MJ18	Jewelry metal pendant	≤7.95	0.98 ± 30	3.67E+05 ± 18	10.8 ± 0.4	6.86E+04 ± 85	1.19 ± 41	17.7 ± 62	100 ± 1	32.7 ± 43	50.6 ± 2
MJ19	Bracelet beads	5.16 ± 2	212 ± 11	8.62 ± 41	7.87 ± 5.4	1.53E+05 ± 2	2.61 ± 22	24.1 ± 13	10.7 ± 23	173 ± 21	≤3.18
MJ20	Bracelet charm	≤5.36	<0.03	57.1 ± 74	1.44 ± 20	9.79E+04 ± 29	1.62 ± 22	≤1.42	<3.61	40.6 ± 21	≤10.09
MJ24/2	Bracelet - metal chain	85.2 ± 5	≤0.57	82.5 ± 3	307 ± 1.9	2.82E+04 ± 3	3.72E+03 ± 1.9	184 ± 3	<3.33	48.2 ± 18	<6.87
MJ25	Necklace - silver plastic	15.7 ± 15	780 ± 9	17.5 ± 3	16.2 ± 17	298 ± 39	19.7 ± 7.9	59.3 ± 47	85.2 ± 4	631 ± 40	<6.59
PL02	Eraser set shaped as food	24.9 ± 33	8.93 ± 57	0.23 ± 23	0.67 ± 22	10.8 ± 32	13.4 ± 4.2	1.37 ± 3	76.5 ± 5	182 ± 1	<1.35
PL05/1	Jewelry design set - very small glass beads	81.2 ± 22	375 ± 50	771 ± 69	4.5 ± 6.7	19.2 ± 75	13.7 ± 27	2.99 ± 35	≤8.03	18.1 ± 39	156 ± 49
PL05/2	Jewelry design set - small glass beads	207 ± 67	347 ± 6	351 ± 19	329 ± 45	338 ± 7	4.17E+03 ± 75	22.3 ± 57	24.2 ± 92	39.8 ± 69	≤5.96
PC05	Carmine red colored metallic paint on metal toy car	<67.7	3.28 [e]± -	<4.10	<6.22	<8.23	3.46 ± -	<19.7	<57.6	370 ± -	<119.1
PC09	Blue colored metallic paint on metal toy car	<66.3	<0.41	<4.01	<6.09	2139 ± -	<0.82	<19.3	<56.5	<53.1	<116.7
PC11	Red colored metallic paint on metal toy car	<61.7	<0.39	<3.73	<5.66	<7.50	<0.76	<18.0	<52.5	131 ± -	<108.5
PC12	Carmine red colored metallic paint on metal toy car	<70.7	<0.44	<4.28	968 ± -	<8.59	10.1 ± -	<20.6	<60.1	<56.5	<124.3
PC19	Maroon colored metallic paint on metal toy car	<56.4	1.69 ± -	<3.41	212 ± -	<6.85	8.49 ± -	<16.4	<48.0	<45.1	<99.1
BP04	Crayon set (tip and coating composite sample)	<1.58	2210 ± 49	0.37 ± 24	12.6 ± 3.4	54.5 ± 17	48.7 ± 7.1	11.4 ± 2	<1.35	<1.27	<2.78
BP05	Oil crayon set (composite sample)	<1.53	319 ± 10	<0.09	3.36 ± 37	216 ± 57	31.4 ± 0	3.3 ± 15	<1.30	<1.22	<2.69
BP18	Crayon set (tip and coating composite sample)	<1.46	1971 ± 2	2.81 ± 10	32.2 ± 4.9	301 ± 15	32.2 ± 4.1	25.1 ± 0	<1.24	<1.17	<2.57
BP19	Crayon set (tip and coating composite sample)	<1.54	3205 ± 14	<0.09	5.78 ± 0.3	0.38 ± 48	17.8 ± 0.9	2.14 ± 2	≤2.47	<1.23	<2.71

[a] *Bold italic values* indicate elevated total concentrations (25% - 100% of EU migration limit).   [b] **Bold underlined values** indicate total concentrations exceeding EU migration limit (see the text for values).
[c] <: Concentrations analyzed in both duplicates are below DL. Average of DL's is given.   [d] ≤: Concentration analyzed in one of the duplicates is below DL and the other is above DL. Average of two measurements is given.
[e] -: RSD not calculated since only one duplicate was analyzed due to limited amount of sample available

Table 4.2 Bioaccessible concentrations of Cd, Cu, Ni and Pb (average, mg.kg-1) in selected children's toys and jewelry after 30, 60 and 120 minutes during modified DIN and RIVM mouthing tests

EU migration limit	23			7700			930			160		
DIN	Cd			Cu			Ni			Pb		
	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
Sample												
MJ01	[a]<0.02	<0.03	<0.03	0.50	0.78	1.37	5.95	9.35	15.8	<0.20	<0.24	<0.27
MJ03	<0.01	<0.01	<0.01	0.71	1.00	1.43	3.58	6.80	15.4	0.86	1.36	3.87
MJ05	0.12	0.13	0.13	1.30	1.36	1.33	2.01	2.25	2.47	<0.13	<0.15	<0.17
MJ06	0.14	0.15	0.14	1.88	1.90	1.91	2.89	3.19	3.44	<0.13	<0.15	<0.17
MJ07	<0.04	<0.05	<0.05	2.88	3.49	5.10	8.20	10.1	12.3	<0.36	<0.42	<0.48
MJ08	<0.03	<0.03	<0.04	2.39	2.65	2.71	1.88	2.16	2.46	<0.24	<0.28	<0.32
MJ09	<0.05	<0.05	<0.06	15.8	23.2	39.8	[b]≤0.23	0.52	0.98	<0.42	<0.49	<0.56
MJ10	<0.04	<0.05	<0.05	20.0	30.4	52.2	0.42	0.65	0.91	<0.37	≤0.48	<0.50
MJ12	<0.05	<0.06	<0.07	2.52	2.73	2.82	5.30	8.45	11.4	<0.44	<0.52	<0.59
MJ13	<0.05	<0.05	<0.06	39.3	49.1	60.3	*391*	*534*	*687*	<0.43	<0.50	<0.57
MJ14	<0.02	<0.02	<0.03	1.47	2.18	2.32	<0.06	<0.08	<0.09	0.53	0.93	1.88
MJ15	5.30	[c]*6.49*	*7.03*	8.86	9.13	9.78	1.66	1.83	2.22	<0.30	<0.36	<0.41
MJ16	4.56	*9.33*	*12.8*	6.75	7.80	8.90	<0.37	<0.43	<0.49	<1.04	<1.21	<1.39
MJ18	[d]*≤22.8*	*≤35.6*	*≤38.4*	4.79	5.85	7.13	≤0.19	<0.12	<0.13	<0.24	<0.28	<0.31
MJ19	<0.04	<0.05	<0.06	8.89	13.2	20.8	<0.14	<0.16	<0.18	<0.39	<0.45	<0.52
MJ24/2	<0.02	<0.02	<0.03	1.46	1.84	2.17	2.93	3.85	5.15	<0.17	<0.20	<0.23
SRM54d	4.14	4.35	4.79	20.1	21.0	22.9	<0.17	<0.20	<0.23	<0.48	<0.56	<0.64
RIVM	Cd			Cu			Ni			Pb		
	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
Sample												
MJ01	<0.13	<0.16	<0.18	<0.23	0.44	1.38	1.78	2.99	6.59	<1.22	<1.42	<1.62
MJ03	<0.03	<0.04	<0.05	0.24	0.54	1.01	1.11	1.62	2.67	1.04	2.48	3.40
MJ05	<0.09	<0.10	<0.12	0.24	0.40	0.95	2.52	4.75	8.19	<0.78	<0.91	<1.04
MJ06	<0.09	<0.10	<0.11	0.35	0.60	1.06	1.67	3.69	7.79	<0.77	<0.90	<1.03
MJ07	<0.24	<0.27	<0.31	9.10	11.0	13.9	2.44	3.33	11.8	<2.13	<2.48	<2.84
MJ08	<0.16	<0.19	<0.22	≤0.72	1.08	1.53	5.19	7.94	12.7	<1.47	<1.71	<1.95
MJ09	<0.28	<0.32	<0.37	32.4	42.4	58.2	≤1.05	1.58	3.08	<2.51	<2.93	<3.35
MJ10	<0.25	<0.29	<0.33	34.5	48.2	68.6	1.44	1.63	3.57	<2.23	<2.60	<2.97
MJ12	<0.29	<0.34	<0.39	<0.50	0.73	1.21	11.5	17.9	30.9	<2.65	<3.09	<3.54
MJ13	<0.28	<0.33	<0.38	25.1	34.9	51.4	16.9	25.6	40.2	<2.56	<2.99	<3.41
MJ14	<0.12	<0.14	<0.16	0.42	0.98	2.20	<0.39	<0.45	<0.52	1.98	4.63	9.10
MJ15	0.76	2.94	*6.48*	2.74	5.07	8.90	0.94	2.53	6.68	<1.83	<2.13	<2.44
MJ16	<0.69	<0.81	≤1.18	<1.18	≤1.69	4.46	<2.22	<2.59	<2.96	<6.25	<7.29	<8.33
MJ18	**33.5**	**63.1**	**202**	4.45	7.52	12.7	<0.50	<0.59	1.08	<1.42	<1.65	<1.89
MJ19	<0.26	<0.30	1.07	4.13	7.04	10.2	<0.83	<0.96	<1.10	<2.32	<2.71	<3.10
MJ24/2	<0.12	<0.14	<0.15	2.45	3.92	5.57	1.31	2.20	3.01	<1.05	<1.22	<1.40
SRM54d	0.68	0.57	0.60	16.5	17.8	21.7	<1.02	<1.19	<1.36	3.76	4.23	19.5

[a] <: Concentrations analyzed in both duplicates are below DL. Average of DL's is given.
[b] ≤: Concentration analyzed in one of the duplicates is below DL and the other is above DL. Average of two measurements is given.
[c] **Bold underlined values** indicate migratable concentrations exceeding EU migration limit (see the text for values).
[d] *Bold italic values* indicate elevated migratable concentrations (25% - 100% of EU migration limit).

4.3 Results and Discussion

4.3.1 Metal contamination in toys

Total and bioaccessible concentrations of metals (Tables 4.1 and 4.2) in 32 toys and jewelry items (articles with significant metal concentrations among the 72 screened items) were compared to migration limits stated in the European Union (EU) Toy Safety Directive (European Council, 2009). The limits defined in this directive were used since the EU Toy Safety directive provides a more comprehensive approach to the chemical safety of toys than North American legislations due to its sound scientific basis, the number of selected contaminants, and the separate limits defined for different material categories (Guney & Zagury, 2012). The EU Toy Safety Directive states migratable concentration limits for three different types of toy materials: in liquid or sticky toy material, in scraped-off toy material (applicable to the categories MJ, PL and PC in this study, in mg.kg^{-1}; As:47, Ba:56,000, Cd:23, Cr(III):460, Cu:7,700, Mn:15,000, Ni:930, Pb:160, Sb:560, Se:460), and in dry, brittle, powder-like or pliable toy material (applicable to the category BP, in mg.kg^{-1}; As:3.8, Ba:4,500, Cd:1.9, Cr(III):37.5, Cu:622.5, Mn:1200, Ni:75, Pb:13.5, Sb:45, Se:37.5). If the total concentration of an element in a toy exceeds the EU migration limit, it can be said that further migration analysis is necessary to determine whether exposure to this toy is safe or not.

The sample category with the highest metal contamination was MJ (20 items with total concentrations exceeding migration limits), where some metal concentrations (i.e. for Pb and Cd) exceeded the EU limits up to several thousand times. The majority of the items in this category had significant concentrations of Cu and Ni, exceeding the stated EU limit in 15 and 11 items, respectively. As, Cd, Pb, and Sb concentrations were also high in numerous items. For example, the samples MJ03 and MJ14 had extremely high Pb concentrations (44% and 65% w/w, respectively). Furthermore, 12 items had a Cd content (up to 37%, w/w) exceeding the EU limit 23 mg.kg^{-1}. Therefore, it was found crucial to determine the migration potential of metals in items belonging to that category.

The level of contamination and the percentage of contaminated items in PL, PC, and BP categories were lower. For the PL category, only PL05 (a jewelry design set) had metal concentrations exceeding the EU limits (for As, Cd) in two sub-samples, and PL02 (food shaped

eraser set) had elevated As, Pb, and Sb concentrations (between 25%-100% of the EU limits). Similarly, for the PC and BP categories, there was only one toy with metal concentrations exceeding the EU limits (PC12, toy car, Cr; BP18, crayon set, Cd). Also, a limited number of items from the PC and BP had elevated metal concentrations between 25%-100% of the EU limits. The PL, PC, and BP categories were found less problematic than the MJ category in terms of metal contamination.

When the U.S. and the Canadian limits are considered (which regulate only Pb and Cd in toys and/or jewelry), it was found that 11 MJ samples exceeded the U.S. Pb limit (100 mg.kg^{-1} in children's products (U.S.CPSC, 2012)) and the Canadian Pb limit (90 mg.kg^{-1} for toys intended for children less than three years of age, as well as other products whose normal pattern of use involves the product being brought into contact with the user's mouth (Government of Canada, 2010)). The U.S. limit for Cd (300 mg.kg^{-1} in children's jewelry (ASTM International, 2011)) has been exceeded by 3 items (MJ15, MJ16 and MJ18), and the Canadian limit (130 mg.kg^{-1} in children's jewelry (Health Canada, 2011)) by 7 items. The categories PL, PC and BP conformed to the U.S. and the Canadian regulations (limits on only total Pb content).

4.3.2 Bioaccessibility of metals in saliva

Results for the oral bioaccessibility of ten elements according to the DIN and RIVM methods showed that the metals with important levels of migration into saliva were Cd, Cu, Ni, and Pb; whereas the migration of As, Ba, Cr, Mn, Sb, and Se was either low or below detection limits, therefore, results for the latter metals are not presented. Sixteen out of 36 items leached significant quantities of Cd and Pb (>1 μg), as well as Cu and Ni (>10 μg). All of the 16 items having significant levels of metal migration belonged to the MJ category.

According to the bioaccessible concentrations (in mg.kg^{-1}) of Cd, Cu, Ni, and Pb presented in Table 4.2 for 16 samples, only one item surpassed the migration limits stated in the EU directive (MJ 18, for Cd, limit: 23 mg.kg^{-1}). For the DIN test after 30 min, one replicate for MJ18 (a jewelry metal pendant) yielded Cd concentrations below the detection limit (<0.025 mg.kg^{-1}), while Cd significantly leached from the second replicate (45.7 mg.kg^{-1}), resulting with an average concentration of 22.8 mg.kg^{-1} (after 60 min: 35.6 mg.kg^{-1}, 120 min: 38.4 mg.kg^{-1}; the EU

migratable concentration limit for Cd is 23 mg.kg^{-1}). Using the RIVM test, bioaccessible concentrations of Cd after 30, 60, and 120 min were found even higher (33.5, 63.1, and 202 mg.kg^{-1}, respectively) for MJ18, with RSD ranging between 58% and 77%. Large differences between the results of replicates for MJ18 in both tests were attributed to the variability of chemical composition of different items of the same type as multiple copies of the same item were used as replicates. Similar observations have been previously reported by Weidenhamer and Clement (2007). Apart from the only sample (MJ18) exceeding the EU regulations, significant amounts of metals (bioaccessible concentrations between 25% - 100% of the values stated in the EU regulation) were mobilized to saliva from MJ13 (Ni for the DIN test), MJ15 (Cd for the DIN and the RIVM tests) and MJ16 (Cd for the DIN test).

4.3.3 Comparison of tests

It was possible to compare results from RIVM and DIN methods because the same replicates of samples were used for both tests. The mouthing compartments of the RIVM and DIN methods gave similar results in terms of Cu bioaccessibility. The slope of linear fit between the two sets of results for Cu was close to 1 (regression equation: $Cu_{RIVM} = 1.097 \times Cu_{DIN} + 0.13$, n=16, p<0.001). The results from both tests were also highly correlated ($R^2 = 0.888$). However, for Ni, the results were not similar, and the RIVM method tended to give higher results (regression equation: $Cu_{RIVM} = 0.343 \times Cu_{DIN} + 6.56$, n=11). Moreover, the measured values by the two methods were not correlated ($R^2 = 0.062$) and the regression yielded a very high p value (p=0.46). The difference between two tests in terms of Ni bioaccessibility might be caused by the differences in synthetic saliva composition. The synthetic saliva from the RIVM method contains much higher amounts of NaHCO$_3$, as well as higher amounts of certain inorganics (i.e. NaCl and Na$_2$SO$_4$) and organics (amylase, urea, and uric acid). This difference in composition also required dilution before the final analysis of samples for metals and thus caused higher test detection limits for the RIVM method. However, as there is neither any validation for these tests nor any certified reference material with known test values, it is not possible at this point to conclude on the accuracy of the results. Finally, due to the small number of measurements above detection limit, it wasn't possible to statistically compare Pb and Cd bioaccessibility results from the two tests.

4.3.4 Comparison of total and bioaccessible concentrations of metals

The total Cu content was moderately correlated to bioaccessible content (n=16, R^2=0.265 and 0.513, p<0.05 and <0.01 for the DIN and the RIVM methods, respectively).Total metal concentrations failed to predict Cu bioaccessible concentrations in many cases (i.e. MJ07 has 57% Cu but leached only 5.1 µg, while MJ13 contains less Cu (3.2%) but leached 60.3 µg). For Ni, there wasn't any apparent relationship between total and bioaccessible Ni concentrations (n=11, R^2=0.001 for both methods, p=0.94 and 0.91 for the DIN and the RIVM methods, respectively). Finally, it wasn't possible to compare bioaccessible and total concentrations for other metals from the two tests due to the small number of measurements above detection limit. Especially for Ni and also for Cu, it can be concluded that total metal concentrations may not accurately predict mouthing bioaccessibility.

4.3.5 Effect of test duration on metals mobilization

Longer extraction times yielded larger bioaccessible amounts and concentrations of metals. The effect of testing time on bioaccessible concentrations was sample-dependent. For some samples, the relationship between extraction time and extracted concentration was linear, while in others, the bioaccessible concentration became higher with time but tended to plateau (for details, see Figure S1.1 of *Annex*). As mouthing frequency and times for children can be highly variable (Xue et al., 2007), in order to estimate exposed amount for children, it can be helpful to calculate a mobilization rate from mouthing test data. For most samples, a simple rate can be calculated by assuming a linear relationship between bioaccessible quantity and mouthing time, and by simple linear regression while taking y-intersection as 0 (i.e. no mobilized amount of metal at t=0 min). Migration rates for Cd, Cu, Ni, and Pb (µg.min^{-1}) for selected nine samples with the highest bioaccessible concentrations show that the rate for a metal was highly specific to the article tested, and may also vary with the type of test used (range: 0.042 – 7.78 µg.min^{-1} for DIN, and <0.005 – 3.20 for µg.min^{-1} for RIVM, additional information is presented in Table S1.2 of *Annex*). For a more accurate evaluation of risk, the migration rates calculated by this approach were used in risk characterization.

4.3.6 Risk characterization

For the selected nine samples with the highest bioaccessible concentrations of Cd, Cu, Ni and Pb (Table 4.2), HI values were calculated via two calculation methods for young children between 6 mo- and 3 y-old. Calculation method #1 assumes that the mouthing time is constant (30 min.d^{-1}), and that the mobilized quantities of contaminants in saliva (in µg) during each exposure are equal to the bioaccessible quantities provided by the DIN and the RIVM protocols following 30 min extraction. Moreover, a more detailed set of calculations were made (calculation method #2, see *section 2.3*) assuming that the mouthing time varies according to the age category (U.S.EPA, 2008b), and that metals are mobilized in calculated rates (in µg.min^{-1}) specific to each item and metal.

According to the results of risk characterization presented in Table 4.3, for Cd (method #1), two jewelry items (MJ15 and MJ18) had HI>1 according to the DIN protocol, and only MJ18 (total metal content of 37% (w/w)) had a HI>1 for the RIVM protocol. HI was also elevated for MJ16, but still below 1. Similarly for method #2, only MJ18 had a HI>1, and HI values for MJ15 and MJ16 were elevated but below 1. As mentioned in *Section 3.2*, only MJ18 reached the migration limits stated in the EU directive (limit for Cd: 23 mg.kg^{-1}, migratable concentration for the DIN test after 30 min: 22.8 mg.kg^{-1}, for the RIVM test: 33.5 mg.kg^{-1}). The U.S.CPSC (2010b) states that chronic acceptable daily intake level of Cd for children is 1.8 µg.d^{-1}. When the results for bioaccessibility for 30 min for Cd for MJ15, MJ16, and MJ18 (DIN: 8.81, 2.26, and ≤46.8 µg.d^{-1}, respectively; RIVM: 0.76, <0.69, and 33.5 µg.d^{-1}, respectively) are taken as daily uptake values based on the assumption of 30 min of exposure per day, they either exceed or are close to the value of 1.8 µg.d^{-1}. Jewelry items MJ15, MJ16 and MJ18 have very high Cd total concentrations (26%, 17% and 37%, respectively [w/w]). Based on the HI values, the EU limits, and the U.S.CPSC-defined chronic acceptable daily intake level value for Cd, it can be concluded that jewelry highly contaminated with Cd can leach quantities of Cd above safe thresholds to saliva when mouthing behavior is present. This finding is similar to the results presented by Weidenhamer et al. (2011), where saline extractable Cd values (saline extraction was used to simulate exposure by mouthing behavior according to the U.S.CPSC) from Cd-contaminated

jewelry were up to 2,189 µg following a 6-h extraction (n=34). The maximum saliva extractable Cd in the present study after 2 h was 420 µg (MJ18, for the RIVM method).

Table 4.3 Hazard index (HI) values from exposure to contaminated metallic toys and jewelry for selected samples via two calculation methods for 6-12 mo-old infants, 1-2 y and 2-3 y-old toddlers

Metal	Sample	HI (DIN)			HI (RIVM)		
		6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y
		Calculation method #1[a]					
Cd	MJ15	**1.92**[c]	**1.55**	**1.28**	0.27	0.22	0.18
	MJ16	0.49	0.40	0.33	<0.07[d]	<0.06	<0.05
	MJ18	**≤10.2**[e]	**≤8.21**	**≤6.78**	**15.2**	**12.3**	**10.1**
Cu	MJ09	0.20	0.16	0.13	0.42	0.34	0.28
	MJ10	0.29	0.24	0.19	0.50	0.40	0.33
	MJ13	0.50	0.40	0.33	0.32	0.26	0.21
Ni	MJ12	0.03	0.03	0.02	0.07	0.06	0.05
	MJ13	**2.47**	**2.00**	**1.65**	0.11	0.09	0.07
Pb	MJ03	0.25	0.21	0.17	0.30	0.25	0.20
	MJ14	0.04	0.03	0.03	0.17	0.13	0.11
		Calculation method #2[b]					
Cd	MJ15	0.70	0.47	0.29	0.48	0.33	0.20
	MJ16	0.33	0.22	0.13	<0.03	<0.02	<0.01
	MJ18	**≤4.54**	**≤3.10**	**≤1.86**	**18.1**	**12.3**	**7.41**
Cu	MJ09	0.12	0.08	0.05	0.19	0.13	0.08
	MJ10	0.17	0.12	0.07	0.24	0.17	0.10
	MJ13	0.20	0.13	0.08	0.16	0.11	0.06
Ni	MJ12	0.02	0.01	0.01	0.04	0.03	0.02
	MJ13	**1.10**	0.75	0.45	0.06	0.04	0.02
Pb	MJ03	0.23	0.16	0.10	0.24	0.16	0.10
	MJ14	0.03	0.02	0.01	0.16	0.11	0.07

[a] Daily exposure considering a constant mouthing time of 30 min.d^{-1}.
[b] Daily exposure based on variable mouthing times recommended by the U.S.EPA (2008) and calculated migration rates (Table S1.2).
[c] **Bold values** indicate HI exceeding 1.
[d] <: Concentrations analyzed in both duplicates are below DL. Result calculated using the average of DL's is given.
[e] ≤: Concentration analyzed in one of the duplicates is below DL and the other is above DL. Result calculated using the average of two measurements is given.

Considering Pb, for both calculation methods, HI values were less than 1 (≤0.30 for method #1, and ≤0.24 for method #2). The concentrations of Pb mobilized by saliva was either low or below

detection limits (Table 4.2) for all samples, including MJ03 and MJ14 with very high Pb levels (total concentrations: 44% and 65%, respectively [w/w]). The U.S.CPSC recommends that children should not ingest more than 175 µg of accessible lead in a short period of time (2005). The maximum amount of Pb mobilized was 10.1 µg for MJ03 (DIN, 30 min), which was well below the CPSC recommended value. Although there is no safe level for Pb exposure as even very low concentrations of Pb in blood (<10 µg,dL^{-1}) may have adverse health effects (Canfield et al., 2003), it can be said that the amount of Pb mobilized via saliva from the toys and jewelry tested in the present study is low.

For Cu, considering both calculation methods, HI values were below 1 (≤0.50 for method #1, and ≤0.24 for method #2). The upper level of exposure to Cu stated by the RIVM (van Engelen et al., 2006) is 0.083 mg.kg^{-1}.d^{-1}. For an infant between 6-12 mo-old and with a body weight of 9.2 kg, according to this value, Cu uptake should be less than 763 µg.d^{-1}, which is highly above the bioaccessible amounts found in saliva (maximum: 70.1 µg for DIN, and 91.5 µg for RIVM; after 120 min). Based on these criteria as well as the calculated HI values, Cu mobilization via mouthing can be considered not posing an important risk for the toys and jewelry tested in the present study. For Ni, HI exceeded 1 only for MJ13 (only for the results of the DIN protocol, calculation method #1). Based on the HI values and the oral toxicological profile of Ni, it can be concluded that bioaccessible Ni is generally safe for infants and toddlers for the saliva exposure for toys and jewelry tested in the present study. However, the highest Ni concentration was 1.4% (w/w, MJ13) which is lower compared to Cd, Cu and Pb contamination. Metallic toys or jewelry having higher concentrations of Ni may yield unsafe levels of bioaccessible quantities in saliva. It should be also noted that the allergic potential of Ni in toys and jewelry and accompanying risks were not taken into account in the present study.

4.4 Conclusions

Overall, the results of our analyses show that the mouthing of toys and jewelry does not cause a release of unacceptable quantities of toxic elements to saliva, with the exception of Cd in metallic toys and jewelry. In general, solubilization in saliva may be less important than solubilization in

the gastro-intestinal system following ingestion due to more favorable conditions for metals mobilization in gastrointestinal system. This is supported by the gastrointestinal oral bioaccessibility results for the jewelry sample MJ14 previously tested by our research team (Guney & Zagury, 2013a). Intestinal bioaccessibility in µg (measured via the IVG protocol) clearly exceeds bioaccessibility in saliva in the present study (via the DIN/RIVM methods) for Pb (705 µg vs. 5.08/25.0 µg, after 120 min) and for Cd (1.34 µg vs. <0.07/<0.44 µg, after 120 min). Bioaccessible concentrations were correlated to extraction time, and calculated migration rates were highly dependent on the sample and the type of test used. Therefore, in assessing exposure, children's actual mouthing time is critical. Total and bioaccessible metal concentrations were different and did not always well correlate, indicating that using bioaccessibility data should be preferred when assessing the risk over the use of total concentrations. Finally, it should be noted that mouthing behavior may also lead to additional physical or chemical risks via increasing the probability of suffocation or ingestion. Specifically, additional research on children's exposure potential to toxic elements in toys and jewelry via the ingestion of toy or jewelry material or small pieces is recommended.

CHAPTER 5   PUBLICATION #4 – BIOACCESSIBILITY OF As, Cd, Cu, Ni, Pb, AND Sb IN TOYS AND LOW-COST JEWELRY

In addition to exposure of children to toxic elements in toys and jewelry via saliva mobilization following mouthing which was discussed in *Chapter 4*, mobilization in gastrointestinal tract following ingestion of toy or jewelry material is another important scenario. Both the literature review in *Chapter 2* on toxic elements in toys and children's jewelry, and the results of the experiments made on selected samples presented in *Chapter 3* already indicated important mobilization potential of toxic elements in gastrointestinal system. In this chapter, gastrointestinal bioavailability of selected elements was estimated in a large set of contaminated articles by using three different *in vitro* bioaccessibility test protocols. The risk for children was also characterized. The work presented in this chapter has been accepted for the publication in journal *Environmental Science and Technology* with minor revisions (on November 10th, 2013 manuscript #: ES-2013-036122), which ranks #1 in total citations in the Environmental Engineering and Environmental Sciences categories and has an Impact Factor of 5.257 according to the 2012 Journal Citation Reports from Thomson Reuters.

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ABSTRACT

Children can be exposed to potentially toxic elements in toys and children's jewelry following ingestion. As, Cd, Cu, Ni, Pb, and Sb gastrointestinal bioavailability was assessed (n=24) via three in vitro bioaccessibility protocols (IVG, PBET, and EN 71-3), and health risk for children was characterized. Cd, Cu, Ni, and Pb were mobilized from 19 metallic toys and jewelry (MJ), and one crayon set. Bioaccessible Cd, Ni, or Pb exceeded EU migratable concentration limits in

four to six MJ, depending on the protocol. Using two-phase (gastric and intestinal) IVG or PBET would be recommended over EN 71-3 since these tests are more conservative and better represent gastrointestinal physiology. Bioaccessible and total metal concentrations were different and not always correlated, encouraging the use of bioaccessibility for more accurate risk characterization. For children (6 mo- to 6y-old), hazard index (HI) values for Pb, Cd, or Ni were >1 for all six MJ exceeding the EU limits. Considering infants' (6-12 mo-old) exposure, ten MJ had HI>1 for Cd, Cu, Ni, or Pb. HI values were up to 75 for Cd and 43 for Pb. Research on prolonged exposure to MJ and more detailed risk characterization for exposure to toys and jewelry is recommended.

5.1 Introduction

Children are particularly sensitive to contaminant exposure due to their physiological and developmental properties, and are exposed to toxic substances including metals via multiple pathways i.e. food, air, water and soil (Glorennec et al., 2007; Trejo-Acevedo et al., 2009; Vogt et al., 2012). For children <6 years old, object mouthing is a common behavior and mouthing frequency and duration are especially high for infants (6-12 mo) and toddlers (1-3 y) (Tulve et al., 2002; U.S.EPA, 2008b; Xue et al., 2007). According to Farmakakis et al. (2007), toys were the most frequent cause of medical emergency situations due to aspiration or ingestion of inedible foreign bodies in Greek children, followed by coins and jewelry.

In the past, children's exposure to lead (Pb) via the ingestion of low-cost jewelry resulted in cases with serious acute or chronic adverse effects, including death (CDC, 2004, 2006; VanArsdale et al., 2004). Recent studies also show contamination with other toxic elements in various toys and especially in jewelry (with cadmium (Cd), and to a lesser extent copper (Cu), nickel (Ni), arsenic (As), and antimony (Sb)) (Guney & Zagury, 2013a), in plastic toys, and in modeling clay (with As, Cd, and Sb) (Korfali, Sabra, Jurdi, & Taleb, 2013). Toys and jewelry sold in North America may contain high levels of metals mainly due to lack of regulations for certain contaminants and inadequate enforcement of current ones (Becker et al., 2010; Guney & Zagury, 2011, 2012). Sources, presence, toxic effects, and mobilization potential of various contaminants in toys and

children's jewelry have been already discussed in the reviews of Becker et al. (2010) and Guney and Zagury (2012). Exposure of children to metals in toys and inexpensive jewelry via ingestion due to mouthing may significantly add to the present carcinogenic and non-carcinogenic risks from other sources.

Metals can be released from toy and jewelry matrices to gastric and intestinal fluids following ingestion. Significant amounts of metals may become bioavailable and harm various organs once having reached systemic circulation. Oral bioavailability is the fraction of a contaminant reaching the systemic circulation from the gastrointestinal tract after ingestion, and oral bioaccessibility is the fraction of the substance that becomes soluble in the gastrointestinal tract and is thus available for absorption (Ruby, Davis, Schoof, Eberle, & Sellstone, 1996). Bioaccessibility can be used as an estimation of bioavailability and, when available, validated *in vitro* bioaccessibility tests might be preferred over *in vivo* bioavailability tests for their cost advantage and ethical considerations. A few studies have investigated the bioaccessibility of metals in toys and jewelry, concentrating mainly on Pb or Cd bioaccessibility in low-cost jewelry. Yost and Weidenhamer (2008b) used the U.S. Consumer Product Safety Commission (U.S.CPSC) test to determine accessible Pb in contaminated plastic jewelry (0.07 M HCl, no digestive enzymes). Total accessible Pb measured in nine items varied from 7.5 to 1,290 μg per item. Weidenhamer et al. (2011) investigated Cd bioaccessibility in contaminated jewelry using saline (representing saliva) and diluted HCl (ingestion) solutions. They found that Cd can leach to saline, and to a larger extent, to HCl solution. Brandon et al. (2006) investigated bioaccessibility of different contaminants in consumer products by using a detailed physiologically-based in *vitro* digestion model. They tested three toys (one finger paint and one chalk spiked with Pb, and one chalk contaminated by Pb) for Pb bioaccessibility (gastrointestinal bioaccessibility reported as 4.5-6.3% for finger paint, <1% for spiked chalk, 0.1-0.3% for contaminated chalk). Finally, Guney and Zagury (2013) recently tested a limited number of samples (n=4) and demonstrated that Pb and Cd in low-cost jewelry can become bioaccessible.

A systematic study investigating metal bioaccessibility in toys and children's jewelry in a large set of contaminated articles is missing in the literature. It is important to assess metal bioaccessibility in toys and children's jewelry and characterize risk for children when the following points are considered: children's significant exposure to metals from other sources, the

extent of toy and jewelry contamination problem in North America, documented incidences and significant potential of exposure to contaminated items, and, leaching potential of toxic elements from toys and children's jewelry in gastrointestinal tract. The objectives of this study are (1) to estimate gastrointestinal bioavailability of elements in selected contaminated items (n=24) using three bioaccessibility protocols, (2) to evaluate and compare performances of protocols, and, (3) to characterize the risks for children via oral exposure following ingestion.

5.2 Experimental

5.2.1 Sample selection

Selected contaminated samples (n=24) from a previously analyzed sample set (Guney & Zagury, 2013a) (n=72) were tested to determine oral bioaccessibility. Samples belonged to the following categories: metallic toys and jewelry (MJ, n=20), plastic toys (PL, n=3), and brittle or pliable toys (BP, n=1). MJ can have very high concentrations of metals. Low-cost jewelry can contain very high amounts of Pb or Cd (Désy, 2012; Guney & Zagury, 2013a; Weidenhamer & Clement, 2007a; Weidenhamer et al., 2011; Yost & Weidenhamer, 2008b), and concentrations exceeding 80% (w/w) have been reported for many samples (Weidenhamer & Clement, 2007a; Weidenhamer et al., 2011). Pb, Sn or Cu may come from leaded electronic waste which can be recycled for use in jewelry production (Weidenhamer & Clement, 2007c). Cd is used as a cheap substitute for banned Pb by manufacturers (Becker et al., 2010). PL covers a wide variety of toys (including teethers and rattles), and may include metals used as stabilizers during plastics production (Kumar & Pastore, 2007). Finally, BP includes toys like play dough, carton puzzle and chalk. One of the 24 items analyzed was selected from a list of previously screened toys for their metal content via XRF analysis (PL05), and five articles were similar to jewelry items previously recalled by Health Canada (samples MJ14-18) (Ecology Center, 2008; Health Canada, 2010d). All items were bought from the North American market: from dollar stores, toy shops, low-cost jewelry stores, retailer chains and on the Internet.

5.2.2 Determination of total metal content

For each sample, a part representative of a section of the item which may be subject to exposure was sampled and acid-digested. For the MJ category, the entire item was tested for MJ01, MJ02, MJ03, MJ05, MJ06, MJ12 and MJ13; while an intact part of an item (i.e. pendant from a jewelry) was used for the remaining articles (See Table 5.1 for detailed sample descriptions). Digestion was performed in duplicates via HNO_3 digestion on a hot plate, followed by optional additional digestion via $HClO_4$ or $HClO_4/HF$ whenever necessary (Standard Method 3030) (Clesceri et al., 1999). Digestates and procedure blanks were centrifuged (5,000×g), filtered (0.45 µm), diluted to 50 mL, and preserved at 4°C until analysis. As, Cd, Cu, Ni, Pb, and Sb concentrations in the digestates were measured by ICP-OES with method detection limits (DL's) as (in $\mu g.L^{-1}$); As: 19.5, Cd: 1.2, Cu: 2.4, Ni: 5.7, Pb: 16.6, Sb: 15.6. The concentrations of elements in toy/jewelry material ($mg.kg^{-1}$) are presented in Table 5.1.

5.2.3 Determination of oral bioaccessibility

Samples were tested by using the *in vitro* gastrointestinal protocol (IVG, n=24), the *in vitro* physiologically based extraction test (PBET, n=12), and the protocol of European Standard on Safety of Toys for migration of elements (EN 71-3, n=24) (See Table 5.2 for test conditions and comparison). IVG (Rodriguez et al., 1999) and PBET (Ruby et al., 1999) are physiologically-based *in vitro* bioaccessibility protocols mimicking the gastrointestinal conditions of infant physiology. These tests are conducted at 37 °C with the presence of digestive compounds, under controlled pH, and with mechanical agitation. The IVG protocol has been validated against *in vivo* studies to assess As, Cd, and Pb bioaccessibility for some soils, and is widely used in the assessment of metal bioaccessibility in soils (Guney, Welfringer, de Repentigny, & Zagury, 2013; Juhasz et al., 2010, 2011; Rodriguez et al., 1999; Schroder et al., 2004; U.S.EPA, 2000). Similarly, the PBET protocol has also been validated for various elements and is widely used to assess the bioaccessibility of metals in soils (Jovanovic, Pan, & Wong, 2012; Juhasz et al., 2013; Lanphear et al., 2000; Vasiluk, Dutton, & Hale, 2011). The EN 71-3 protocol (BSI, 2006) is a relatively more simple test (no *in vivo/in vitro* validation, only HCl is added, no mechanical agitation for testing glass/ceramic/metallic materials, no intestinal phase) used to determine the migration of As, Ba, Cd, Cr, Hg, Pb, Sb, and Se in different types of toy materials. It is a part of

European Standard EN 71 which specifies safety requirements for toys. EN 71-3 is widely used for toy testing especially in Europe since this test is designed to verify compliance to migration limits stated in the European Toy Safety Legislation (European Council, 2009) (see *Results and Discussion* for values).

For all bioaccessibility tests, an entire item was tested for MJ01-06, MJ12, MJ13, and PL02 (varying mass). A 1 g sample was tested for PL05 and BP18, and an intact part of each item was tested for the remaining samples (i.e. pendant from jewelry, varying mass). The tests were performed in triplicates (except in duplicates for the samples MJ12-18) and six procedure blanks for each test were processed. Ten mL of sample was taken from each replicate and from the procedure blank both at the end of gastric phase (for gastric (G) bioaccessibility) and intestinal phase (for gastrointestinal (GI) bioaccessibility). After centrifugation (5000×g) and filtration (0.45 μm), 1 mL of HNO_3 was added to the samples and they were preserved at 4 °C until analysis. The concentrations of As, Cd, Cu, Ni, Pb, and Sb were measured via ICP-OES. Then, migrated amounts (in μg) of elements (= elemental concentration × volume of gastric or intestinal liquid), as well as their migratable concentrations (in $mg.kg^{-1}$) of metals in toy/jewelry material (= migrated amount ÷ sample mass) were calculated (Tables 5.3 and 5.4).

5.2.4 Risk characterization

Chemical daily intake (CDI, μg.kg bodyweight^{-1}.d^{-1}) was calculated assuming one time exposure to jewelry or toy sample (via the ingestion of an entire item, an intact part from entire item, or a mass of 1 g; depending on the sample) for the elements having significant bioaccessibility values (Cd, Cu, Ni, and Pb) according to the following formula:

$$CDI = \frac{BAcc \times EF}{BW} \qquad (1)$$

Where BAcc (μg) is the GI bioaccessible quantity of an element via one time exposure determined via the IVG or PBET protocols (Table 5.3), EF is exposure frequency (1 d^{-1}, representing one time exposure), and BW is the mean body weight (9.2 kg for 6−12 mo-old infants, 11.4 kg for 1-2 yr-old toddlers, 13.8 kg for 2-3 yr-old toddlers, 18.6 kg for 3-6 yr-old young children[5]).

A hazard index (HI, no unit) for oral exposure to elements via ingestion was calculated according to the following formula:

$$HI = {CDI}/{RfD} \qquad (2)$$

Where RfD is the reference dose (RfD for Cd: 0.5 $\mu g.kg^{-1}.d^{-1}$, suggested limit value for Pb exposure from toys: 3.6 $\mu g.kg^{-1}.d^{-1}$, minimal risk level for Cu: 10 $\mu g.kg^{-1}.d^{-1}$, RfD for Ni: 20 $\mu g.kg^{-1}.d^{-1}$) (ATSDR, 2011; U.S.EPA, 1994, 1996a; van Engelen et al., 2006).

5.2.5 Quality assurance/quality control

In order to assess the accuracy of the digestion method, one standard reference material (SRM54d, tin-base bearing metal) was analyzed in duplicate (reference values (%w/w): 7.04 for Sb, 3.62 for Cu, 0.62 for Pb and 0.002 for Ni). The obtained results were consistent with the certified values (%w/w; 8.98 for Sb, 3.50 for Cu, 0.65 for Pb, and 0.004 for Ni; within 20% of the certified values for Sb, Cu and Pb). In order to assess the reproducibility of the bioaccessibility protocols, the bioaccessibilities of selected elements (As and Cu for the IVG protocol, As and Pb for PBET protocol) in one standard reference material (SRM2710, Montana highly elevated trace element concentration soil) was determined in triplicate by using the IVG and PBET protocols. GI bioaccessibility of As and Cu according to the IVG protocol were 29.2% and 57.6%, respectively (relative standard deviation [RSD]: ±4.9% and ±7.1%, respectively). These values are comparable to the values reported by Pouschat and Zagury (As: 25.2%; Cu: 46.5%; standard deviations [SD]: ±0.3% and ±2.8%, respectively) (Pouschat & Zagury, 2006, 2008). The GI bioaccessibility of As and Pb according to PBET were 23.0% and 22.1%, respectively (RSD: ±7.4% and ±0.9%, respectively). These values are at the lower end of the As and Pb bioaccessibilities measured using PBET by five different laboratories as reported by Koch et al (2013). According to this study, the inter-laboratory variability of As and Pb bioaccessibility for SRM2710 was high, accompanied by high reproducibility RSDs (22 – 44% for As, 45 – 83% for Pb). SRM54d was also tested via the IVG, PBET and EN 71-3 protocols to provide a basis of comparison to future studies. This reference material is mentioned in Health Canada's protocol C-08 (Determination of migratable lead in consumer products) to be used as control sample

(Health Canada, 2008) and therefore was considered suitable in this study to be analyzed via bioaccessibility protocols (results given in Tables 5.3 and 5.4).

Total metal concentrations in blanks from the digestion and bioaccessibility experiments were always below or very close to the method DLs. The RSDs of duplicates for each analyzed sample were calculated (see Table 5.1 for digestions, the RSD values for the bioaccessibility protocols were calculated but are not reported). The RSD values were less than 25% for the majority of the analyses. However, for some samples, results from duplicates showed larger differences. This was attributed to sample heterogeneity during sampling (samples had to be prepared from different sections of the same toy, or similar sections of two or more identical jewelry items were used) and/or to the variability of the chemical composition of different items of the same type. It has been already shown that individual samples of the same type of jewelry can vary greatly in terms of metals concentrations (i.e. individual samples tested for the same type of bracelet by different laboratories showed analyses of 99.1%, 67.0% and 0.07% Pb [w/w]), indicating opportunistic use of source materials for jewelry[20] and resulting in varying concentrations for different production batches.

Table 5.1 Concentrations of As, Cd, Cu, Ni, Pb, and Sb (average [mg.kg^{-1}] ± RSD [%]) in low-cost jewelry and toys

#	Item	Description	As	As±	Cd	Cd±	Cu	Cu±	Ni	Ni±	Pb	Pb±	Sb	Sb±
1	MJ01	Pewter ornament for scrapbooking	<4.51		[a]9.58	± 1	[b]2.68E+04	± 20	2.50E+03	± 24	102	± 16	139	± 49
2	MJ02	Smiley face brads for scrapbooking	10.1	± 3	180	± 5	142	± 3	265	± 8	325	± 9	211	± 10
3	MJ03	Pewter embellishments for scrapbooking	431	± 36	187	± 4	1.25E+04	± 7	5.05E+03	± 6	4.36E+05	± 4	1.02E+03	± 58
4	MJ05	Pewter embellishments for scrapbooking	[c]≤1.59		9.45	± 26	1.30E+04	± 2	2.28E+03	± 52	157	± 4	25.0	± 6
5	MJ06	Pewter embellishments for scrapbooking	1.57	± 0	15.2	± 21	1.61E+04	± 12	3.23E+03	± 3	119	± 7	17.5	± 1
6	MJ07	Decorative metal beads for craft	3.56	± 12	1.40	± 6	5.68E+05	± 2	9.20E+03	± 4	163	± 10	28.8	± 5
7	MJ08	Bracelet with metal beads	43.5	± 1	88.2	± 0	1.34E+03	± 19	1.40E+04	± 1	86.1	± 46	53.0	± 0
8	MJ09	Decorative metal beads for craft	≤4.75		263	± 4	7.10E+05	± 21	1.10E+04	± 28	469	± 4	27.6	± 7
9	MJ10	Bracelet with metal beads	[d]<2.86		8.00	± 84	5.75E+05	± 0	8.24E+03	± 7	106	± 13	23.2	± 27
10	MJ12	Decorative pins for scrapbooking	20.7	± 4	73.8	± 5	1.73E+03	± 11	4.38E+03	± 3	31.8	± 38	39.3	± 16
11	MJ13	Decorative pins for scrapbooking	56.8	± 46	76.0	± 1	3.16E+04	± 15	5.29E+03	± 36	≤5.82		38.8	± 33
12	MJ14	Jewelry piece - metal rose	62.4	± 0	203	± 4	4.35E+03	± 1	5.94	± 25	6.53E+05	± 11	272	± 33
13	MJ15	Jewelry piece - metal key	<4.76		2.59E+05	± 14	1.36E+05	± 18	1.03E+03	± 69	1.08E+03	± 7	60.7	± 1
14	MJ16	Metal earring	<4.41		1.66E+05	± 13	9.50E+04	± 8	1.87	± 23	37.0	± 16	35.7	± 16
15	MJ17	Jewelry metal pendant	<5.25		12.7	± 70	1.58E+04	± 6	≤1.74		88.5	± 0	38.6	± 17
16	MJ18	Jewelry metal pendant	≤7.95		3.67E+05	± 18	6.86E+04	± 85	17.7	± 62	100	± 1	32.7	± 43
17	MJ19	Bracelet beads	5.16	± 2	8.62	± 41	1.53E+05	± 2	24.1	± 13	10.7	± 23	173	± 21
18	MJ20	Bracelet charm	≤5.36		57.1	± 74	9.79E+04	± 29	≤1.42		<3.61		40.6	± 21
19	MJ24/2	Bracelet - metal chain	85.2	± 5	82.5	± 3	2.82E+04	± 3	184	± 3	<3.33		48.2	± 18
20	MJ25	Necklace - silver plastic	15.7	± 15	17.5	± 3	298	± 39	59.3	± 47	85.2	± 4	631	± 40
21	PL02	Eraser set shaped as food	24.9	± 33	0.23	± 23	10.8	± 32	1.37	± 3	76.5	± 5	182	± 1
22	PL05/1	Jewelry design set - very small glass beads	81.2	± 22	771	± 69	19.2	± 75	2.99	± 35	≤8.03		18.1	± 39
23	PL05/2	Jewelry design set - small glass beads	207	± 67	351	± 19	338	± 7	22.3	± 57	24.2	± 92	39.8	± 69
24	BP18	Crayon set (tip and coating composite sample)	<1.46		2.81	± 10	301	± 15	25.1	± 0	<1.24		<1.17	

[a] *Italic bold values* indicate elevated total concentrations between 25% and 100% of the EU migration limit.
[b] **Underlined bold values** indicate total concentration exceeding EU migration limit (see the text for limit values).
[c] ≤: Concentration analyzed in one or more of the replicates is below DL and the rest above DL. Average of measurements is given.
[d] <: Concentrations analyzed in all replicates are below DL. Average of DL's is given.

Table 5.2 Comparison of three in vitro bioaccessibility tests used to assess gastrointestinal bioavailability of As, Cd, Cu, Ni, Pb, and Sb

	[a]IVG	[b]PBET	[c]EN 71-3
Test temperature	37 °C	37 °C	37 °C
Presence of HCl	Yes	Yes	Yes
Presence of digestive salts and enzymes	Yes	Yes	No
Mechanical agitation	Yes (padded stirrer, at 100 rpm)	Yes (padded stirrer, at 100 rpm)	No
Volume of gastric solution	150 mL	100 mL	50 times (in mL) of sample's mass (in g)
Gastric phase	Yes	Yes	Yes
Duration	1 h	1 h	2 h
pH of gastric phase	1.8	2.5	1.8
Digestive compounds	HCl, NaCl, pepsin	HCl, pepsin, malate, citrate, lactic acid, acetic acid	HCl
Intestinal phase	Yes	Yes	No
Duration	1 h	4 h	-
pH of intestinal phase	5.5	7.0	-
Digestive compounds	Bile, pancreatin, Na_2CO_3	Bile, pancreatin, Na_2CO_3	-

[a] Rodriguez et al. (1999)
[b] adapted from Ruby et al. (1999)
[c] British Standards (2006)

Table 5.3 Gastric/gastrointestinal bioaccessible quantities (in µg, mean (n=3)) of Cd, Cu, Ni, and Pb in selected samples measured via IVG, PBET, and EN 71-3 protocols

	EN 71-3 (Gastric)				IVG (Gastrointestinal)				PBET (Gastrointestinal)			
	Cd	**Cu**	**Ni**	**Pb**	**Cd**	**Cu**	**Ni**	**Pb**	**Cd**	**Cu**	**Ni**	**Pb**
MJ01	[a]<0.11	17.7	112	<0.87	<0.31	81.7	384	<2.50	<0.21	15.5	747	≤1.75
MJ02	1.13	<0.18	2.48	<0.85	5.53	1.41	15.2	<2.50	14.5	1.49	78.2	<1.67
MJ03	<0.33	[b]≤0.84	95.0	1.63E+03	<0.31	21.3	535	1.43E+03	≤0.22	29.4	533	1.26E+03
MJ05	0.80	≤0.40	54.0	<1.05	<0.31	≤0.66	124	<2.49	≤0.26	2.52	231	<1.67
MJ06	<0.13	0.85	15.7	<1.04	<0.31	<0.51	83.3	<2.49	0.42	1.64	227	≤2.56
MJ07	<0.07	88.7	39.7	<0.57	<0.31	451	243	<2.50	<0.21	99.1	1.47E+03	<1.67
MJ08	0.27	201	73.2	<0.83	<0.31	≤1.58	8.88	<2.49	-	-	-	-
MJ09	1.66	774	10.5	<0.83	≤0.37	338	9.71	<2.50	≤0.49	624	18.0	<1.67
MJ10	0.44	298	97.6	<0.82	0.65	645	46.2	<2.50	0.44	731	73.1	<1.67
MJ12	0.80	0.67	19.8	<0.90	≤0.33	2.86	21.1	<2.50	-	-	-	-
MJ13	≤0.12	18.5	17.9	<0.95	<0.31	41.6	55.9	<2.50	-	-	-	-
MJ14	1.60	≤1.49	<0.50	112	<0.19	1.42	<0.37	705	0.45	11.7	<0.52	756
MJ15	96.5	2.54	2.53	<1.33	128	1.75	4.92	<2.50	139	9.89	13.0	<1.67
MJ16	13.0	29.2	0.49	<0.39	12.1	1.49	<0.78	<2.50	21.6	3.14	<0.52	<1.66
MJ17	<0.12	0.36	<0.32	<1.01	<0.31	5.08	≤1.56	≤2.83	-	-	-	-
MJ18	172	5.31	1.21	<1.71	347	9.69	≤1.58	<2.49	286	34.0	1.43	<1.66
MJ19	<0.12	478	<0.29	<0.93	<0.31	217	≤1.72	<2.50	-	-	-	-
MJ20	<0.07	1.91	<0.18	<0.58	<0.31	6.23	≤2.12	<2.50	-	-	-	-
MJ24-2	<0.28	2.96	20.3	<2.24	<0.31	12.2	50.0	<2.49	-	-	-	-
BP18	0.23	0.67	0.94	≤1.09	0.34	1.25	1.58	≤2.65	-	-	-	-
SRM54d	21.8	12.9	<0.26	392	28.9	19.1	≤1.09	329	12.9	31.6	≤0.60	353

[a]<: Concentrations analyzed in all replicates are below DL. Average of DL's is given.
[b]≤: Concentration analyzed in one or more of the replicates is below DL and the rest above DL. Average of measurements is given.

Table 5.4 Gastric/gastrointestinal bioaccessible concentrations (in mg.kg^{-1}, mean (n=3)) of Cd, Cu, Ni, and Pb in selected samples measured via IVG, PBET, and EN 71-3 protocols

	EN 71-3 (Gastric)				IVG (Gastrointestinal)				PBET (Gastrointestinal)			
	Cd	**Cu**	**Ni**	**Pb**	**Cd**	**Cu**	**Ni**	**Pb**	**Cd**	**Cu**	**Ni**	**Pb**
MJ01	[a]<0.10	16.0	106	<0.82	<0.32	83.0	***396***	<2.56	<0.21	15.7	***757***	[b]≤1.77
MJ02	1.09	<0.17	2.39	<0.82	5.35	1.34	14.6	<2.40	[c]***13.7***	1.40	73.8	<1.59
MJ03	<0.10	≤0.26	29.9	[d]**<u>502</u>**	<0.10	6.88	172	**<u>423</u>**	≤0.07	9.31	171	**366**
MJ05	0.63	≤0.32	42.5	<0.82	<0.24	≤0.51	97.1	<1.96	≤0.20	1.97	182	<1.31
MJ06	<0.10	0.67	12.4	<0.82	<0.24	<0.40	65.5	<1.96	0.33	1.28	178	≤2.00
MJ07	<0.10	128	57.6	<0.82	<0.45	652	***353***	<3.62	<0.30	143	**<u>2.12E+03</u>**	<2.40
MJ08	0.26	195	70.2	<0.82	<0.30	≤1.55	8.88	<2.47	[e]-	-	-	-
MJ09	1.55	733	10.3	<0.82	≤0.38	351	9.71	<2.67	≤0.46	567	17.4	<1.78
MJ10	0.44	301	97.7	<0.82	0.65	645	45.9	<2.52	0.44	734	73.7	<1.67
MJ12	0.73	0.61	18.4	<0.82	≤0.34	2.89	21.8	<2.60	-	-	-	-
MJ13	≤0.10	15.9	15.3	<0.82	<0.27	35.7	48.3	<2.15	-	-	-	-
MJ14	0.83	≤0.82	<0.26	***57.9***	<0.18	1.34	<0.34	**<u>647</u>**	0.23	5.93	<0.27	**389**
MJ15	**<u>60.2</u>**	1.55	1.56	<0.82	**<u>80.0</u>**	1.07	3.05	<1.54	**<u>85.5</u>**	5.99	7.96	<1.03
MJ16	**<u>26.7</u>**	63.3	1.01	<0.82	**<u>24.8</u>**	3.10	<1.64	<5.25	**<u>44.0</u>**	6.64	<1.09	<3.49
MJ17	<0.10	0.29	<0.26	<0.82	<0.25	4.12	≤2.12	≤2.27	-	-	-	-
MJ18	**<u>81.5</u>**	2.50	0.58	<0.82	**<u>165</u>**	4.60	≤0.77	<1.19	**<u>136</u>**	16.2	0.68	<0.79
MJ19	<0.10	420	<0.26	<0.82	<0.27	191	≤1.54	<2.19	-	-	-	-
MJ20	<0.10	2.70	<0.26	<0.82	<0.43	8.30	≤2.81	<3.49	-	-	-	-
MJ24-2	<0.10	1.06	7.56	<0.82	<0.11	4.39	18.2	<0.90	-	-	-	-
BP18	0.22	0.66	0.92	≤1.07	0.34	1.23	1.56	≤2.62	-	-	-	-
SRM54d	21.7	12.9	<0.26	390	28.9	19.0	≤1.09	328	12.9	31.6	≤0.60	353

[a] <: Concentrations analyzed in all replicates are below DL. Average of DL's is given.
[b] ≤: Concentration analyzed in one or more of the replicates is below DL and the rest above DL. Average of measurements is given.
[c] *Italic bold values* indicate elevated total concentrations between 25% and 100% of the EU migration limit.
[d] **<u>Underlined bold values</u>** indicate total concentration exceeding EU migration limit (see the text for limit values).   [e] (-): Sample not tested

5.3 Results & Discussion

5.3.1 Metal contamination in toys and jewelry

Total (Table 5.1) and bioaccessible concentrations of elements (Table 5.4) in 20 MJ, 3 PL and 1 BP samples were compared to migration limits stated in the European Union (EU) Toy Safety Directive (European Council, 2009). These limits were used since the EU Toy Safety Directive provides a more comprehensive approach to the chemical safety of toys than North American legislations due to its scientific basis, the large number of selected contaminants, and separate limits defined for different categories (Guney & Zagury, 2012). Migratable concentration limits defined in this directive are (in mg.kg^{-1}); As:47, Cd:23, Cu:7,700, Ni:930, Pb:160, Sb:560, (in scraped-off toy material, applicable to the categories MJ and PL in this study); and, As:3.8, Cd:1.9, Cu:622.5, Ni:75, Pb:13.5, Sb:45 (lower limits defined for dry, brittle, powder-like or pliable toy material; applicable to the category BP).When the total concentration of an element in a toy exceeds the EU migration limit, it can be said that further migration analysis is necessary to determine whether exposure to this toy is safe or not. Also, in the following discussion, the total concentration of an element was stated as elevated if it was between 25 and 100% of the limit value.

The samples analyzed in this study were mainly contaminated by highly toxic Cd (number of samples with elevated concentrations: 22, number of samples exceeding the EU limit: 15) and Pb (elevated: 15 samples, exceeding: 6 samples). As, Cu, Ni and Sb contamination was also present in some samples (elevated: 10, 17, 13, and 6 samples, respectively; exceeding: 6, 15, 11, and 2 samples, respectively). In some items, the total Pb and Cd concentrations exceeded the EU limits up to several thousand times (Pb concentrations up to 65% (w/w) and Cd as high as 37% (w/w) in jewelry). In addition to Pb and Cd, some MJ items also had very high concentrations of Cu and Ni. All samples except PL02 (eraser shaped as food) had at least one elemental concentration among six exceeding the EU limit. The sample PL02 could also be categorized in the BP category. If done so, the contamination in this item exceeds the lower EU limits specially defined for the BP category.

When the U.S. and the Canadian limits are considered (which regulate only total Pb and Cd concentrations in toys and/or jewelry), it was found that 11 MJ samples exceeded the U.S. and

the Canadian Pb limit (100 and 90 mg.kg^{-1}, respectively) (Government of Canada, 2010; U.S.CPSC, 2012). The U.S. limit for total Cd (300 mg.kg^{-1} in children's jewelry, (ASTM International, 2011)) was exceeded in three articles (jewelry items MJ15, MJ16 and MJ18), and the Canadian limit (130 mg.kg^{-1} in children's jewelry (Health Canada, 2011)) in seven items.

5.3.2 Bioaccessibility of As, Cd, Cu, Ni, Pb, and Sb

According to the results for the oral bioaccessibility of six elements (Tables 5.3 and 5.4); Cd, Cu, Ni, and Pb were present above DLs in gastric and intestinal fluids from 20 of 24 samples. Arsenic was below DL in all gastric and intestinal extracts, and Sb was present in low concentrations in two samples (MJ02 and MJ05, maximum bioaccessible concentration: 15.7 mg.kg^{-1} according to IVG, value well below the EU limit). Therefore, the results for these elements were not presented. Also, results for MJ25 (plastic jewelry), PL02 (eraser set), PL05/1 and PL05/2 (glass beads from a children's jewelry design set) were not included in Tables 5.3 and 5.4 since the concentrations of all six elements in gastric and intestinal fluids were below DLs.

The results from the IVG protocol showed that the bioaccessible concentrations of Cd exceeded the EU limit of 23 mg.kg^{-1} in MJ15, MJ16, and MJ18 (jewelry items, having the highest total Cd concentrations among all samples). Pb bioaccessible concentrations were above the EU limit of 160 mg.kg^{-1} in MJ03 and MJ14 (one metallic embellishment and one jewelry, having the highest Pb total concentrations among all samples). Ni bioaccessible concentrations were elevated (25-100% of the EU limit of 930 mg.kg^{-1}) in MJ01 and MJ07 (two metallic toys).The values for bioaccessible Cu were well below the EU limit. In summary, five items were found to exceed the EU standard limit values for Cd or Pb.

Twelve items were tested using the PBET protocol, and they gave similar results to the ones obtained via the IVG protocol. GI bioaccessibility values exceeded the EU limits in the same five articles for Cd (MJ15, MJ16, and MJ18) and Pb (MJ03 and MJ14). Similarly for Ni, bioaccessible concentration was elevated for MJ01. Furthermore, it exceeded the EU limit for MJ07. Bioaccessible Cu in all samples was well below the EU limit. Overall, six items had bioaccessible concentrations exceeding the EU limits for Cd, Ni, or Pb.

The more simple gastric-only EN 71-3 protocol generally yielded bioaccessible metal concentrations lower than IVG and PBET protocols. However, for Cd, the same articles (MJ15, MJ16, and MJ18) had bioaccessible concentrations exceeding the EU limit, with values being generally lower than the ones provided by the other two protocols. For Pb, bioaccessible concentration in MJ03 exceeded the limit, but not in MJ14 (elevated concentration). Bioaccessible Ni and Cu in all samples were well below the EU limits. In summary, four samples exceeded the EU standard limits for Cd or Pb, when the EN 71-3 protocol was used. Lower results in terms of bioaccessible concentrations could be attributed to the absence of mechanical agitation, intestinal phase, and digestive enzymes in this protocol.

For the IVG and PBET protocols, the G and GI bioaccessibilities of four metals were compared via paired-sample t-test using the results for samples having bioaccessibility values above DL. For the IVG protocol, the difference between G and GI bioaccessibilities was significant for Ni (average, in mg.kg^{-1}: 96.5 for GI vs. 79.2 for G; n=13; p<0.05), and for Cu (average, in mg.kg^{-1}: 134 vs. 89.4; n=15; p<0.1). For the PBET protocol, GI bioaccessibility exceeded G for all four metals, but the difference was significant only for Cu (average, in mg.kg^{-1}: 168.8 vs. 40.6, n=9; p<0.1). Although the differences were not statistically significant for all samples, GI bioaccessibility was higher than G in many samples for IVG and especially for PBET. Therefore, it can be said that conducting a two-phase (gastric and intestinal) bioaccessibility test could be safer than using only the gastric phase of that test while assessing oral metal bioaccessibility from jewelry and toys.

5.3.3 Comparison of tests

A regression analysis was performed to compare the EN 71-3, IVG, and PBET protocols by using the overall bioaccessibility data for sample pairs with values above DL (Figure 5.1). Also, two-tailed paired-sample t-test was used to check the null hypothesis that the mean of dataset pairs from different protocols are statistically similar. These analyses were done for the results of Cd, Cu, Ni, and Pb between IVG – EN 71-3, PBET – EN 71-3, and PBET – IVG pairs (a total of 12 analyses for four metals and three test pairs). Overall, the tests were compared below only considering the results for Cd, Cu and Ni since the degrees of freedom for Pb was low (n=2).

The regression analysis yielded fits between test pairs with moderate to very high R^2 values and low y-intercepts (with the only exception of PBET – EN 71-3 pair for Ni), indicating a good fit and related results between test pairs. Also, overall, none of the *t* values between test pairs were large enough to show a significant difference, meaning that tests did not differ from each other when predicting bioavailabilities (with the only exception of PBET – EN 71-3 pair for Ni, p <0.1).
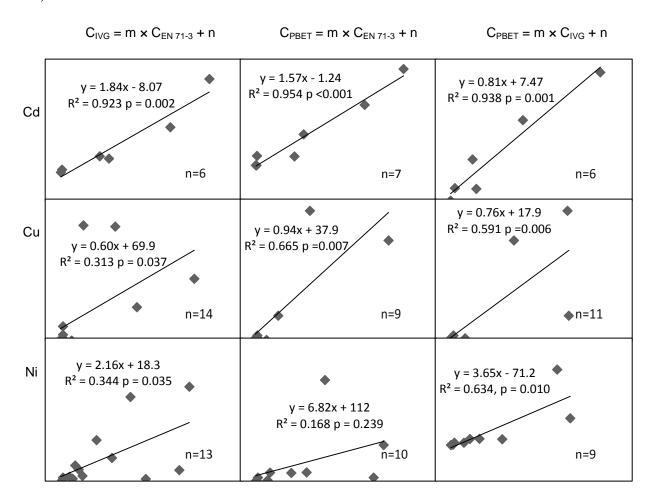
Figure 5.1 Comparison of results from EN 71-3, IVG, and PBET bioaccessibility tests for Cd, Cu, and Ni

The individual results for selected samples and metals were also compared by using student's *t*-test and considering the values from replicate analyses (for Cd: MJ15, MJ16, MJ18; Cu: MJ07, MJ09, MJ10; Ni: MJ01, MJ03, MJ07; Pb: MJ03, MJ14; samples with high metal bioaccessibility leading to HI>1 as discussed in the next section). The analyses were performed between IVG –

EN 71-3, PBET – EN 71-3, and PBET – IVG pairs (results for eleven samples used, three test pairs, a total of 33 analyses done). The results indicated significant differences (p<0.05) between the measured bioaccessibilities of Cu (MJ07 for EN-71-3 – IVG and IVG – PBET pairs, MJ10 for EN71-3 – PBET pair), Ni (MJ01 and MJ07, all three pairs), and Pb (MJ14, all three pairs). In total, 12 of 33 tests indicated a significant difference between measured concentrations via different bioaccessibility tests in the same sample for the same metal. In other words, different bioaccessibility tests predicted different bioavailability for some of the selected metallic toys and low-cost jewelry. Since no study has been conducted on the use and validation of *in vitro* bioaccessibility tests regarding toy and low-cost jewelry testing, it is not possible to conclude on the accuracy of the measured values or to recommend one test among the others. This being said, EN 71-3 is not physiologically based and has not been subjected to *in vivo – in vitro* validation studies. Moreover, since EN 71-3 has the potential to underestimate bioaccessibility as discussed above, it could be safer to use a two-phase bioaccessibility test like IVG or PBET for the assessment of bioaccessibility of potentially toxic elements in jewelry and toys.

5.3.4 Comparison of total and bioaccessible concentrations of metals

A regression analysis was performed by using the total metal and the bioaccessibility data from the IVG protocol for samples with values above DL (Figure 5.2). For Cd and Cu, total and bioaccessible metal concentrations were correlated (R^2 = 0.833 and 0.804; p <0.05 and <0.001; n = 6 and 17, respectively). However, it should be noted that total metal concentration data distribution is definitely far from normal distribution, and has values with unproportionately higher weight in regression analysis which can affect the quality of the regression and the subsequent comparison (Table 5.1). For example, for Cu, when the highest three total metal concentration and its corresponding bioaccessibility values were removed from the regression, R^2 decreased to 0.230.

For Ni, total and bioaccessible metal concentrations had no apparent relationship (R^2 = 0.0001, p = 0.970, n = 13). Especially for Ni, and also for Cd and Cu in some samples, total concentrations failed to accurately predict bioaccessible concentrations. Finally for Pb, it was not appropriate to draw conclusions due to low number of data points (n=3). Since total and bioaccessible concentrations are different and do not always well correlate, total concentration may not be a

good indicator of metal bioavailability for toys and low-cost jewelry. Therefore, bioaccessibility testing is recommended to estimate bioavailability when assessing risk from exposure to toys and low-cost jewelry rather than evaluating the risk based on total concentrations.
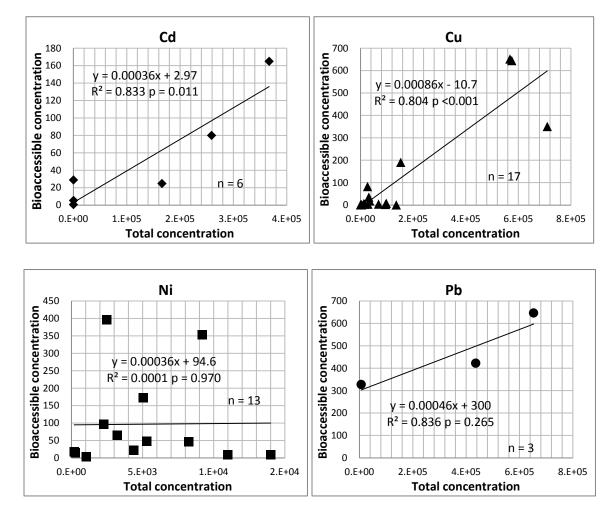
Figure 5.2 Comparison of total and bioaccessible (IVG) concentrations of metals (mg.kg^{-1})
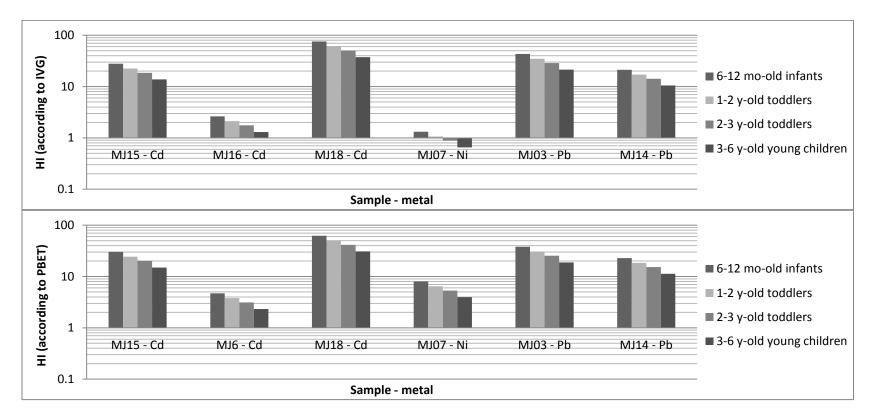
Figure 5.3 Hazard index (HI) values for selected samples and metals (n=6, according to IVG and PBET protocols) from oral exposure to contaminated metallic toys and jewelry for 6-12 mo-old infants, 1-2 yr-old and 2-3 yr-old toddlers, and 3-6 yr-old young children

5.3.5 Risk characterization

The results of the risk characterization (Figure 5.3) performed for six samples with total metal concentrations exceeding the EU migration limits for Cd, Ni, and Pb showed that one time exposure to these items may lead to HI values exceeding 1. For items contaminated by Cd (jewelry articles MJ15, MJ16 and MJ18) and Pb (metallic toy MJ03 and jewelry piece MJ14), HI values calculated using the bioaccessible quantities measured via the IVG and PBET protocols were both similar and very high. For Ni-contaminated MJ07 (craft beads), HI values were higher than 1 according to the results of both tests for 6-12 mo old infants and 1-2 y old toddlers. For 2-3 y old toddlers and 3-6 y old young children, HI values were above 1 according to the results from PBET, but not from IVG. Due to their lower mean body weight, 6-12 mo old infants had the highest HI values among the four age groups.

Back calculations using the formula (1), accounting for the mean body weight of 6-12 mo old infants, and taking HI = 1 yielded limit bioaccessible quantities of 4.6 µg for Cd, 92 µg for Cu, 184 µg for Ni and 33.1 µg for Pb. In other words, exceeding these bioaccessible quantities will yield HI values >1 for 6-12 mo old infants in case of oral exposure via ingestion. When compared to the bioaccessible quantities measured via the IVG and PBET bioaccessibility protocols (Table 5.3), these values (for both tests) were exceeded in four items for Cd (for MJ02, in addition to previously discussed MJ15, MJ16 and MJ18 which were identified as hazardous according to the EU limit), three items for Cu (for MJ07, MJ09, and MJ10), three items for Ni (for MJ01 and MJ03, in addition to previously identified MJ07), and two items for Pb (for previously identified MJ03 and MJ14). In summary, ten of 20 articles in MJ category had HI>1 and thus could be hazardous to 6-12 mo old infants in case of oral exposure following ingestion.

It should be finally noted that HI values presented here are appropriate when based on the assumption that exposure duration to ingested items is around typical average digestion time for food for children, resulting in an orocoecal (from ingestion to the large intestine) transit time close to 5 h (Ruby et al., 1999). This exposure time is roughly represented by one bioaccessibility test. In other words, following ingestion, small contaminated item rests in the gastrointestinal tract for a certain limited time, and then is eliminated from the body, However, in some cases, contaminated jewelry can stay in the gastrointestinal tract for longer times following ingestion (CDC, 2006; VanArsdale et al., 2004). In this case of prolonged exposure, the risk will be higher.

Research on prolonged exposure to contaminated metallic toys and jewelry is therefore recommended. Furthermore, a more detailed risk assessment for exposure to contaminated toys and jewelry considering additional pathways (i.e. the mobilization of metals in saliva) as well as variability and uncertainty of different exposure parameters (i.e. mouthing time, body weight etc.) is also recommended.

Acknowledgements

CHAPTER 6   PUBLICATION #5 – CHILDREN'S EXPOSURE TO HARMFUL ELEMENTS IN TOYS AND LOW-COST JEWELRY: CHARACTERIZING RISKS AND DEVELOPING A COMPREHENSIVE APPROACH

This chapter aims to finalize the work conducted within the scope of the present dissertation by providing an integrated detailed risk characterization considering children's exposure to contaminated toys and low-cost jewelry under three exposure scenarios. Also, in order to overcome drawbacks and complexities accompanying different approaches for toy safety, a comprehensive methodology based on contaminant bioavailability has been developed and presented. This approach provides definitions, testing recommendations, and guidelines for risk characterization which permit to effectively evaluate children's potential exposure to contaminated toys and jewelry; and includes bioaccessible limits for different exposure scenarios and for eight priority elements. This work has been submitted to the journal *Science of the Total Environment* (on October 7, 2013; manuscript #: STOTEN-D-13-03258) which is one of the leading journals in the category of Environmental Science with an Impact Factor of 3.258 as of 2012.

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ABSTRACT

Contamination problem in jewelry and toys, and the possibility of children's exposure to these items have been previously demonstrated. In the present study, risk from oral exposure has been characterized for highly contaminated metallic toys and jewelry (n=16) considering ingestion of parts/pieces, ingestion of scraped-off material, and saliva mobilization scenarios. To resolve shortcomings of current approaches to chemical safety of jewelry and toys, a comprehensive approach including bioaccessible limits for eight priority elements was developed. Total and bioaccessible concentrations of Cd, Cu, Ni, and Pb were high in selected metallic toys and jewelry. Ingestion of parts or pieces caused unacceptable risk (Hazard Index [HI]>1) for eight items for Cd, Ni, and Pb (HI as high as 75, 5.8, and 43, respectively). Second, HI caused by ingestion of scraped-off material was always <1 (up to 0.287 for Cd, 0.156 for Pb). Finally, saliva mobilization scenario caused HI>1 in three samples (two for Cd, one for Ni). Risk characterization identified different potentially hazardous items when compared to U.S., Canadian, and EU regulatory approaches. A comprehensive approach has been developed to deal with complexity and drawbacks caused by various toy and jewelry definitions, test methods, exposure scenarios, and elements considered in different regulatory approaches. Further research is recommended on metals bioaccessibility determination in toys and jewelry, development of an in vitro bioaccessibility test with in vivo validation, more accurate estimation of play time and toy material ingestion rates, presence of Cr(VI) and Sn(org), and assessment of prolonged exposure to metallic toys and jewelry.

6.1 Introduction

Children can be exposed to contaminants including potentially toxic elements via different sources, and economical costs and adverse health outcomes of children's exposure to environmental contaminants are difficult to oversee. For example, the burden associated with childhood lead exposure in low- and middle-income countries is estimated as 1.2% of world gross domestic product in 2011, and lead-attributable economic costs are predicted as 51 and 55 billion USD for the U.S. and Europe, respectively (Attina & Trasande, 2013). In addition to

cognitive deficits related to blood Pb levels even in low concentrations previously accepted as safe (Lanphear et al., 2000), exposure to As, Mn, and Cd has also been associated with neurodevelopment and behavioral disorders in children (Rodríguez-Barranco et al., 2013).

Inexpensive children's jewelry and toys may contain high levels of toxic metals. The use of metals as stabilizers in plastics during manufacturing, application of paint containing metal pigments to toys and low-cost jewelry, and the use of contaminated recycled plastics or metals in production are the main reasons for the metal contamination in toys and jewelry (Becker et al., 2010; Guney & Zagury, 2012). Due to chemical safety hazards, more than 100 children's products recalls were issued in North America alone in 2007 and 2008, and recalls comprising millions of items have been made during the last decade (Morrisson, 2009; U.S.CPSC, 2010a). Contamination problem has been previously demonstrated in plastic toys (Greenway & Gerstenberger, 2010), toys with paint or coating (Kawamura et al., 2006), brittle or pliable toys (Rastogi & Pritzl, 1996), liquid paint (Rastogi, 1992), and jewelry (MJ) (Maas et al., 2005; Weidenhamer & Clement, 2007a; Weidenhamer et al., 2011; Yost & Weidenhamer, 2008b). Recent research also showed the ongoing problem of contamination with toxic elements in metallic toys and jewelry (MJ) (with Pb and Cd, and to a lesser extent Cu, Ni, As, and Sb (Guney & Zagury, 2013a)).

Mouthing behavior plays an important role in children's exposure to environmental contaminants (Tulve et al., 2002). Children are increasingly likely to mouth non-food items starting from 6-mo old, and the high behavioral frequency of mouthing hands and objects (1 to 2 y) begins to moderate between 2 y- and 3 y-old (Firestone, Moya, Cohen-Hubal, Zartarian, & Xue, 2007), then becomes less significant after 3 y-old (Xue et al., 2007). According to Farmakakis et al. (2007), toys were the most frequent cause of medical emergency situations due to aspiration or ingestion of inedible foreign bodies in Greek children, followed by coins and jewelry. Mouthing contaminated jewelry or toys may cause the release of contaminants via contact with saliva, or lead to ingestion of toy or jewelry material. These may result in contaminant release into digestive fluids, as shown by laboratory essays simulating saliva extraction and gastro-intestinal

digestion (Brandon et al., 2006; Guney, Nguyen, & Zagury, 2013; Guney & Zagury, 2013a, 2013b; Weidenhamer et al., 2011; Yost & Weidenhamer, 2008b). Also in the past, children's exposure to Pb via ingestion of low-cost jewelry resulted in cases with serious acute or chronic adverse effects, including death (CDC, 2006; VanArsdale et al., 2004). Therefore, it can be hypothesized that especially for young children up to the age of 3, mouthing behavior can significantly contribute to exposure to toxic elements from low-cost jewelry and toys.

Oral bioavailability is the fraction of a contaminant reaching the systemic circulation from the gastro-intestinal tract after ingestion, and oral bioaccessibility is the fraction of the substance that becomes soluble in the gastro-intestinal tract and is thus available for absorption (Ruby et al., 1996). Bioaccessibility can be used as an estimation of bioavailability and, when available, validated *in vitro* bioaccessibility tests might be preferred over *in vivo* bioavailability tests for their cost advantage and ethical considerations. Incorporating contaminant bioavailability in terms of bioaccessibility and using bioaccessible concentrations instead of total concentrations in risk characterization may prevent risk overestimation and provide more accurate results. Although the extent of jewelry and toy metal contamination as well as the mobilization potential of elements has been demonstrated, there is no comprehensive risk characterization study in the literature considering children's exposure to contaminated jewelry and toys.

Despite the fact that elemental contamination in inexpensive jewelry and toys is not limited to Pb and Cd, the U.S. and Canadian regulations provide limits (Table 6.1) only for these metals (in addition, migratable limits for additional elements are only applicable to paints or coatings) (ASTM International, 2008, 2011; Health Canada, 2012, 2013; U.S.CPSC, 2012). These regulations should be ideally based on bioaccessible content rather than on total contaminant concentrations (Guney & Zagury, 2013a), and should include a larger number of potentially toxic elements. In Europe, migration limits for 19 elements (Table 6.1) are already stated in the EU Toy Safety Directive (European Council, 2009). These limits have been determined assuming that children's exposure to elements should not exceed a certain percentage of their TDI. However, background exposure to elements from other sources was not explicitly considered while determining the limits. Furthermore, some of these 19 elements are not expected to be found in toys and/or have low toxicity. Finally, there is a need for a special set of limits regarding toys or jewelry considering ingestion of parts or pieces (carrying a potential of ingestion in the

order of grams per exposure) in addition to the current limits based on scraped-off , brittle/pliable, and liquid/sticky material (based on ingestion amounts assumed as 8, 100, and 400 mg.d^{-1}, respectively).

The present study's objective is to characterize risk from oral exposure to Cd, Cu, Ni, and Pb in selected contaminated inexpensive jewelry (n=16) and toys considering three scenarios: ingestion of parts or pieces of a jewelry or toy, ingestion of scraped-off toy or jewelry material, and mobilization via saliva following contact with mouth. A comprehensive approach is developed and presented with bioaccessible quantity limits for eight priority elements (As, Cd, Cr, Cu, Hg, Ni, Pb, and Sb).

6.2 Material and Methods

6.2.1 Sample selection

Previously, a set of toys and inexpensive jewelry has been analyzed for its total concentrations of ten elements (Guney & Zagury, 2013a) (n=72), and contaminated items were further tested to assess saliva mobilization and gastro-intestinal bioaccessibility via different *in vitro* protocols (Guney, Nguyen, et al., 2013; Guney & Zagury, 2013b). In the present study, the results for selected MJ (n=16) were used to conduct risk characterization. Sample descriptions with total and bioaccessible concentrations of Cd, Cu, Ni, and Cd are presented in Table 6.2. MJ can have very high concentrations of metals. Especially, inexpensive jewelry has been shown to contain high amounts of toxic elements (Désy, 2012; Guney & Zagury, 2013a; Weidenhamer & Clement, 2007a; Weidenhamer et al., 2011; Yost & Weidenhamer, 2008b), where Pb and Cd concentrations exceeding 80% (w/w) have been reported for many samples (Weidenhamer & Clement, 2007a; Weidenhamer et al., 2011). Analyzed items were bought from the North American market: from dollar stores, toy shops, low-cost jewelry stores, retailer chains; and, on the Internet.

6.2.2 Total metal content and oral bioaccessibility determination

For each sample, a part representative of a section of the item which may be subject to exposure was sampled and acid-digested. Digestion was performed in duplicates via HNO_3 digestion on a hot plate, followed by optional additional digestion via $HClO_4$ or $HClO_4/HF$ whenever necessary (Standard Method 3030 (Clesceri et al., 1999)). Digestates and procedure blanks were centrifuged (5,000×g), filtered (0.45 µm), diluted to 50 mL, and preserved at 4°C until analysis. Cd, Cu, Ni, and Pb concentrations in the digestates were measured by ICP-OES with method DLs as (in µg.L^{-1}); Cd: 1.2, Cu: 2.4, Ni: 5.7, Pb: 16.6.

To determine bioaccessibility for the case of ingestion, the IVG protocol (Rodriguez et al., 1999), which is a physiologically-based *in vitro* bioaccessibility protocol simulating the gastro-intestinal conditions of an infant, was used. Tests are conducted at 37 °C with the presence of digestive compounds, under controlled pH, and with mechanical agitation. For bioaccessibility tests, an entire item was tested for MJ01-06, MJ12, and MJ13 (varying sample mass), and an intact part of each item was tested for the remaining samples (i.e. pendant from jewelry, varying mass). The bioaccessibility tests were performed in triplicates (except in duplicates for the samples MJ12-18). Ten mL of sample was taken from each replicate and from the procedure blank at the end of intestinal phase to determine gastro-intestinal bioaccessibility. After centrifugation (5,000×g) and filtration (0.45 µm), 1 mL of HNO_3 was added to the samples and they were preserved at 4 °C until analysis. Metal concentrations were measured via ICP-OES with method DLs as (in µg.L^{-1}); Cd: 1.2, Cu: 2.4, Ni: 5.7, Pb: 16.6.

Table 6.1 Tolerable daily intake (TDI) and limit values for potentially toxic elements in toys and jewelry: Current (U.S., Canadian, and EU) and proposed limits

	TDI values			U.S. limits (total and soluble concentrations)			Canadian limits (total and soluble concentrations)			EU limits (migratable concentrations)			Proposed bioaccessible amount limits	
													Acute exposure	Chronic exposure
	TDI [a]	Background exposure [a]	TDI$_{toy}$	Children's products [e] (total)	Surface coating material in toy and jewelry [f,g] (soluble)	Jewelry [f] (total/soluble)	Toys [h] (total)	Surface coating material in toys [h] (soluble)	Jewelry [i] (total/soluble)	Scraped off toy material [j]	Brittle or pliable toy material [j]	Liquid or sticky toy material [j]	Ingestion of an entire toy or jewelry part/piece [k]	Ingestion of toy/jewelry material, or saliva mobilization [l]
	(μg.kg^{-1}.d^{-1})	(μg.kg^{-1}.d^{-1})	(μg.kg^{-1}.d^{-1})	(mg.kg^{-1})	(mg.kg^{-1})	(mg.kg^{-1}/μg)		(mg.kg^{-1})	(mg.kg^{-1})	(mg.kg^{-1})	(mg.kg^{-1})	(mg.kg^{-1})	(μg)	(μg)
As	1	up to 0.7	0.05[b]	-	25	-	-	1000	-	47	3.8	0.9	**8**	**0.4**
Cd	0.5	0.45	0.025[b]	-	75	300/200	-	1000	130/-	23	1.9	0.5	**4**	**0.2**
Cr	5	1	0.5[c]	-	60	-	-	-	-	460	37.5	9.4	**40**	**4**
Cu	83	60	4.15[d]	-	-	-	-	-	-	7700	622.5	156	**664**	**33.2**
Hg	2	0.1	0.1[b]	-	60	-	prohibited	-	-	94	7.5	1.9	**16**	**0.8**
Ni	10	8	0.5[d]	-	-	-	-	-	-	930	75	18.8	**80**	**4**
Pb	3.6	2	0.18[b]	100	90	100/-	90	-	600/90	160	13.5	3.4	**28.8**	**1.4**
Sb	6	0.53	1.27[c]	-	60	-	-	1000	-	560	45	11.3	**48**	**10.2**

[a] Values of TDI and background exposure taken from van Engelen et al. (2006)   [b] TDI$_{toy}$ taken as 5% of TDI due to high elemental toxicity (see text).
[c] TDI$_{toy}$ + Background exposure = 30% of TDI (see text).   [d] TDI$_{toy}$ taken as 5% of TDI due to background exposure exceeding 30% of TDI (see text).
[e] U.S.CPSC (2012)   [f,g] ASTM (2008,2011) (also provide limits for soluble Ba and Se in surface coating material)
[h] Health Canada (2012) (also provides limits for soluble Ba and Se in surface coating material)   [i] Health Canada (2013)
[j] European Council (2009) (also provides migratable limits for Al, B, Ba, Cr(VI), Co, Mn, Se, Sr, Sn, Sn(org), and Zn)
[k] Based on 100% of TDI considering short-term exposure, and and a lower percentile body weight of 8.0 kg for a female infant (6 - 12 mo-old; U.S.EPA (2011))
[l] Based on TDI$_{toy}$ considering long-term exposure, and a lower percentile body weight of 8.0 kg for a female infant (6 - 12 mo-old; U.S.EPA (2011))

Table 6.2 Total and bioaccessible (saliva and gastro-intestinal) concentrations of Cd, Cu, Ni, and Pb (in mg.kg-1) in contaminated toys and jewelry

Sample	Description	Total concentration (via total digestion)				Saliva bioaccessible concentration (via the DIN protocol)				Gastro-intestinal bioaccessible concentration (via the IVG protocol)			
		Cd	Cu	Ni	Pb	Cd	Cu	Ni	Pb	Cd	Cu	Ni	Pb
MJ01	Pewter ornament for scrapbooking	9.58	2.68E+04	2.50E+03	102	<0.02	0.5	5.95	<0.20	<0.32	83.0	396	<2.56
MJ03	Pewter embellishments for scrapbooking	187	1.25E+04	5.05E+03	4.36E+05	<0.01	0.71	3.58	0.86	<0.10	6.88	172	423
MJ05	Pewter embellishments for scrapbooking	9.45	1.30E+04	2.28E+03	157	0.12	1.30	2.01	<0.13	<0.24	≤0.51	97.1	<1.96
MJ06	Pewter embellishments for scrapbooking	15.2	1.61E+04	3.23E+03	119	0.14	1.88	2.89	<0.13	<0.24	<0.40	65.5	<1.96
MJ07	Decorative metal beads for craft	1.40	5.68E+05	9.20E+03	163	<0.04	2.88	8.20	<0.36	<0.45	652	353	<3.62
MJ08	Bracelet with metal beads	88.2	1.34E+03	1.40E+04	86.1	<0.03	2.39	1.88	<0.24	<0.30	≤1.55	8.88	<2.47
MJ09	Decorative metal beads for craft	263	7.10E+05	1.10E+04	469	<0.05	15.8	≤0.23	<0.42	≤0.38	351	9.71	<2.67
MJ10	Bracelet with metal beads	8.00	5.75E+05	8.24E+03	106	<0.04	20.0	0.42	<0.37	0.65	645	45.9	<2.52
MJ12	Decorative pins for scrapbooking	73.8	1.73E+03	4.38E+03	31.8	<0.05	2.52	5.3	<0.44	≤0.34	2.89	21.8	<2.60
MJ13	Decorative pins for scrapbooking	76.0	3.16E+04	5.29E+03	≤5.82	<0.05	39.3	391	<0.43	<0.27	35.7	48.3	<2.15
MJ14	Jewelry piece - metal rose	203	4.35E+03	5.94	6.53E+05	<0.02	1.47	<0.06	0.53	<0.18	1.34	<0.34	647
MJ15	Jewelry piece - metal key	2.59E+05	1.36E+05	1.03E+03	1.08E+03	5.30	8.86	1.66	<0.30	80.0	1.07	3.05	<1.54
MJ16	Metal earring	1.66E+05	9.50E+04	1.87	37.0	4.56	6.75	<0.37	<1.04	24.8	3.10	<1.64	<5.25
MJ18	Jewelry metal pendant	3.67E+05	6.86E+04	17.7	100	≤22.8	4.79	≤0.19	<0.24	165	4.60	≤0.77	<1.19
MJ19	Bracelet beads	8.62	1.53E+05	24.1	10.7	<0.04	8.89	<0.14	<0.39	<0.27	191	≤1.54	<2.19
MJ24-2	Bracelet - metal chain	82.5	2.82E+04	184	<3.33	<0.02	1.46	2.93	<0.17	<0.11	4.39	18.2	<0.90

<: Concentrations analyzed in both duplicates are below DL. Average of DL's is given.

≤: Concentration analyzed in one of the duplicates is below DL and the other is above DL. Average of two measurements is given.

To determine bioaccessibility via saliva mobilization, a slightly modified version of the saliva extraction compartment of the three-phase bioaccessibility test DIN method 19738 (DIN, 2004) was used. This test approximates real exposure case via mechanical agitation and synthetic saliva at 37 °C with the presence of various inorganic and organic chemical compounds. The saliva composition per 30 mL was 15 mg NaSCN, 55 mg Na_2SO_4, 50 mg NaCl, 15 mg $NaHCO_3$, 45 mg KCl, 60 mg KH_2PO_4, 15 mg $CaCl_2.2H_2O$, 75 mg mucin, 25 mg α-amylase, 10 mg urea, and 1 mg uric acid. For each sample, a part representative of a section of the children's jewelry or toy (~10 cm^2 whenever possible and applicable) which may be subject to mouthing was sampled, transferred to a 125 mL erlenmeyer, and was agitated at 100 rpm for 30 min following the addition of 30 mL of synthetic saliva. The aliquots taken after 30 min were centrifuged (5,000×g, 15 min), filtered (0.45 µm), acidified by adding 0.5 mL of HNO_3, and preserved at 4 °C until analyzed. Concentrations were measured by ICP-OES (method DLs in $µg.L^{-1}$ were Cd: 1.6, Cu: 2.8, Ni: 5.3, Pb: 14.9). Analyses were made in duplicates.

The bioaccessibility of each element was calculated and expressed in terms of quantity ($Q_{bioacc} = C_{aliq} \times V$, in µg) and concentration ($C_{bioacc} = C_{aliq} \times V \div m$, in $mg.kg^{-1}$) where C_{aliq} ($mg.L^{-1}$) is the concentration in aliquot of digestive fluid, V (mL) is the volume of digestive fluid, and m (g) is the sample mass tested.

6.2.3 Risk characterization

Exposure was assessed by considering oral pathway and considering these scenarios: ingestion of parts or pieces of a toy or jewelry, ingestion of scraped-off toy or jewelry material, and saliva mobilization following mouth contact. CDI was calculated for each scenario by using the formulas and selected parameter values given in Table 6.3. Three different age categories were considered: 6-12 mo-old toddlers, 1-2 y-old infants, and 2-3 y-old infants. Corresponding mean body weights for these age categories (9.2 kg for 6−12 mo-old infants, 11.4 kg for 1-2 yr-old toddlers, and 13.8 kg for 2-3 yr-old toddlers) were used in calculations (U.S.EPA, 2011).

For mouthing (solubilization in saliva), an age-specific exposure duration ($min.d^{-1}$) was calculated for 6−12 month-old infants, 1-2 yr-old toddlers, and 2-3 yr-old toddlers based on their mean play and mouthing times (U.S.EPA, 2011). For the scenario of ingestion of parts or pieces, one-time acute exposure was assumed. For the ingestion of scraped-off material and saliva mobilization scenarios, chronic daily exposure was assumed. For the ingestion of scraped-off

material, an ingestion rate was assumed as 8 mg.d^{-1} for all samples (scraped-off toy material (van Engelen et al., 2006)). A HI (no unit) for oral exposure to elements was calculated according to the following formula:

$$HI = {}^{CDI}\!/_{RfD} \qquad (1)$$

RfD values were taken from the TDI list compiled by van Engelen et al. (2006): Cd: 0.5 µg.kg^{-1}.d^{-1}, Cu: 83 µg.kg^{-1}.d^{-1}, Ni: 10 µg.kg^{-1}.d^{-1}, Pb: 3.6 µg.kg^{-1}.d^{-1}. Calculated HI values for three different exposure scenarios are presented in Tables 6.4-6.6.

6.2.4 Quality assurance/quality control

Total metal concentrations in blanks from the digestion and bioaccessibility experiments were always below or very close to the method DLs. Two certified reference materials (SRM2710 and SRM54d) were analyzed in order to assess the accuracy of the digestion method and the reproducibility of the bioaccessibility protocols. RSD values were also calculated to track the variability of results. Quality control of reagents, analytical processes, analytical proficiency; method accuracy and reproducibility; and analysis variability was satisfactory (results given in detail in previous articles (Guney, Nguyen, et al., 2013; Guney & Zagury, 2013a, 2013b)).

Table 6.3 Formulas and related exposure parameters with selected values and tests used to assess children's exposure to toys and low-cost jewelry

Formula	Parameter		Value
Oral pathway via ingestion of parts or pieces $CDI_{whole} =$ $Q_{bioacc} \times EF \div BW$	CDI_{whole}	Chemical daily intake via whole ingestion ($\mu g.kg^{-1}.d^{-1}$)	To be determined
	Q_{bioacc}	Bioaccessible quantity (μg)	Determined via the IVG protocol by testing an ingestible part or piece, can be also determined via other appropriate protocols i.e. the PBET protocol, or the EN 71-3 method.
	EF	Exposure frequency (d^{-1})	$1\ d^{-1}$, assuming one-time exposure
	BW	Body weight (kg)	9.2 kg for 6-12 mo-old infants, 11.4 kg for 1-2 y-old toddlers, and 13.8 kg for 2-3 y-old toddlers
Oral pathway via ingestion of scraped-off, brittle/pliable, or liquid/sticky material $CDI_{partial} =$ $IngR \times C_{bioacc} \times CF \div BW$	$CDI_{partial}$	Chemical daily intake via partial ingestion ($\mu g.kg^{-1}.d^{-1}$)	To be determined
	IngR	Ingestion rate ($mg.d^{-1}$)	$8\ mg.d^{-1}$ for scraped-off material, $100\ mg.d^{-1}$ for brittle or pliable material, $400\ mg.d^{-1}$ for liquid or sticky material
	C_{bioacc}	Bioaccessible concentration ($mg.kg^{-1}$)	Determined via the IVG protocol by testing an ingestible part or piece, can be also determined via other appropriate protocols i.e. the PBET protocol, or the EN 71-3 method.
	BW	Body weight (kg)	9.2 kg for 6-12 mo-old infants, 11.4 kg for 1-2 y-old toddlers, and 13.8 kg for 2-3 y-old toddlers
	CF	Unit conversion factor	10^{-3}
Oral pathway via saliva mobilization $CDI_{saliva} =$ $Q_{bioacc} \times ED \div BW$	CDI_{saliva}	Chemical daily intake via mouthing ($\mu g.kg^{-1}.d^{-1}$)	To be determined
	Q_{bioacc}	Bioaccessible quantity ($\mu g.30\ min^{-1}$)	Determined via the mouthing compartment of the DIN protocol by testing a mouthable part or piece ($\sim 10\ cm^2$) for 30 min, can be also determined via other suitable protocols i.e. the RIVM protocol, etc.
	ED	Age-specific exposure duration ($min.d^{-1}$)	Calculated for 6−12 month-old infants, 1-2 yr-old toddlers, and 2-3 yr-old toddlers by taking play time as 1.1, 3.2, and 2.6 $h.d^{-1}$, respectively; and mean mouthing time as 9, 7, and 10 $min.h^{-1}$, respectively.
	BW	Body weight (kg)	9.2 kg for 6-12 mo-old infants, 11.4 kg for 1-2 y-old toddlers, and 13.8 kg for 2-3 y-old toddlers

6.3 Results and Discussion

6.3.1 Metal contamination and bioaccessibility

The tested samples include six metallic toys and ten jewelry, and total and bioaccessible concentrations of Cd, Cu, Ni, and Pb in 16 selected MJ were high (sample descriptions and metal concentrations are given in Table 6.2). In certain items, total Pb and Cd total concentrations exceeded the U.S., Canadian, and EU limits up to several thousand times (Pb concentrations up to 65% (w/w) and Cd as high as 37% (w/w) in jewelry). In terms of bioaccessible concentrations, four samples did not comply with the EU limit for migratable Cd (measured via the EN 71-3 method (BSI, 2013), results not shown). In particular, Cd bioaccessibility exceeded the EU limit in samples MJ15 (jewelry piece from a necklace), MJ16 (metal earring), and MJ18 (jewelry pendant piece); and, Pb bioaccessibility exceeded the migration limit in MJ03 (pewter embellishment for scrapbooking). When the U.S. regulations are considered, nine items (MJ01-07, MJ09, MJ10, MJ14, MJ15, and MJ18) exceeded the total Pb standard (100 mg.kg^{-1}). For Cd, MJ15, MJ16, and MJ18 exceeded the total Cd limit (300 mg.kg^{-1}), but not the soluble limit (200 μg, when tested via EN 71-3), therefore these samples were in compliance with the Cd regulation. Finally, when Canadian regulations are taken into account, nine items (MJ01-06, MJ09, MJ14-18) did not comply with the standards due to total/soluble Pb or total Cd content.

6.3.2 Risk characterization

The results for the scenario of ingestion of parts or pieces (Table 6.4) showed that eight samples may pose significant risk to children's health in terms of Cd, Ni, and/or Pb exposure if ingested. HI values were especially high for the samples MJ03 (pewter embellishments for scrapbooking) and MJ14 (jewelry piece shaped as metal rose) for Pb, and for the samples MJ15 (jewelry piece shaped as metal key) and MJ18 (jewelry metal pendant) for Cd. HI also exceeded 1 for MJ16 (Cd), MJ01, MJ03, MJ05, and MJ07 (Ni). Furthermore, for many samples, Cd, Cu, and Ni exposure was below the critical threshold but HI can still be considered as high (between 0.1 and 1). Therefore, it can be concluded that children's exposure to MJ via the ingestion of parts or pieces for the samples included in this study cause unacceptable hazard indices.

Table 6.4 Hazard Index (HI) values calculated for 6 mo- to 3 y-old children's oral exposure (ingestion of parts or pieces) to contaminated metallic toys and jewelry

Sample	Cd			Cu			Ni			Pb		
	6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y
MJ01	-	-	-	*0.107*	0.086	0.071	**4.17**	**3.37**	**2.78**	-	-	-
MJ03	-	-	-	0.028	0.023	0.019	**5.82**	**4.69**	**3.88**	**43.2**	**34.8**	**28.8**
MJ05	-	-	-	-	-	-	**1.35**	**1.09**	*0.899*	-	-	-
MJ06	-	-	-	-	-	-	*0.905*	*0.731*	*0.604*	-	-	-
MJ07	-	-	-	*0.591*	*0.477*	*0.394*	**2.64**	**2.13**	**1.76**	-	-	-
MJ08	-	-	-	-	-	-	0.097	0.078	0.064	-	-	-
MJ09	-	-	-	*0.443*	*0.357*	*0.295*	*0.106*	0.085	0.070	-	-	-
MJ10	*0.141*	*0.114*	0.094	*0.845*	*0.682*	*0.563*	*0.502*	*0.405*	*0.335*	-	-	-
MJ12	-	-	-	0.004	0.003	0.002	*0.229*	*0.185*	*0.153*	-	-	-
MJ13	-	-	-	0.054	0.044	0.036	*0.608*	*0.490*	*0.405*	-	-	-
MJ14	-	-	-	0.002	0.002	0.001	-	-	-	**21.3**	**17.2**	**14.2**
MJ15	**27.8**	**22.5**	**18.6**	0.002	0.002	0.002	0.053	0.043	0.036	-	-	-
MJ16	**2.63**	**2.12**	**1.75**	0.002	0.002	0.001	-	-	-	-	-	-
MJ18	**75.4**	**60.9**	**50.3**	0.013	0.010	0.008	-	-	-	-	-	-
MJ19	-	-	-	*0.284*	*0.229*	*0.189*	-	-	-	-	-	-
MJ24-2	-	-	-	0.016	0.013	0.011	*0.543*	*0.439*	*0.362*	-	-	-

Bold values show HI > 1.
Italic values show 0.1 < HI < 1.
-: Not calculated since concentrations analyzed in one or more replicates are below DL

The scenario of ingestion of scraped-off material (Table 6.5) yielded much lower HI values in comparison to ingestion of parts or pieces scenario. HI values were lower than 1 for all samples, metals, and age categories. For a limited number of samples, Cd and Pb exposure was relatively elevated (HI between 0.1 and 0.3). The lower HI values can be attributed to the low ingestion rate value (taken as 8 mg.d^{-1} based on the estimation of van Engelen et al. (2006) for scraped-off toy material). There is an important need for studies observing and estimating children's toy ingestion rates; and yet, for the moment, the estimation taken from van Engelen et al. (2006) seems reasonable when general properties of MJ (hardness, difficulty of scraping, etc.) are taken into account. As a result, it can be concluded that ingestion of scraped-off material from MJ does not pose unacceptable risk to children, compared to the ingestion of parts or pieces for this category. It should be noted, however, that risk values for the exposure would be significantly higher for other toy categories (namely brittle or pliable toys, and liquid or sticky toys) due to higher possible ingestion rates for these toy categories (assumed as 100 and 400 mg.d^{-1}, respectively (van Engelen et al., 2006)). Finally, for both ingestion scenarios, calculated risk values for the age category 6-12 mo-old were the highest, followed by the 1-2 y-old and 2-3 y-old

categories. This is due to the fact that the mean body weight used in the calculations for the 6-12 mo-old infants is the lowest, exceeded by the values for 1-2 y-old and 2-3 y-old toddlers.

Table 6.5 Hazard Index (HI) values calculated for 6 mo- to 3 y-old children's oral exposure (ingestion of scraped-off material) to contaminated metallic toys and jewelry

Sample	Cd			Cu			Ni			Pb		
	6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y
MJ01	-	-	-	0.001	0.001	0.001	0.034	0.027	0.023	-	-	-
MJ03	-	-	-	0.000	0.000	0.000	0.015	0.012	0.010	*0.102*	0.082	0.068
MJ05	-	-	-	-	-	-	0.008	0.006	0.005	-	-	-
MJ06	-	-	-	-	-	-	0.006	0.005	0.004	-	-	-
MJ07	-	-	-	0.007	0.006	0.005	0.031	0.025	0.021	-	-	-
MJ08	-	-	-	-	-	-	0.001	0.001	0.001	-	-	-
MJ09	-	-	-	0.004	0.003	0.003	0.001	0.001	0.001	-	-	-
MJ10	0.001	0.001	0.001	0.007	0.006	0.005	0.004	0.003	0.003	-	-	-
MJ12	-	-	-	0.000	0.000	0.000	0.002	0.002	0.001	-	-	-
MJ13	-	-	-	0.000	0.000	0.000	0.004	0.003	0.003	-	-	-
MJ14	-	-	-	0.000	0.000	0.000	-	-	-	*0.156*	*0.126*	*0.104*
MJ15	*0.139*	*0.112*	0.093	0.000	0.000	0.000	0.000	0.000	0.000	-	-	-
MJ16	0.043	0.035	0.029	0.000	0.000	0.000	-	-	-	-	-	-
MJ18	*0.287*	*0.232*	*0.191*	0.000	0.000	0.000	-	-	-	-	-	-
MJ19	-	-	-	0.002	0.002	0.001	-	-	-	-	-	-
MJ24-2	-	-	-	0.000	0.000	0.000	0.002	0.002	0.001	-	-	-

Italic values show $0.1 < HI < 1$.
-: Not calculated since concentrations analyzed in one or more replicates are below DL

HI values calculated for saliva mobilization scenario (Table 6.6) were lower in comparison to the scenario of ingestion of parts or pieces; but higher than the values from scenario of ingestion of scraped-off material. Calculations for the samples MJ13 (for Ni), MJ15, and MJ18 (for Cd) yielded HI values larger than 1. Also for some samples, HI values were relatively high (i.e. between 0.1 and 1) for Cd, Ni, and Pb. The calculated values were the highest for 1-2 y-old toddlers since they have the highest exposure duration (product of mean mouthing and play times) compared to the other age categories.

Overall, the ingestion of parts or pieces was found as the most problematic scenario, followed by saliva mobilization and ingestion of scraped-off material scenarios. This was mainly because the amount of toy or jewelry material to be exposed was much higher (in the order of grams) in the scenarios of ingestion of parts or pieces and saliva mobilization following mouth contact, compared to the exposure via the ingestion of scraped-off material. The eight samples identified via the risk characterization as potentially hazardous to children were not exactly the same items

that did not comply with the U.S., Canadian, and EU regulations (Table 6.7). When the limits for migratable concentrations of elements stated in the EU Toy Safety Directive are used (scraped-off toy material category, applicable to the 16 samples analyzed in this study, the strictest limits among the three toy categories in the directive), only four samples (MJ03, MJ15, MJ16, and MJ18) has been considered as hazardous, for their migratable Cd concentrations (measured via the EN 71-3 method (BSI, 2013), results not presented). However, risk characterization indicated four additional potentially hazardous samples (MJ01, MJ05, MJ07, and MJ14). Similarly, the limits from the U.S. regulations set apart nine potentially hazardous samples due to their Pb content (six samples when the Canadian regulations are considered), while according to the results of risk characterization, only two of eight potentially harmful samples are classified as such because of Pb contamination and mobility.

Table 6.6 Hazard Index (HI) values calculated for 6 mo- to 3 y-old children's oral exposure (saliva mobilization) to contaminated metallic toys and jewelry

Sample	Cd			Cu			Ni			Pb		
	6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y	6-12 mo	1-2 y	2-3 y
MJ01	-	-	-	0.001	0.002	0.002	0.052	0.095	0.091	-	-	-
MJ03	-	-	-	0.003	0.005	0.005	*0.124*	*0.226*	*0.217*	0.084	*0.153*	*0.147*
MJ05	0.033	0.060	0.058	0.002	0.004	0.004	0.027	0.049	0.047	-	-	-
MJ06	0.039	0.071	0.068	0.003	0.005	0.005	0.040	0.073	0.070	-	-	-
MJ07	-	-	-	0.002	0.004	0.004	0.041	0.075	0.072	-	-	-
MJ08	-	-	-	0.002	0.004	0.004	0.014	0.026	0.025	-	-	-
MJ09	-	-	-	0.008	0.015	0.014	-	-	-	-	-	-
MJ10	-	-	-	0.012	0.022	0.021	0.002	0.004	0.004	-	-	-
MJ12	-	-	-	0.001	0.002	0.002	0.021	0.038	0.037	-	-	-
MJ13	-	-	-	0.020	0.037	0.035	**1.63**	**2.98**	**2.85**	-	-	-
MJ14	-	-	-	0.002	0.004	0.004	-	-	-	0.014	0.026	0.025
MJ15	*0.632*	**1.15**	**1.11**	0.006	0.011	0.011	0.010	0.018	0.018	-	-	-
MJ16	*0.162*	*0.296*	*0.284*	0.001	0.002	0.002	-	-	-	-	-	-
MJ18	**3.36**	**6.14**	**5.88**	0.004	0.007	0.007	-	-	-	-	-	-
MJ19	-	-	-	0.005	0.009	0.009	-	-	-	-	-	-
MJ24-2	-	-	-	0.002	0.004	0.004	0.030	0.055	0.053	-	-	-

Bold values show HI > 1.
Italic values show 0.1 < HI < 1.
-: Not calculated since concentrations analyzed in one or more replicates are below DL

Table 6.7 Comparison of total and migratable concentrations of Cd, Cu, Ni, and Pb in selected metallic toys and jewelry to the U.S., Canadian, and EU regulations; and results from risk characterization

Item	*Type*	*U.S. regulations*	*Canadian regulations*	*EU regulations*	*Risk characterization (whole ingestion)*
MJ01	Toy	**Does not comply (total Pb)**	**Does not comply (total Pb)**	Complies	**Poses significant risk i.e. HI > 1 (Ni)**
MJ03	Toy	**Does not comply (total Pb)**	**Does not comply (total Pb)**	**Does not comply (migratable Cd)**	**Poses significant risk (Ni, Pb)**
MJ05	Toy	**Does not comply (total Pb)**	**Does not comply (total Pb)**	Complies	**Poses significant risk (Ni)**
MJ06	Toy	**Does not comply (total Pb)**	**Does not comply (total Pb)**	Complies	Does not pose significant risk
MJ07	Jewelry	**Does not comply (total Pb)**	Complies	Complies	**Poses significant risk (Ni)**
MJ08	Jewelry	Complies	Complies	Complies	Does not pose significant risk
MJ09	Jewelry	**Does not comply (total Pb)**	**Does not comply (total Cd)**	Complies	Does not pose significant risk
MJ10	Jewelry	**Does not comply (total Pb)**	Complies	Complies	Does not pose significant risk
MJ12	Toy	Complies	Complies	Complies	Does not pose significant risk
MJ13	Toy	Complies	Complies	Complies	Does not pose significant risk
MJ14	Jewelry	**Does not comply (total Pb)**	**Does not comply (total Pb and Cd, soluble Pb)**	Complies	**Poses significant risk (Pb)**
MJ15	Jewelry	**Does not comply (total Pb)**	**Does not comply (total Pb and Cd)**	**Does not comply (migratable Cd)**	**Poses significant risk (Cd)**
MJ16	Jewelry	Complies	**Does not comply (total Cd)**	**Does not comply (migratable Cd)**	**Poses significant risk (Cd)**
MJ18	Jewelry	**Does not comply (total Pb)**	**Does not comply (total Cd)**	**Does not comply (migratable Cd)**	**Poses significant risk (Cd)**
MJ19	Jewelry	Complies	Complies	Complies	Does not pose significant risk
MJ24/2	Jewelry	Complies	Complies	Complies	Does not pose significant risk

COMPREHENSIVE APPROACH	
Step	**Notes**
Step 1. Assessing possibility of exposure: Can the sample be classified as toy (a product that is intended for use in learning or play by a child of <14 y-old), or children's jewelry (jewelry that appeals primarily to children <15 y-old)?	If yes, sample should be evaluated via the following steps. If no, no evaluation is needed.
Step 2. Determining suitable exposure scenario(s): 2a. Acute exposure via whole ingestion of parts or pieces: Does the toy or jewelry contain small parts or pieces that can lead to ingestion, determined as explained in ASTM F963-08 or ASTM F2923-11?	If yes, apply step 3, then 4a (skip 2b and 2c). If no, go through step 2b.
2b. Chronic exposure via partial ingestion of toy or jewelry material: Is the toy or jewelry brittle or pliable (classified as category I in BS EN 71-3 2013), does it contain liquid or sticky material (classified as category II), or can it be scraped-off (classified as category III)?	If yes, apply step 3, then 4b. If no, no testing is needed for whole ingestion. In both cases, also go through step 2c.
2c. Chronic exposure via saliva mobilization following mouthing: Does the toy or jewelry contain large non-ingestible sections, parts, or pieces that may come into contact with saliva in a child's mouth?	If yes, apply step 3, then 4b. If no, no testing is needed for mouthing.
Step 3. Determining bioaccessible quantities of eight elements: Based on the exposure scenario, test sample by using an appropriate bioaccessibility test and calculate the bioaccessible quantities (Q_{bioacc}, in µg) of As, Cd, Cr, Cu, Hg, Ni, Pb, and Sb.	Protocol selection based on the exposure scenario and additional test details are given in sections *3.3.2.* and *2.2.*
Step 4: Evaluating risk: 4a. Do the bioaccessible quantities of one or more elements exceed proposed limits for acute exposure (whole ingestion, step 2a) stated in Table 6.1?	If yes, the sample may pose risk in case of acute exposure. If no, it may be deemed as chemically safe.
4b. Do the bioaccessible quantities of one or more elements exceed proposed limits for chronic exposure (partial ingestion or mouthing, steps 2b or 2c) stated in Table 6.1?	If yes, the sample may pose risk in case of chronic exposure. If no, it may be deemed as chemically safe.

Figure 6.1 A comprehensive approach for evaluating risk from children's oral exposure to harmful elements in toys and low-cost jewelry

6.3.3 Comprehensive methodology

A comprehensive methodology was developed in order to deal with shortcomings of current approaches to chemical safety of jewelry and toys (Figure 6.1), including proposed bioaccessible amount limits for eight priority elements (Table 6.1).

6.3.3.1 Definitions of toy and jewelry

The definitions of toy and children's jewelry presented in various regulations differ from each other, and are generally accompanied by particular interpretations, age categories, and list of excluded items. For the comprehensive methodology (Figure 6.1), the following concise definitions are used: A toy is a product that is intended for use in learning or play by a child less than 14 y of age, and children's jewelry is jewelry that appeals primarily to children less than 15 y of age (Health Canada, 2012, 2013). More detailed explanations, examples, and excluded items are given by Health Canada.

6.3.3.2 Exposure scenarios and testing

The most preoccupying scenario in children's exposure to contaminated toys and jewelry is the ingestion of parts or pieces, since toys and jewelry (especially MJ) can be highly contaminated (Guney & Zagury, 2013a; Weidenhamer & Clement, 2007a; Weidenhamer et al., 2011). Moreover, ingested quantity can reach several g in this scenario (instead of several mg for the ingestion of scraped-off material, brittle/pliable, and liquid/sticky material (van Engelen et al., 2006)), and potential negative outcomes are severe (CDC, 2006; VanArsdale et al., 2004). This scenario is expected to represent a one-time ingestion incidence which may lead to acute exposure to the elements found in a piece of contaminated jewelry or toy. Therefore, it should first be determined if a sample contains easily detachable or breakable smalls parts that can be ingested, which can be done by using a small parts cylinder (ASTM International, 2008, 2011). If positive, the ingestible part or piece should be tested to estimate gastro-intestinal bioavailability of elements via an appropriate bioaccessibility protocol. Using two-phase (gastric and intestinal) physiologically based protocols like IVG (Rodriguez et al., 1999) or PBET (Ruby et al., 1996) would be recommended over simpler protocols like the gastric-only EN 71-3 method (BSI, 2013) since the former are more conservative and also better represent gastrointestinal physiology (Guney & Zagury, 2013b). Since the ingestion of parts or pieces from toys or jewelry is the most

problematic scenario, when it is considered as relevant, there is no need to examine other two scenarios which pose less risk.

If the toy or jewelry does not contain small loose parts that are ingestible, and therefore ingestion of parts or pieces is not an issue; children still can be exposed to contaminants in toys and jewelry via ingestion of some toy or jewelry material, or via saliva mobilization. In this case, the sample must be categorized into one of the following categories: brittle or pliable (classified as category I in EN 71-3 2013 (BSI, 2013)), liquid or sticky (classified as category II), or toy or jewelry that can be scraped-off (classified as category III). For the scenario of ingestion, depending on the exact category, it is suggested to test 8 mg of material toy or jewelry that can be scraped-off, 100 mg of brittle or pliable material, or 400 mg of liquid or sticky material (van Engelen et al., 2006) by using one of the bioaccessibility tests or an equivalent mentioned in the above paragraph.

For saliva mobilization scenario, it should be first checked if the toy or jewelry contains large sections that are not ingestible but may come into contact with saliva during child's mouthing activities. If this is the case, testing a mouthable part or piece (~10 cm^2) should be done for 30 min. The mouthing compartment of the DIN or RIVM bioaccessibility protocols (Brandon et al., 2006; DIN, 2004) or any other equivalent test can be used for bioaccessibility determination.

6.3.3.3 Selection of elements

The U.S. and Canadian legislations only regulate the content of Pb in toys, and Pb and Cd in jewelry (Table 6.1). The limits for other potentially toxic elements are only present for surface coating materials (a total of eight elements in the U.S. standards, five elements in the Canadian standard). In addition, Hg is prohibited in toys according to the Canadian standard. However, it has been recently shown that toys and jewelry may contain other toxic metals like As, Cu, Ni, and Sb, and their presence is not limited to only paints or coatings (Guney & Zagury, 2013a). On the contrary, the EU Directive provides limits for a large set of elements (19 metals and metalloids). This detailed list of elements presented in the directive could be shortened for more efficient testing while maintaining the same level of protection, because, certain elements included in this regulation have low toxicity or are not expected to be present in toys and jewelry. For example, Ba and Mn are toxic only in relatively high concentrations (U.S.EPA, 1996b, 2005), and like Se, their presence in various toys and jewelry was not a concern based on 72

samples tested (Guney & Zagury, 2013a). Exposure to Se is also expected to be limited (via diet, specific industries, and contaminated sites (ATSDR, 2003)). Similarly, Zn is an essential nutrient with a relatively high RfD, and, no cases of home exposure to Zn for children could be found in the literature (ATSDR, 2005a). B exposure is relevant only for inhalation pathway from industrial occupational sources, except for limited exposure from food and naturally occurring forms of this element (U.S.EPA, 2004). Co is an essential nutrient (van Engelen et al., 2006), is naturally abundant, is not commercially produced, most children are exposed to it largely through their diet (ATSDR, 2004), and there isn't any indication showing its possible presence in toys or jewelry. Cr(VI) is highly toxic (U.S.EPA, 1998), however, no information could be found regarding Cr(VI) presence in toys and jewelry therefore it has been excluded from the list of priority elements in the present study. However, Cr(VI) presence in toys and jewelry, and related exposure should be further investigated and limits must be defined if necessary. Sn(org) is also highly toxic, its major use is in antifouling paints (followed by its use as heat stabilizer in PVC), and other commercial applications (disinfectant, biocide, pesticide etc.), and most commercially used Sn(org) compounds are relatively immobile in environmental media (ATSDR, 2005b). As the toxicity of Sn(org) is high and Sn(org) compounds are numerous, its toxicity and presence in toys and jewelry (especially in plastic items) should be separately investigated and corresponding limits must be defined together with other organic contaminants in toys. Finally, Al, inorganic Sn, and Sr have very low toxicity. In conclusion, within the scope of the present study, only limits for As, Cd, Cr, Cu, Hg, Ni, Pb, and Sb in toys and jewelry have been defined, and additional research is suggested for Cr(VI) and Sn (org).

6.3.3.4 Defining limit values

The limits for 19 elements stated in the EU Toy Safety Directive (European Council, 2009) have been determined assuming that children's exposure to elements should not exceed a certain percentage of their TDI. Assuming that the ingestion is the primary exposure pathway, migratable concentration limits corresponding to 5%, 10% and 20% of TDI's have been proposed for the case of ingestion (van Engelen et al., 2006), and selected values were later used in the new Directive (European Council, 2009). Background exposure was not explicitly taken into consideration when determining the present limits. Since children may already be exposed to

significant quantities of contaminants via other pathways, considering background exposure while defining limits for potentially toxic elements in toys and jewelry is important as doing so would provide an additional safety margin.

By using a similar approach to that of van Engelen et al. (2006) while taking background exposure into account, bioaccessible limits were calculated for the scenarios of partial ingestion of toy or jewelry material (scraped-off, brittle/pliable, and liquid/sticky material), and saliva mobilization following mouthing for eight priority elements (As, Cd, Cr, Cu, Hg, Ni, Pb, and Sb). TDI and background exposure values for elements compiled from different studies by van Engelen et al. were used in calculations. For Cr and Sb, it was assumed that combined exposure from toys (TDI_{toy}) and background exposure should not exceed 30% (preliminary suggested value) of the TDI for a certain element. For Cu and Ni, background exposure already exceeds 30% of the corresponding TDI, therefore maximum allowed exposure from toys (TDI_{toy}) was conservatively selected as 5% of TDI when determining limits. For highly toxic As, Cd, Hg, and Pb, regardless of background exposure, the conservative value of 5% of TDI was taken as TDI_{toy}.

Exposure to toys and jewelry via whole ingestion of parts or pieces, which may lead to very high hazard indices, is not considered in the approach of van Engelen (van Engelen et al., 2006) and no corresponding limits are included in the EU toy safety directive regarding this exposure scenario (European Council, 2009). In the present comprehensive approach, specifically for the whole ingestion of parts or pieces, limits were calculated based on the 100% of TDI without considering the background exposure. A higher TDI percentage could be taken since this scenario is considered as an acute exposure case representing a one-time exposure incidence instead of chronic exposure.

All limit values were calculated and proposed as bioaccessible quantities (in µg). Bioaccessible quantities rather than bioaccessible concentrations are presented, because setting concentration limits in terms of concentrations requires assuming a single constant quantity of exposed toy or jewelry material in calculations, which may not represent the real exposure cases.

The present comprehensive approach is suggested with the purpose of dealing with shortcomings and complexities accompanying different approaches on chemical safety of toys and jewelry. By providing clear guidelines about the definitions of toy and children's jewelry, the number and

types of elements to be considered, the exposure scenarios to be taken into account, the test methods to be employed, and the limits to be used, it may help evaluating risk more efficiently for children's oral exposure to contaminated toys and jewelry.

6.4 Conclusions

High elemental contamination in children's low-cost jewelry and toys (especially in metallic toys and jewelry) is an important concern for children's health. Metals in children's low-cost jewelry and toys can be solubilized in gastro-intestinal tract following ingestion, or in saliva following mouthing. Risk characterization performed for 16 highly contaminated metallic toys and jewelry showed that ingestion of jewelry or toy parts or pieces may lead to unacceptable risk in terms of hazard index (HI) values for Cd, Ni, and Pb. Saliva mobilization posed less but still significant risk (HI>1) for some samples, while risk from ingestion of scraped-off toy or jewelry material was the lowest (HI<1). Furthermore, a comprehensive approach has been developed to assess chemical safety of toys and jewelry for eight priority elements. It was meant to overcome the shortcomings and complexity of current approaches (U.S., Canada, and EU); particularly on the definitions of toy and children's jewelry, the scope of elements, exposure scenarios, test methods, and the limit values.

More research on oral bioavailability of potentially toxic elements in toys and jewelry is needed. Testing different types and samples of low-cost jewelry and toys can reveal specific items and sample sub-categories that could be of special concern in case of exposure. Furthermore, an *in vitro* test designed for jewelry and toy testing which is validated against *in vivo* studies is missing, therefore additional research is recommended. Also, more information is especially needed on the quantity of ingested toy or jewelry material and on the frequency of ingestion, as better estimates may significantly improve the results of risk characterization. Another kind of study that is missing in the literature is the average play duration with different toys, specific to age categories. The presence of Cr(VI) and Sn(org) and children's potential exposure to these substances should also be further investigated. Finally, prolonged exposure to contaminated jewelry or toy pieces is possible following ingestion, since it is known that an ingested foreign

object can stay in gastro-intestinal tract for days and even weeks. In these cases, even higher risk can be present due to continuous release of elements in gastro-intestinal tract. Therefore, prolonged exposure should be tested using modified *in vitro* tests, and corresponding risk should be evaluated.

Acknowledgements

CHAPTER 7   GENERAL DISCUSSION

7.1 Contamination of toys and jewelry with potentially toxic elements

Total metal concentration analyses of ten potentially toxic elements made on 72 toys bought on the North American market indicated widespread contamination in the category MJ. Total concentrations of As, Cd, Cu, Ni, Pb, and Sb in the majority of the 24 items in this category exceeded the migratable concentration limits stated in the EU Toy Safety Directive, and particularly, the concentrations of Cd, Cu, Ni, and Pb were very high in certain samples. These articles could be potentially dangerous for children and therefore have been later subjected to migration analysis via different saliva and gastro-intestinal bioaccessibility tests. Metallic jewelry has been found having much higher contamination than plastic jewelry.

In contrary to the category MJ, the extent of contamination was much smaller in the PL, PC, and BP categories (one item in each of these categories having total concentrations exceeding the migratable concentration limits). In summary, investigation on presence of potentially toxic elements in a large set of toy and jewelry samples has helped to indicate potentially problematic sample types (metallic samples) and categories (the category of MJ), as well as elements with common presence (Cd, Cu, Ni, Pb). The overall results have led to the testing of selected items with elevated concentrations of potentially toxic elements (mainly from the category MJ) for the determination of saliva and gastro-intestinal bioaccessibility.

7.2 Bioavailability of toxic elements in toys and jewelry

Gastro-intestinal bioaccessibilities of Cd, Cu, Ni, and Pb were high in an important fraction of the 24 samples tested, and bioaccessible concentrations of Cd, Ni, or Pb exceeded EU migratable concentration limits in four to six MJ, depending on the *in vitro* bioaccessibility protocol used. Cd, Cu, Ni, and Pb from contaminated items were also mobilized to saliva, but saliva

bioaccessibility of metals were lower than their gastro-intestinal bioaccessibility. This can be attributed to low pH of the gastric phase in oral bioaccessibility tests, which favors the mobilization of metals.

In terms of test performances, considering saliva bioaccessibility tests, the mouthing compartments of the RIVM and DIN methods gave similar results for Cu bioaccessibility, but not for Ni (it wasn't possible to compare other metals due to small number of measurements above detection limit). Considering gastro-intestinal bioaccessibility tests, tests did not statistically differ from each other when predicting bioavailabilities. This has been found notable given the fact that these tests have different final pH values (5.5 for IVG, 7.0 for PBET, and 1.8 for EN 71-3). Lower metal solubility for the IVG and PBET protocols could be expected at the end of intestinal phase due to higher end pH, in comparison to the metal concentrations at the end of the gastric-only EN 71-3 protocol. The presence of organic molecules in gastrointestinal fluids in IVG and PBET may be responsible for sustained metals solubilization during the intestinal phase via organic ligands. Investigation of metals speciation in gastro-intestinal fluids as a result of exposure to contaminated toys and jewelry could be interesting, and may provide further understanding on mobility and toxicity. Chemical equilibrium models (i.e. MINEQL+, VMINTEQ) could particularly be of use for predicting speciation distribution of metals in gastro-intestinal tract.

Finally, a good correlation between total and bioaccessible concentrations was not always available (valid for both saliva and gastro-intestinal bioaccessibility results); therefore, total concentrations may not always predict metal bioaccessibility in tested toys and jewelry. This makes the use of bioaccessibility data in risk characterization more preferable than the total concentrations data.

7.3 Risk for children from oral exposure to toxic elements in contaminated toys and jewelry

Among the three scenarios considered for risk characterization, the scenario of ingestion of parts of pieces yielded the highest risk (HI>1 for eight of 16 MJ samples included in the

characterization), which has been followed by the saliva mobilization scenario (HI>1 for three articles), and the scenario of ingestion of scraped-off material (HI<1 for all samples). The order of results can be explained by the change in exposed quantity of toy or jewelry material in different scenarios. The quantity of material to be exposed in the scenarios of ingestion of parts and pieces and saliva mobilization (scenarios with higher risk) is in the order of grams, while the quantity assumed for the ingestion of scraped-off material (scenario with the lowest risk) is 8 mg. This relatively low ingested quantity in comparison to saliva exposure influences the results of calculations and thus leads to lower risk values for the scenario of ingestion of scraped-off material than saliva mobilization scenario, although gastric conditions are normally more favorable for metals mobilization than exposure via saliva.

While the quantity of mouthed or ingested material seems very important in quantifying risk, the exact mouthable mass or ingestible quantity is highly dependent of the type of sample and its properties. It should be noted that for the scenarios of ingestion of parts or pieces and saliva exposure, exposed quantities from different items can be highly variable. Therefore, limits based on bioaccessible concentrations (in mg.kg^{-1}) as given by in the EU Directive may not be always appropriate to use as they are calculated based on the assumption of a constant quantity of exposure. Setting limits in terms of bioaccessible quantities (in µg) rather than bioaccessible concentrations seems more suitable as bioaccessible quantity determined via testing already includes the effect of exposed quantity (it is indeed the equivalent of bioaccessible concentration × ingestion rate). It should be also noted for the scenario of ingestion of scraped-off material that the assumed ingested quantity of 8 mg for this scenario seems reasonable, but also carries high uncertainty as it is based on the sole assumption of van Engelen et al. (2006), and therefore should be updated if necessary following more research (behavioral studies or tracer research on children) in this area.

Finally, a last oral exposure scenario that is not considered in the present work is the scenario of prolonged exposure to an ingested item. The scenarios and the testing methods used in the present work represent the case that exposure duration to ingested items is around typical average digestion time for food for children (a transit time close to 5 h). However, it is known that in some cases, ingested objects can stay in the gastro-intestinal tract for a long duration (up to weeks). In this case, a child is exposed to ingested object for longer times in daily alternating

conditions of fasted and fed states. This scenario of prolonged exposure, which may result in very high risk, requires a specially designed laboratory testing scheme and risk characterization approach to be properly evaluated.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The main conclusion of the present work is that children's oral exposure to toys and low-cost jewelry contaminated with toxic elements may pose significant health risk. For the sample set analyzed in the present study, the level of contamination, metal bioaccessibility, and risk values were found elevated, particularly for the category of metallic toys and jewelry (MJ). Calculated hazard index (HI) values indicated unacceptable risk levels for certain samples. The level of contamination and the accompanying risk values were much lower for other three categories (plastic toys (PL), toys with paint or coating (PC), and brittle or pliable toys (BP)).

The following specific conclusions can be drawn from this study:

- The U.S. and Canadian regulations mainly focus on Pb (and to a lesser extent Cd) exposure prevention, while additional elements are regulated only in paints and coating. However, this approach and accompanying defined limits stated in these legislations may be insufficient to ensure the chemical safety of toys and jewelry for children, as it was shown that other potentially toxic elements can be present in toys and low-cost jewelry which can become bioaccessible following exposure.

- An important fraction of samples from the analyzed set of toys and jewelry (n=72) bought from the North American market for this study are contaminated with Pb and Cd, and to a lesser extent with As, Cu, Ni, and Sb, as shown by the total elemental concentrations analysis. The category of MJ is particularly problematic since the majority of the samples are contaminated and concentrations of certain elements are very high. On the contrary, samples from the PL, PC, and BP categories had less contamination with lower concentrations.

- Mobilization of elements via saliva following mouthing may be an important concern. Particularly, the results of the saliva extraction compartments of *in vitro* bioaccessibility tests RIVM and DIN show that Cd, Cu, Ni, and Pb mobilized from 16 of 32 tested samples in significant quantities (>1 µg for highly toxic Cd and Pb, >10 µg for Cu and Ni). Mobilized quantities and calculated risk values were high particularly for Cd.

- The ingestion of contaminated articles can result in the mobilization of large quantities of metals in gastro-intestinal tract as shown by the two-phase (gastric and intestinal) *in vitro* bioaccessibility tests IVG and PBET, and gastric-only protocol EN 71-3. The results from the EN 71-3 protocol were comparable to the ones from the IVG and PBET protocols. And yet, for the assessment of bioavailability, using physiologically-based two-phase *in vitro* bioaccessibility tests like IVG or PBET would be recommended over one-phase EN 71-3 since the former tests are more conservative and better represent gastro-intestinal physiology. In particular, mobilized quantities and the accompanying risk were high for Cd, Ni, or Pb in some MJ.

- The use of bioaccessibility concept to assess chemical safety of toys and jewelry should be preferred to the use of total concentrations for screening of potentially hazardous items and performing risk evaluations. This is because the experimental results have shown total and bioaccessible concentrations of toxic elements are not equal and do not always correlate. Toy safety approaches that utilize the concept of contaminant bioavailability should therefore be preferred.

- A detailed risk characterization done for children considering three exposure scenarios for 16 selected samples showed that the scenario of ingestion of parts or pieces of toy or jewelry yielded the highest risk to children (HI may exceed 1, with values up to 75), followed by the scenario of saliva mobilization due to mouthing (HI larger than 1, but accompanied with lower values compared to whole ingestion), and the scenario of ingestion of scraped-off material (HI<1 for all samples). Screening of potentially hazardous samples via risk characterization yielded different potentially dangerous samples than the screening done using the U.S., Canadian and EU toy safety approaches.

- A comprehensive methodology has been developed considering different exposure scenarios, and including testing and risk characterization recommendations as well as bioaccessible limits for eight priority elements based on children's tolerable daily intake values and background exposure. Its future use for the assessment of chemical safety of toys and jewelry is suggested.

Recommendations

Based on the conclusions presented, the main recommendations of this study are as follows:

- Further research on the determination of contaminant bioaccessibility and assessing children's exposure to potentially toxic elements is recommended by considering exposure to additional toy and jewelry samples. This is important to determine potentially problematic sub-categories of toy or jewelry material. A particular importance should be given to the ingestion of toy or jewelry material, and to the samples belonging to the category MJ.

- Research on prolonged exposure to MJ and an accompanying detailed risk characterization is needed. This is the case in which ingested items may stay in gastro-intestinal tract for a long duration (days or weeks). In this case, exposure dynamics (gastro-intestinal exposure via repeating alternating cycles of fasted and fed states) are different than the one simulated by the bioaccessibility protocols used in this study (one cycle of exposure in fasted state, representing the worst-case scenario for metals mobilization). Therefore, research on prolonged exposure requires a specially designed and well-justified laboratory setup and an accompanying risk characterization approach.

The following additional recommendations are also made based on the findings from this dissertation:

- The development of an *in vitro* bioaccessibility test with *in vivo* validation for bioaccessibility testing of jewelry and toys is recommended.

- The presence of Cr(VI) and Sn(org) in toys and jewelry, and children's potential exposure to these elements should be further investigated, and limits for these elements need to be established if found necessary.

- Exposure parameter estimates used in risk characterization carry high uncertainty. Therefore, more research on the estimation of play time for children, mouthing frequency and times, and toy material ingestion rate is needed.

- Metals speciation in toys and jewelry has not been investigated in this study. Moreover, metals speciation in saliva and gastro-intestinal fluids following exposure has not been examined. Investigating metals speciation could be beneficial for providing a better understanding of metals mobility and toxicity. Particularly, laboratory determination of species in toys and jewelry via specialized analytical methods, and prediction of metals speciation in synthetic body fluids via chemical equilibrium models can be recommended.

- In this study, only new toy and jewelry samples were tested. The effect of biting behavior of children and damage from normal use on bioaccessibility should be investigated via testing damaged samples in addition to the new ones.

REFERENCES

ASTM International. Standard Consumer Safety Specification for Toy Safety - Designation: F 963 - 08 (2008). West Conshohocken, PA.

ASTM International. Standard Specification for Consumer Product Safety for Children's Jewelry - Designation: F2923 - 11 (2011). West Conshohocken, PA.

ATSDR. (2003). *Toxicological Profile for Selenium*. Clifton Road, NE.

ATSDR. (2004). *Toxicological Profile for Cobalt*. Clifton Road, NE.

ATSDR. (2005a). *Toxicological Profile for Zinc*. Clifton Road, NE.

ATSDR. (2005b). *Toxicological Profile for Tin and Tin compounds*. Clifton Road, NE.

ATSDR. (2011). Minimal risk levels (MRLs) list. Retrieved from http://www.atsdr.cdc.gov/mrls/mrllist.asp#37tag

Attina, T. M., & Trasande, L. (2013). Economic costs of childhood lead exposure in low- and middle-income countries. *Environmental Health Perspectives*, *In press*. doi:10.1289/ehp.1206424

Barboza, D. (2007). Why Lead in Toy Paint? It's Cheaper. *The New York Times*. Retrieved from http://www.nytimes.com/2007/09/11/business/worldbusiness/11lead.html?_r=1.

Becker, M., Edwards, S., & Massey, R. I. (2010). Toxic chemicals in toys and children's products : Limitations of current responses and recommendations for government and industry. *Environmental Science and Technology*, *44*(21), 7986–7991.

Borling, P., Engelund, B., & Sørensen, H. (2006). *Survey, migration and health evaluation of chemical substances in toys and childcare products produced from foam plastic - Danish EPA report Survey # 70 2006*.

Bouma, K., & Reus, H. R. (2004). *Market surveillances on toy safety - VWA report ND04o063/01*.

Brandon, E. F. A., Oomen, A. G., Rompelberg, C. J. M., Versantvoort, C. H. M., van Engelen, J. G. M., & Sips, A. J. A. M. (2006). Consumer product in vitro digestion model: Bioaccessibility of contaminants and its application in risk assessment. *Regulatory Toxicology and Pharmacology*, *44*(2), 161–171.

Braun, J., Kahn, R. S., Froehlich, T., Auinger, P., & Lanphear, B. P. (2006). Exposures to environmental toxicants and attention deficit hyperactivity disorder in US children. *Environmental Health Perspectives*, *114*(12), 1904–1909.

Bremmer, H. J., & van Veen, M. P. (2002). *Children's toys fact sheet - RIVM report 612810012/2002*.

BSI. (2006). *Safety of toys — Part 3: Migration of elements, method BS EN 71-3* (Vol. 3).

BSI. (2013). *Safety of toys - Part 3: Migration of certain elements, method BS EN 71-3*.

Canfield, R. L., Henderson, C. R., Cory-Slechta, D. A., Cox, C., Jusko, T. A., & Lanphear, B. P. (2003). Intellectual impairment in children with blood lead concentrations below 10 µg per deciliter. *The New England Journal of Medicine*, *348*(16), 1517–1526.

CBC. (2010). Toxic Miley Cyrus jewelry recalled by Wal-Mart. Retrieved from http://www.cbc.ca/news/story/2010/05/20/con-cadmium-recall.html

CDC. (2004). Brief Report: Lead Poisoning from Ingestion of a Toy Necklace - Oregon, 2003. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5323a5.htm

CDC. (2006). Death of a Child After Ingestion of a Metallic Charm - Minnesota, 2006. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5512a4.htm

CDC. (2009). *Fourth National report on human exposure to environmental chemicals*. Buford Highway, NE.

CDC. (2013). *Fourth National report on human exposure to environmental chemicals - Updated tables, March, 2013*. Buford Highway, NE.

Clesceri, L. S., Greenberg, A. E., & Eaton, A. D. (1999). *Standard Methods for the Examination of Water and Wastewater* (20th ed.). APHA.

Cox, C., & Green, M. (2010). Reduction in the prevalence of lead-containing jewelry in California following litigation and legislation. *Environmental Science and Technology*, *44*(16), 6042–6045.

Dargan, P. I., Evans, P. H., House, I. M., & Jones, A. L. (2000). A case of lead poisoning due to snooker chalk. *Archives of Disease in Childhood*, *83*(6), 519–520.

Désy, C. (2012). Bijoux pour enfants - Peut contenir du cadmium. *Protégez-vous Consumer Magazine, December 2012*, 23–27.

Dietrich, K. N., Ris, M. D., Succop, P. A., Berger, O. G., & Bornschein, R. L. (2001). Early exposure to lead and juvenile delinquency. *Neurotoxicology and Teratology*, *23*(6), 511–518.

DIN. (2004). *Soil quality - Bioaccessibility of organic and inorganic pollutants from contaminated soil material, method DIN 19738*. Berlin, Germany.

Ecology Center. (2008). HealthyStuff.org reports with products tagged as « toys ». Retrieved from http://www.healthystuff.org/get-stuff.php?group-report=Toys

Esernio-Jenssen, D., Donatelli-Guagenti, A., & Mofenson, H. C. (1996). Severe lead poisoning from an imported clothing accessory: "Watch" out for lead. *Clinical Toxicology*, *34*(3), 329–333.

European Commission. (2004). *Opinion of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on « Assessment of the bioavailability of certain elements in toys »*. Brussels, Belgium.

European Council. Council Directive of 3 May 1988 on the approximation of the laws of the Member States concerning the safety of toys (1988). Brussels, Belgium.

European Council. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures (2008). Brussels, Belgium.

European Council. Directive 2009/48/EC of the European Parliament and of the Council of 18 June 2009 on the safety of toys (2009). Brussels, Belgium.

Farmakakis, T., Dessypris, N., Alexe, D.-M., Frangakis, C., Petoussis, G., Malliori, M., & Petridou, T. E. (2007). Magnitude and object-specific hazards of aspiration and ingestion injuries among children in Greece. *International Journal of Pediatric Otorhinolaryngology*, *71*(2), 317–324.

Fergusson, J. A. E., Malecky, G., & Simpson, E. (1997). Lead foreign body ingestion in children. *Journal of Paediatrics and Child Health*, *33*(6), 542–544.

Firestone, M., Moya, J., Cohen-Hubal, E., Zartarian, V., & Xue, J. (2007). Identifying childhood age groups for exposure assessments and monitoring. *Risk Analysis*, *27*(3), 701–714.

Glorennec, P., Bemrah, N., Tard, A., Robin, A., Le Bot, B., & Bard, D. (2007). Probabilistic modeling of young children's overall lead exposure in France: Integrated approach for various exposure media. *Environment International*, *33*(7), 937–945.

Government of Canada. Surface Coating Materials Regulations (SOR/2005-109) (2005). Ottawa, ON. Retrieved from ttp://laws-lois.justice.gc.ca/eng/regulations/SOR-2005-109/index.html

Government of Canada. Children's Jewellery Regulations (SOR/2005-132) (2005). Ottawa, ON. Retrieved from http://laws-lois.justice.gc.ca/eng/regulations/SOR-2005-132/

Government of Canada. Consumer Products Containing Lead (Contact with Mouth) Regulations SOR/2010-273 (2010). Ottawa, ON. Retrieved from http://laws-lois.justice.gc.ca/eng/regulations/SOR-2010-273/page-1.html

Greenway, J. A., & Gerstenberger, S. (2010). An evaluation of lead contamination in plastic toys collected from day care centers in the Las Vegas Valley, Nevada, USA. *Bulletin of Environmental Contamination and Toxicology*, *85*(4), 363–366.

Guney, M., Nguyen, A., & Zagury, G. J. (2013). Estimating children's exposure to toxic elements in toys and children's jewelry via saliva mobilization. *Chemosphere*, *submitted*.

Guney, M., Welfringer, B., de Repentigny, C., & Zagury, G. J. (2013). Children's exposure to mercury-contaminated soils: Exposure assessment and risk characterization. *Archives of Environmental Contamination and Toxicology*, *65*(2), 345–355.

Guney, M., & Zagury, G. J. (2011). Toxic chemicals in toys and children's products. *Environmental Science and Technology*, *45*(9), 3819.

Guney, M., & Zagury, G. J. (2012). Heavy metals in toys and low-cost jewelry: Critical review of U.S. and Canadian legislations and recommendations for testing. *Environmental Science & Technology*, *46*(8), 4265–4274.

Guney, M., & Zagury, G. J. (2013a). Contamination by ten harmful elements in toys and children's jewelry bought on the North American market. *Environmental Science & Technology*, *47*(11), 5921–5930.

Guney, M., & Zagury, G. J. (2013b). Bioaccessibility of As, Cd, Cu, Ni, Pb, and Sb in toys and children's jewelry. *Environmental Science and Technology*, *submitted*.

Health Canada. (2008). *Determination of migratable lead in consumer products, method C-08*. Ottawa, ON.

Health Canada. (2010a). Consumer Product Recalls - "Best Friends" Charm Bracelet Sets. Retrieved from http://cpsr-rspc.hc-sc.gc.ca/PR-RP/recall-retrait-eng.jsp?re_id=1033

Health Canada. (2010b). Consumer Product Recalls - Various Children's Jewellery. Retrieved from http://cpsr-rspc.hc-sc.gc.ca/PR-RP/recall-retrait-eng.jsp?re_id=1052

Health Canada. (2010c). News Release 2010-178 - Government of Canada Calls on Industry to Voluntarily Stop the Use of Cadmium in Children's Jewellery. Retrieved from http://www.hc-sc.gc.ca/ahc-asc/media/nr-cp/_2010/2010_178-eng.php

Health Canada. (2010d). Consumer Product Recalls - Garage Beaded String Bracelet and Antique Silver Bracelet. Retrieved from http://cpsr-rspc.hc-sc.gc.ca/PR-RP/recall-retrait-eng.jsp?re_id=1011

Health Canada. (2011). Consultation on a Proposed Guideline for Cadmium in Children's Jewellery. Retrieved from http://www.hc-sc.gc.ca/cps-spc/legislation/consultation/_2011cadmium/index-eng.php

Health Canada. (2012). *Industry Guide to Health Canada's safety requirements for children's toys and related products, 2012*. Ottawa, ON.

Health Canada. (2013). *Industry Guide to children's jewellery*. Ottawa, ON.

Jovanovic, S. V, Pan, P., & Wong, L. (2012). Bioaccessibility of uranium in soil samples from Port Hope, Ontario, Canada. *Environmental Science & Technology*, *46*(16), 9012–9018.

Juberg, D. R., Alfano, K., Coughlin, R. J., & Thompson, K. M. (2001). An observational study of object mouthing behavior by young children. *Pediatrics*, *107*(1), 135–142.

Juhasz, A. L., Smith, E., Weber, J., Rees, M., Kuchel, T., Rofe, A., … Naidu, R. (2013). Predicting lead relative bioavailability in peri-urban contaminated soils using in vitro bioaccessibility assays. *Journal of Environmental Science and Health. Part A, Toxic/hazardous substances & environmental engineering*, *48*(6), 604–611.

Juhasz, A. L., Weber, J., Naidu, R., Gancarz, D., Rofe, A., Todor, D., & Smith, E. (2010). Determination of cadmium relative bioavailability in contaminated soils and its prediction using in vitro methodologies. *Environmental Science and Technology*, *44*(13), 5240–5247.

Juhasz, A. L., Weber, J., & Smith, E. (2011). Predicting arsenic relative bioavailability in contaminated soils using meta analysis and relative bioavailability-bioaccessibility regression models. *Environmental Science & Technology*, *45*(24), 10676–10683.

Juhasz, A. L., Weber, J., Smith, E., Naidu, R., Marschner, B., Rees, M., … Sansom, L. (2009). Evaluation of SBRC-gastric and SBRC-intestinal methods for the prediction of in vivo relative lead bioavailability in contaminated soils. *Environmental Science and Technology*, *43*(12), 4503–4509.

Juhasz, A. L., Weber, J., Smith, E., Naidu, R., Rees, M., Rofe, A., … Sansom, L. (2009). Assessment of four commonly employed in vitro arsenic bioaccessibility assays for predicting in vivo relative arsenic bioavailability in contaminated soils. *Environmental Science and Technology*, *43*(24), 9487–9494.

Kalnicky, D. J., & Singhvi, R. (2001). Field portable XRF analysis of environmental samples. *Journal of Hazardous Materials*, *83*(1-2), 93–122.

Kawamura, Y., Kawasaki, C., Mine, S., Mutsuga, M., & Tanamoto, K. (2006). Contents of eight harmful elements in baby toys and their migration tests. *Shokuhin Eiseigaku Zasshi*, *47*(2), 51–57.

Kawamura, Y., Mutsuga, M., Yamauchi, T., Ueda, S., & Tanamoto, K. (2009). Migration tests of cadmium and lead from paint film of baby toys. *Shokuhin Eiseigaku Zasshi*, *50*(2), 93–96.

Koch, I., Reimer, K. J., Bakker, M. I., Basta, N. T., Cave, M. R., Denys, S., … Zagury, G. J. (2013). Variability of bioaccessibility results using seventeen different methods on a standard reference material, NIST 2710. *Journal of Environmental Science and Health. Part A, Toxic/hazardous substances & environmental engineering*, *48*, 641–655.

Korfali, S. I., Sabra, R., Jurdi, M., & Taleb, R. I. (2013). Assessment of toxic metals and phthalates in children's toys and clays. *Archives of Environmental Contamination and Toxicology*, *In press*. doi:10.1007/s00244-013-9925-1

Kumar, A., & Pastore, P. (2007). Lead and cadmium in soft plastic toys. *Current Science*, *93*(6), 818–822.

Lanphear, B. P., Dietrich, K., Auinger, P., & Cox, C. (2000). Cognitive deficits associated with blood lead concentrations <10 µg/dL in US children and adolescents. *Public Health Reports*, *115*, 521–529.

Lithner, D., Damberg, J., Dave, G., & Larsson, K. (2009). Leachates from plastic consumer products - Screening for toxicity with Daphnia magna. *Chemosphere*, *74*(9), 1195–1200.

Lynett, A. (2008). Tests find toxic toys in stores across GTA. *Toronto Star*. Retrieved from http://www.thestar.com/article/511758

Maas, R. P., Patch, S. C., Pandolfo, T. J., Druhan, J. L., & Gandy, N. F. (2005). Lead content and exposure from children's and adult's jewelry products. *Bulletin of Environmental Contamination and Toxicology*, *74*(3), 437–444.

Mattel & Fisher-Price. (2007). Customer service: Recall & safety alerts. Retrieved from http://service.mattel.com/us/recall.aspx

McKinney, P. E. (2000). Acute elevation of blood lead levels within hours of ingestion of large quantities of lead shot. *Journal of Toxicology - Clinical Toxicology*, *38*(4), 435–440.

Merritt, T. A. (2005). Lead poisoning from a toy necklace. *Pediatrics*, *116*(4), 1050–1051.

Morrisson, W. M. (2009). *Health and safety concerns over U.S. imports of Chinese products: An overview*. Washington, DC.

Mowad, E., Haddad, I., & Gemmel, D. J. (1998). Management of lead poisoning from ingested fishing sinkers. *Archives of Pediatrics & Adolescent Medicine*, *152*(5), 485–488.

Moya, J., Bearer, C. F., & Etzel, R. A. (2004). Children's behavior and physiology and how it affects exposure to environmental contaminants. *Pediatrics*, *113*(4), 996–1006.

Oomen, A. G., Rompelberg, C. J. M., Bruil, M. A., Dobbe, C. J. G., Pereboom, D. P. K. H., & Sips, A. J. A. M. (2003). Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. *Archives of Environmental Contamination and Toxicology*, *44*(3), 281–287.

Oomen, A. G., van Twillert, K., Hofhuis, M. F. A., Rompelberg, C. J. M., & Versantvoort, C. H. M. (2003). *Development and suitability of in vitro digestion models in assessing bioaccessibility of lead from toy matrices - RIVM report 320102001/2003*.

Oomen, A. G., Versantvoort, C. H. M., Duits, M. R., van de Kamp, E., & van Twillert, K. (2004). *Application of in vitro digestion models to assess release of lead and phthalate from toy matrices and azo dyes from textile - RIVM report 320102003/2004*.

Pentland, W. (2008). The most toxic toys. *The Forbes*. Retrieved from http://www.forbes.com/2008/12/16/toys-product-safety-biz-commerce-cx_wp_1216toxictoys.html

Pouschat, P., & Zagury, G. J. (2006). In vitro gastrointestinal bioavailability of arsenic in soils collected near CCA-treated utility poles. *Environmental Science and Technology*, *40*(13), 4317–4323.

Pouschat, P., & Zagury, G. J. (2008). Bioaccessibility of chromium and copper in soils near CCA-treated wood poles. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, *12*(3), 216–223.

Rastogi, S. C. (1992). Cadmium, chromium, lead, and mercury residues in finger-paints and make-up paints. *Bulletin of Environmental Contamination and Toxicology*, *48*(2), 289–294.

Rastogi, S. C., & Pritzl, G. (1996). Migration of some toxic metals from crayons and water colors. *Bulletin of Environmental Contamination and Toxicology*, *56*(4), 527–533.

Rodriguez, R. R., Basta, N. T., Casteel, S. W., & Pace, L. W. (1999). An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. *Environmental Science & Technology*, *33*(4), 642–649.

Rodríguez-Barranco, M., Lacasaña, M., Aguilar-Garduño, C., Alguacil, J., Gil, F., González-Alzaga, B., & Rojas-García, A. (2013). Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. *Science of the Total Environment*, *454-455*, 562–577.

Ruby, M. V., Davis, A., Schoof, R., Eberle, S., & Sellstone, C. M. (1996). Estimation of lead and arsenic bioavailability using a Physiologically Based Extraction Test. *Environmental Science & Technology*, *30*(2), 422–430.

Ruby, M. V., Schoof, R., Brattin, W., Goldade, M., Post, G., Harnois, M., … Chappell, W. (1999). Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. *Environmental Science and Technology*, *33*(21), 3697–3705.

Schroder, J. L., Basta, N. T., Casteel, S. W., Evans, T. J., Payton, M. E., & Si, J. (2004). Validation of the In Vitro Gastrointestinal (IVG) method to estimate relative bioavailable lead in contaminated soils. *Journal of Environmental Quality*, *33*, 513–521.

Sexton, K., Adgate, J. L., Fredrickson, A. L., Ryan, A. D., Needham, L. L., & Ashley, D. L. (2005). Using biologic markers in blood to assess exposure to multiple environmental chemicals for inner-city children 3–6 years of age. *Environmental Health Perspectives*, *114*(3), 453–459.

Smith, S. A., & Norris, B. (2003). Reducing the risk of choking hazards: mouthing behaviour of children aged 1 month to 5 years. *International Journal of Injury Control and Safety Promotion*, *10*(3), 145–154.

Soares, E. P., Saiki, M., & Wiebeck, H. (2005). Determination of inorganic constituents and polymers in metallized plastic materials. *Journal of Radioanalytical and Nuclear Chemistry*, *264*(1), 9–13.

Trejo-Acevedo, A., Díaz-Barriga, F., Carrizales, L., Domínguez, G., Costilla, R., Ize-Lema, I., … Pérez-Maldonado, I. N. (2009). Exposure assessment of persistent organic pollutants and metals in Mexican children. *Chemosphere*, *74*(7), 974–980.

Tulve, N. S., Suggs, J. C., McCurdy, T., Hubal, E. A. C., & Moya, J. (2002). Frequency of mouthing behavior in young children. *Journal of Exposure Analysis and Environmental Epidemiology*, *12*(4), 259–264.

U.S.CPSC. (2005a). *Standard operating procedure for determining lead (Pb) and its availability in children's metal jewelry*. Gaithersburg, MD.

U.S.CPSC. Interim enforcement policy for children's metal jewelry containing lead - 2/3/2005 (2005). Washington, DC.

U.S.CPSC. (2007). RC2 Corp. Recalls Various Thomas & Friends[TM] Wooden Railway Toys Due to Lead Poisoning Hazard. Retrieved from http://www.cpsc.gov/cpscpub/prerel/prhtml07/07212.html

U.S.CPSC. (2009). CPSIA - Section 106. Mandatory Toy Safety Standards. Retrieved from http://www.cpsc.gov/en/Regulations-Laws--Standards/CPSIA/CPSIA-Topics/Section-106-Mandatory-Toy-Safety-Standards/

U.S.CPSC. (2010a). McDonald's Recalls Movie Themed Drinking Glasses Due to Potential Cadmium Risk. Retrieved from http://www.cpsc.gov/cpscpub/prerel/prhtml10/10257.html

U.S.CPSC. (2010b). Tween Brands Recalls Children's Metal Jewelry Due to High Levels of Cadmium. Retrieved from http://www.cpsc.gov/cpscpub/prerel/prhtml10/10297.html

U.S.CPSC. (2010c). Claire's Recalls Children's Metal Charm Bracelets Due to High Levels of Cadmium. Retrieved from http://www.cpsc.gov/cpscpub/prerel/prhtml10/10227.html

U.S.CPSC. (2010d). FAF Inc. Recalls Children's Necklaces Sold Exclusively at Walmart Stores Due to High Levels of Cadmium. Retrieved from http://www.cpsc.gov/cpscpub/prerel/prhtml10/10127.html

U.S.CPSC. (2010e). *Staff Report - Cadmium in children's metal jewelry*. Bethesda, MD.

U.S.CPSC. (2011). *Test Method: CPSC-CH-E1004-11 - Standard operating procedure for determining cadmium (Cd) extractability from children's metal jewelry*. Gaithersburg, MD.

U.S.CPSC. (2012). CPSIA Section 101. Children's Products Containing Lead; Lead Paint Rule. Retrieved from http://www.cpsc.gov/en/Regulations-Laws--Standards/CPSIA/CPSIA-Topics/Section-101-Childrens-Products-Containing-Lead-Lead-Paint-Rule/

U.S.EPA. (1994). Integrated Risk Information System: Cadmium (CARSN 7440-43-9). Retrieved from http://www.epa.gov/iris/subst/0141.htm

U.S.EPA. (1996a). Integrated Risk Information System: Nickel, soluble salts (CASRN various). Retrieved from http://www.epa.gov/iris/subst/0271.htm

U.S.EPA. (1996b). Integrated Risk Information System: Manganese (CASRN 7439-96-5). Retrieved from http://www.epa.gov/iris/subst/0373.htm

U.S.EPA. (1998). *Toxicological Review of Hexavalent Chromium (CAS No. 18540-29-9)*. Washington, DC.

U.S.EPA. (2000). *Development of chemical methods to assess the bioavailability of arsenic in contaminated media - Grant No. R825410*. Washington, DC.

U.S.EPA. (2004). *Toxicological Review of Boron and Compounds (CAS No. 7440-42-8)*. Washington, DC.

U.S.EPA. (2005). Integrated Risk Information System: Barium and Compounds (CASRN 7440-39-3). Retrieved from http://www.epa.gov/iris/subst/0010.htm.

U.S.EPA. (2008a). *Standard operating procedure for an in vitro bioaccessibility assay for lead in soil - EPA 9200.1-86*. Washington, DC.

U.S.EPA. (2008b). *Child-specific exposure factors handbook - EPA/600/R-06/096F*. Washington, DC.

U.S.EPA. (2011). *Exposure Factors Handbook: 2011 Edition - EPA/600/R-090/052/F*. Washington, DC.

U.S.PIRG. (2012). *Trouble In Toyland - The 27th annual survey of toy safety*. Washington, DC.

UNEP - WHO. (2006). *Environmental Health Criteria 234 - Elemental speciation in human health risk assessment*. Geneva, Switzerland.

Van Engelen, J. G. M., Park, M. V. D. Z., Janssen, P. J. C. M., Oomen, A. G., Brandon, E. F. A., Bouma, K., … van Raaij, M. T. M. (2006). *Chemicals in toys - A general methodology for assessment of chemical safety of toys with a focus on elements - RIVM/SIR Revised Advisory Report 0010278A02*.

VanArsdale, J. L., Leiker, R. D., Kohn, M., Merritt, T. A., & Horowitz, B. Z. (2004). Lead poisoning from a toy necklace. *Pediatrics*, *114*(4), 1096–1099.

Vasiluk, L., Dutton, M. D., & Hale, B. (2011). In vitro estimates of bioaccessible nickel in field-contaminated soils, and comparison with in vivo measurement of bioavailability and identification of mineralogy. *Science of the Total Environment*, *409*(14), 2700–2706.

Vogt, R., Bennett, D., Cassady, D., Frost, J., Ritz, B., & Hertz-Picciotto, I. (2012). Cancer and non-cancer health effects from food contaminant exposures for children and adults in California: a risk assessment. *Environmental Health*, *11*(83), 1–14.

Weidenhamer, J. D. (2009). Lead contamination of inexpensive seasonal and holiday products. *Science of the Total Environment*, *407*(7), 2447–2450.

Weidenhamer, J. D., & Clement, M. L. (2007a). Widespread lead contamination of imported low-cost jewelry in the US. *Chemosphere*, *67*(5), 961–965.

Weidenhamer, J. D., & Clement, M. L. (2007b). Evidence of recycling of lead battery waste into highly leaded jewelry. *Chemosphere*, *69*(10), 1670–1672.

Weidenhamer, J. D., & Clement, M. L. (2007c). Leaded electronic waste is a possible source material for lead-contaminated jewelry. *Chemosphere*, *69*(7), 1111–1115.

Weidenhamer, J. D., Miller, J., Guinn, D., & Pearson, J. (2011). Bioavailability of cadmium in inexpensive jewelry. *Environmental Health Perspectives*, *119*(7), 1029–1033.

Welfringer, B., & Zagury, G. J. (2009). Evaluation of two in vitro protocols for determination of mercury bioaccessibility: influence of mercury fractionation and soil properties. *Journal of Environmental Quality*, *38*(6), 2237–2244.

Xue, J., Zartarian, V., Moya, J., Freeman, N., Beamer, P., Black, K., … Shalat, S. (2007). A meta-analysis of children's hand-to-mouth frequency data for estimating nondietary ingestion exposure. *Risk Analysis*, *27*(2), 411–20.

Yost, J. L., Tao, S. H., Egan, S. K., Barraj, L. M., Smith, K. M., Tsuji, J. S., … Rachman, N. J. (2004). Estimation of dietary intake of inorganic arsenic in U.S. children. *Human and Ecological Risk Assessment: An International Journal*, *10*(3), 473–483.

Yost, J. L., & Weidenhamer, J. D. (2008a). Accessible and total lead in low-cost jewelry items. *Integrated Environmental Assessment and Management*, *4*(3), 358–361.

Yost, J. L., & Weidenhamer, J. D. (2008b). Lead contamination of inexpensive plastic jewelry. *The Science of the Total Environment*, *393*(2-3), 348–350.

Yu, S., Du, J., Luo, T., Huang, Y., & Jing, C. (2012). Evaluation of chromium bioaccessibility in chromite ore processing residue using in vitro gastrointestinal method. *Journal of Hazardous Materials*, *209-210*, 250–255.

APPENDICES

APPENDIX 1 - Supplementary Data for Publication #3

Estimating children's exposure to toxic elements via saliva mobilization from contaminated toys and children's jewelry

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S1.1. Details of digestion protocol

For each sample, a part that is representative of a section of the toy or children's jewelry which may be subject to exposure was sampled and acid digested according to the Standard Method 3030F (Clesceri et al., 1999) via HNO_3 digestion on a hot plate, followed by optional additional digestion via $HClO_4$ or $HClO_4/HF$ whenever necessary. Digestates and procedure blanks were filtered (0.45 μm PVDF filter) following centrifugation (5,000 g, 15 min), diluted to 50 mL, and preserved at 4°C until analysis. Concentrations of elements were measured by ICP-OES (method detection limits in $μg.L^{-1}$ were As: 19.5, Ba: 0.12, Cd: 1.2, Cr: 1.8, Cu: 2.4, Mn: 0.24, Ni: 5.7, Pb: 16.6, Sb: 5.6, and Se: 34.3).

S1.2. Details of saliva bioaccessibility protocols

DIN 19738 is a three-phase bioaccessibility test originally designed to measure the bioaccessibility of organic and inorganic pollutants in contaminated soil. The three-phase RIVM method aims to determine the bioaccessibility of contaminants in consumer products. The saliva composition per 30mL was: 15 mg NaSCN, 55 mg Na_2SO_4, 50 mg NaCl, 15 mg $NaHCO_3$, 45 mg KCl, 60 mg KH_2PO_4, 15 mg $CaCl_2.2H_2O$, 75 mg mucin, 25 mg α-amylase, 10 mg urea and 1 mg

uric acid (DIN); and 386 mg KCl, 86 g KSCN, 382 g NaH$_2$PO$_4$, 245 mg Na$_2$SO$_4$, 128 mg NaCl, 729 mg NaHCO$_3$, 86 mg urea, 11 mg mucin, 125 mg α-amylase and 6 mg uric acid (RIVM).

Modifications to these tests were the amount of saliva used for extraction (30 mL instead of 21 mL [RIVM], no change for the DIN method), shaker type and speed (100 rpm in a horizontal orbital shaker) and time (30, 60, and 120 min in order to monitor the effect of time on metal mobilization, instead of 30 min [DIN] or variable [RIVM]). All experiments were conducted in an incubator at 37 °C. For each sample, a part representative of a section of the toy or children's jewelry (~10 cm^2 whenever possible and applicable) which may be subject to mouthing was sampled and transferred to a 125 mL erlenmeyer. Following the addition of saliva, the mixture was shaken. Five mL samples were taken at 30, 60 and 120 min from each Erlenmeyer, followed by the addition of 5 mL of fresh synthetic saliva to Erlenmeyers. The aliquots were centrifuged (5,000×g, 15 min), filtered (0.45 μm), acidified by adding 0.5 mL of HNO$_3$, and preserved at 4 °C until analyzed. Concentrations of elements were measured by ICP-OES (method detection limits in μg.L^{-1} were As: 12.8, Ba: 3.3, Cd: 1.6, Cr: 1.2, Cu: 2.8, Mn: 0.22, Ni: 5.3, Pb: 14.9, Sb: 25.5 and Se: 19.1).

S1.3. Details of quality assurance/quality control

The reference values (%w/w) for SRM54d are 7.04 for Sb, 3.62 for Cu, 0.62 for Pb and 0.002 for Ni. The results obtained were consistent with the certified values (%w/w, 8.98 for Sb, 3.50 for Cu, 0.65 for Pb and 0.004 for Ni; within 20% of the certified values for Sb, Cu and Pb). The bioaccessible quantities and concentrations for SRM54d according to the DIN and RIVM methods are also given in Table S1.1 and Table 3.2, respectively.

The RSD values were generally small (<25%) for the majority of the analyses. However, for some samples, results from duplicates showed greater difference. This was attributed to (1) sample heterogeneity during sampling (samples had to be prepared from different sections of the same toy/jewelry, or similar sections of two or more identical toys/jewelry items were used) and/or to (2) the variability of the chemical composition of different items of the same type. For example, individual samples of the same type of jewelry can vary greatly in terms of Pb concentrations (i.e. individual samples tested by different laboratories showed analyses of 99.1%, 67.0% and 0.07% Pb [w/w] for the same type of bracelet sold in the market), indicating opportunistic use of source materials for jewelry (Weidenhamer & Clement, 2007a) and resulting in varying concentrations between different production batches.

Table S1.1 Quantities of Cd, Cu, Ni and Pb mobilized to saliva (average, µg) from selected children's toys and jewelry after 30, 60 and 120 minutes during modified DIN and RIVM mouthing tests

DIN	Cd			Cu			Ni			Pb		
Sample	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
MJ01	[a]<0.05	<0.06	<0.07	1.22	1.90	3.35	14.5	22.8	38.6	<0.50	<0.58	<0.66
MJ03	<0.05	<0.06	<0.07	6.89	9.66	13.8	34.7	66.0	149	8.42	13.3	38.1
MJ05	0.46	0.51	0.49	4.92	5.14	5.05	7.62	8.55	9.38	<0.50	<0.58	<0.66
MJ06	0.54	0.56	0.53	7.23	7.32	7.34	11.1	12.3	13.3	<0.50	<0.58	<0.66
MJ07	<0.05	<0.06	<0.07	3.99	4.84	7.07	11.4	14.0	17.1	<0.50	<0.58	<0.66
MJ08	<0.05	<0.06	<0.07	4.88	5.42	5.55	3.85	4.42	5.03	<0.50	<0.58	<0.66
MJ09	<0.05	<0.06	<0.07	18.6	27.3	47.0	≤0.27	0.61	1.15	<0.50	<0.58	<0.66
MJ10	<0.05	<0.06	<0.07	26.8	40.5	69.6	0.56	0.86	1.21	<0.50	<0.58	<0.66
MJ12	<0.05	<0.06	<0.07	2.82	3.06	3.16	5.94	9.47	12.8	<0.50	<0.58	<0.66
MJ13	<0.05	<0.06	<0.07	45.6	57.0	70.1	455	622	801	<0.50	<0.58	<0.66
MJ14	<0.05	<0.06	<0.07	4.03	6.05	6.37	<0.18	<0.21	<0.24	1.39	2.45	5.08
MJ15	8.81	10.8	11.7	14.6	15.1	16.1	2.74	3.02	3.67	<0.50	<0.58	<0.66
MJ16	2.26	4.61	6.32	3.21	3.71	4.24	<0.18	<0.21	<0.24	<0.50	<0.58	<0.66
MJ18	[b]≤46.8	≤72.8	≤78.6	9.96	12.1	14.8	≤0.39	<0.24	<0.27	<0.50	<0.58	<0.66
MJ19	<0.05	<0.06	<0.07	11.4	16.9	26.6	<0.18	<0.21	<0.24	<0.50	<0.58	<0.66
MJ24/2	<0.05	<0.06	<0.07	4.14	5.21	6.15	8.25	10.9	14.5	<0.50	<0.58	<0.66
SRM54d	4.28	4.49	4.94	20.7	21.6	23.6	<0.18	<0.21	<0.24	<0.50	<0.58	<0.66
RIVM	Cd			Cu			Ni			Pb		
Sample	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
MJ01	<0.33	<0.38	<0.44	<0.56	1.09	3.37	4.34	7.29	16.1	<2.97	<3.47	<3.96
MJ03	<0.33	<0.38	<0.44	2.37	5.22	9.76	10.8	15.8	26.0	10.1	23.9	32.8
MJ05	<0.33	<0.38	<0.44	0.91	1.50	3.61	9.62	18.1	31.2	<2.97	<3.47	<3.96
MJ06	<0.33	<0.38	<0.44	1.36	2.30	4.10	6.43	14.2	30.0	<2.97	<3.47	<3.96
MJ07	<0.33	<0.38	<0.44	12.7	15.3	19.5	3.41	4.66	16.4	<2.97	<3.47	<3.96
MJ08	<0.33	<0.38	<0.44	≤1.48	2.21	3.12	10.4	16.0	25.6	<2.97	<3.47	<3.96
MJ09	<0.33	<0.38	<0.44	38.3	50.2	68.8	≤1.24	1.87	3.63	<2.97	<3.47	<3.96
MJ10	<0.33	<0.38	<0.44	46.0	64.3	91.5	1.92	2.18	4.77	<2.97	<3.47	<3.96
MJ12	<0.33	<0.38	<0.44	<0.56	0.82	1.36	12.9	20.1	34.7	<2.97	<3.47	<3.96
MJ13	<0.33	<0.38	<0.44	29.1	40.4	59.6	19.8	30.0	47.0	<2.97	<3.47	<3.96
MJ14	<0.33	<0.38	<0.44	1.17	2.70	6.00	<1.06	<1.23	<1.41	5.47	12.8	25.0
MJ15	1.24	4.83	10.7	4.49	8.31	14.6	1.53	4.12	10.8	<2.97	<3.47	<3.96
MJ16	<0.33	<0.38	<0.44	<0.56	≤0.81	2.15	<1.06	<1.23	<1.41	<2.97	<3.47	<3.96
MJ18	70.0	132	420	9.31	15.8	26.7	<1.06	<1.23	2.26	<2.97	<3.47	<3.96
MJ19	<0.33	<0.38	1.36	5.27	9.01	13.1	<1.06	<1.23	<1.41	<2.97	<3.47	<3.96
MJ24/2	<0.33	<0.38	<0.44	6.95	11.1	15.8	3.69	6.22	8.52	<2.97	<3.47	<3.96
SRM54d	0.71	0.59	0.62	17.1	18.4	22.5	<1.06	<1.23	<1.41	3.90	4.39	20.1

[a] <: Concentrations analyzed in both duplicates are below DL. Average of DL's is given.

[b] ≤: Concentration analyzed in one of the duplicates is below DL and the other is above DL. Average of two measurements is given.

Table S1.2 Calculated migration rates (µg.min^{-1}) from experimental mouthing bioaccessibility data with coefficients of determination (R^2) for selected samples and metals

Metal		Sample description	DIN protocol		RIVM protocol	
			Rate	R^2	Rate	R^2
Cd	MJ15	Jewelry piece - metal key	0.123	0.451	0.085	0.971
	MJ16	Metal earring	0.058	0.914	<0.005[a]	0.483
	MJ18	Jewelry metal pendant	≤0.804[b]	0.626	3.20	0.945
Cu	MJ09	Decorative metal beads for craft	0.415	0.955	0.657	0.778
	MJ10	Bracelet with metal beads	0.613	0.960	0.858	0.841
	MJ13	Decorative pins for scrapbooking	0.698	0.633	0.553	0.864
Ni	MJ12	Decorative pins for scrapbooking	0.121	0.853	0.305	0.967
	MJ13	Decorative pins for scrapbooking	7.78	0.743	0.425	0.926
Pb	MJ03	Pewter embellishments for scrapbooking	0.298	0.967	0.300	0.927
	MJ14	Jewelry piece - metal rose	0.042	0.998	0.208	0.998

[a] <: Concentrations analyzed in both duplicates are below DL. Result calculated using the average of DL's is given.
[b] ≤: Concentration analyzed in one of the duplicates is below DL and the other is above DL. Result calculated using the average of two measurements is given.

Figure S1.1 Bioaccessible quantities of Cd, Cu, Ni and Pb (average, µg) from selected samples expressed as a function of extraction time via modified DIN and RIVM mouthing tests