HEALTH RISKS ASSOCIATED WITH HEAVY METAL EXPOSURE IN GEOPHAGIC PRODUCTS (*EMUMBWA*) CONSUMED DURING PREGNANCY

BY

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DECLARATION

I Justine NABUUMA (17/U/14720/GMFT/PE), declare that this dissertation is original work and has never been submitted to any University or Higher Institution of Learning for a similar or any other academic award.

Signature……………………… Date…………………………….

APPROVAL

This is to certify that the work presented herein is the student’s own, done by her under our supervision and guidance, and is now ready for submission to the Graduate Board of Kyambogo University.

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DEDICATION

Dedicated to my parents; Mr. Joseph Lwevuze and Mrs. Margaret Nyiramataza Lwevuze for the love and parental care offered to me during the course of my education.

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LIST OF ABBREVIATIONS

BMDL	Benchmark Dose Lower Limit
EFSA	European Food Safety Authority
EPO	Euedaphic Pathogenic Organisms
EU	European Union
FFQ	Food Frequency Questionnaire
GFT	Glucose Tolerance Factor
HM	Heavy Metal(s)
IARC	International Agency for Research on Cancer
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
IQ	Intelligence Quotient
LB	Lower Boundary
LOD	Limit of Detection
LOQ	Limit of Quantification
MAAIF	Ministry of Agriculture Animal Industry and Fisheries
MB	Medium Boundary
MOE	Margin of Exposure
ND	Non-detect
PMTDI	Provisional Maximum Tolerable Daily Intake
TDI	Tolerable Daily Intake
UB	Upper Boundary
UBOS	Uganda National Bureau of Statistics

UDHS	Uganda Demographic and Health Survey
UNBS	Uganda National Bureau of Standards
WHO	World Health Organization

ABSTRACT

Geophagic products (*Emumbwa*) consumed by some pregnant women in Uganda may be a source of heavy metal poisoning. However, there is paucity of information regarding the composition, safety and consumption patterns of these products. This study aimed to assess the levels of heavy metals; lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), copper (Cu), chromium (Cr), nickel (Ni), manganese (Mn) and zinc (Zn), and the health risks due to Pb, As, Cr and Ni in geophagic products consumed by pregnant women in Kampala city and the surrounding areas. A total of 60 geophagic clay samples obtained from five markets in Kampala city, were analyzed for the 10 elements using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Geophagic products consumption patterns of 280 pregnant women (18 to 60 years of age) were determined using a set of pre-tested questionnaires. Means were separated by two-way analysis of variance (ANOVA) using XLSTAT, version 2020. Health risk assessment was done using Chi square statistics of the Microsoft Excel in built @risk software (Version 8.1, USA). Iron had the highest concentration (1500 to 22900 mg/kg) followed by Mn (0.68 to 8.99 mg/kg), Zn (0.48 to 2.44 mg/kg), Cr (0.51 to 1.57 mg/kg), Cu (0.14 to 1.20 mg/kg), Pb (0.29 to 0.66 mg/kg), Ni (0.08 to 0.39 mg/kg) and Cd (0.02 to 0.06 mg/kg). Mercury and As were not detected in any of the samples of *Emumbwa* analyzed. Most respondents were 35 to 54 yrs and had body weight between 60 and 80 kg. The majority (83.81%) earned less than one million Uganda shillings per month. The mean clay ingestion rate was 17.08±7.32 g per day. According to this study findings, both consumers and the total population are at a risk of developing chronic kidney disease, combined adenomas and carcinomas and post implantation fetal loss due to lead, chromium and nickel exposure. While geophagia can contribute significantly towards the intakes of useful elements such as Fe, Cu and Zn, the practice poses potential health risks to the consumers due to Pb, Mn, Cd, Ni and Cr poisoning. Sensitization of the population by health workers about the potential health risks of heavy metals in geophagic products is recommended.

Key words: Heavy metal, *Emumbwa*, geophagia, health risk, risk assessment, consumption pattern

CHAPTER 1: INTRODUCTION

1.1 Background

Heavy metals (HM) are a group of elements with atomic density 5 times or more greater than the density of water (Egwurugwu *et al*., 2007). The HM are considered a major environmental problem (Mudassir, Asrar, Arsala, & AFarooqui, 2005). Heavy metals are ubiquitous in nature, and cadmium (Cd), zinc (Zn), copper (Cu), arsenic (As), nickel (Ni), mercury (Hg), chromium (Cr), and lead (Pb) are the metals most frequently detected in the environment (Rodríguez & Mandalunis, 2018). They are non-biodegradable and cumulative poisons that are potentially toxic (Oribhabor & Ogbeibu, 2010). Heavy metals are endocrine-disrupting chemicals (EDC) whose actions may lead to adverse developmental, reproductive, neurological, immunological, teratogenicity and genotoxic effects in mammals (Lavicoli, Fontana, & Bergamaschi, 2009; Tchounwou, Yedjou, Patlolla, & Sutton, 2012; Zhuang, ZA, McBride, Zou, & Wang, 2013). Humans are exposed to HM in soils through inhalation of dust particles and through oral ingestion as is the case in geophagia (Egwurugwu *et al*., 2007). Geophagia is a universally recognized habit characterized by the deliberate consumption of earthy materials particularly sediments, soils and clays (Unarine *et al.,* 2021: Ekosse, Ngole-Jeme, & Diko, 2017). It is most commonly found among pregnant and breastfeeding women in sub-Saharan Africa with the highest prevalence during pregnancy (Odewumi, 2013). Geophagia has also been reported among school children and people with psychiatric disorders (Bisi-Johnson, Obi & Ekosse, 2010).

Geophagia has been reported in a number of continents including Africa, Asia, America and Europe. According to Kawai, Saathoff, Antelman, Msamanga, & Fawzi (2009), the prevalence of geophagia among pregnant women in Africa ranges between 28 and 84%. The highest prevalence has been reported in Kenya, Ghana, Rwanda, Nigeria, Tanzania, and South Africa (Kambunga, Candeias, Hasheela, & Mouri, 2019). Geophagia is also thought to boost fertility, ensure beautiful offspring, reduce stress, and prevent morning sickness, nausea, and vomiting during pregnancy (Bisi-Johnson, Obi, & Ekosse, 2010). It can also be practiced for religious, cultural, micronutrient nutrition and medicinal purposes, famine, and enhancement of personal appearance. Probably it is for such reasons that many men also eat soil (Seim *et al*., 2013). The nature of geophagic material varies widely from the type to the composition (Kambunga, Candeias, Hasheela, & Mouri, 2019). The positive effects notwithstanding, geophagic materials can also be a source of physical, biological, and chemical hazards such as HM implying that the ingestion of geophagic products poses a public health risk to the consumers (Kutalek *et al*., 2010).

Health risk assessment is the method of evaluating the probability of occurrence of any given probable amount of harmful health impacts over a determined time period (Mohammadi *et al*., 2019). It is a valuable tool to support decision-making processes on a wide range of areas, from economic development to environmental protection (Zolezzi, Cattaneo, & Tarazona, 2005; Lee *et al*., 2014). The health risk assessment of a contaminant is normally based on the estimation of the risk level and is classified as cancer or non-cancer (Mohammadi *et al*., 2019). Risk assessment employs a deterministic or a probabilistic approach. Deterministic risk assessment consists of assigning a single representative value to each exposure parameter in a risk equation leading to a unique risk value. This assessment often leads to conservative decisions that are reflected in higher remediation costs (Rivera-Velasquez, Fallico, Guerra, & Straface, 2013). Probabilistic risk assessment on the other hand considers the degree of variability and uncertainty of each parameter in the risk equation leading to more realistic risk outputs (Zolezzi, Cattaneo, & Tarazona, 2005). Probabilistic approaches for generic risk assessments are included in regulatory-based tiered processes in several parts of the world (Zolezzi, Cattaneo, & Tarazona, 2005). The method that employs stochastic methods such as *Monte Carlo* simulations, is therefore increasingly becoming popular (Uusitalo, Lehikoinen, Helle, & Myrberg, 2015) and was employed in the proposed study.

1.2 Problem Statement and Justification

High levels of heavy metals have been reported in geophagic products in many countries. Woode & Hackman-Duncan (2014) reported levels of As (0.6 to 10.6 mg/kg), Pb (10.7 to 18.3 mg/kg), Hg (0.3-4.2 mg/kg), Cd (0.7 to 1.6 mg/kg) and Co (15 to 32.4 mg/kg) in geophagic products from major markets in Ghana. Similarly, Nkansah, Korankye, Darko & Dodd (2016) reported As and Pb levels in the range of 8.11 to 14.18 mg/kg and 549.34 to 622.92 mg/kg, respectively. According to Nkansah *et al.* (2016), As, Cd and Pb would induce both cancer and non-cancer risks among clay consumers. Soil ingestion during pregnancy puts the fetus at risk of early exposure to HM poisoning (Kutalek *et al*., 2010). Heavy metals lead to developmental, reproductive, neurological, immunological, teratogenicity and genotoxic effects in humans (Morais, Costa, & De Lourdes Pereira, 2012). Soil consumption also limits the absorption of essential nutrients such as Fe and Zn, and impairs uptake of medications by the mother (Gomes, 2017). Presence of high levels of divalent metal cations (Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}), and zero valent Hg^0 and trivalent/hexavalent $Cr^{3+/6+}$ in the environment has in addition been reported to stimulate the enrichment of bacteria which bear antibiotic resistance genes (Ţugui, Szekeres, & Baricz, 2017).

In Uganda, up to 50% of pregnant women engage in geophagia during gestation largely for pregnancy-related cravings, enjoyment of the taste, texture or smell of the substance consumed (Seim *et al*., 2013). In Kampala, different clays are available for purchase from street and market vendors, and some are consumed as traditional medicine commonly called *emumbwa* (Abrahams, 1997). However, information regarding the composition, safety and consumption patterns of geophagic products in Uganda is scarce. High concentrations of heavy metals have been reported in soils and plants from wetlands around Kampala (Nabulo, Oryem-Origa, Nasinyama, & Cole, 2008; Fuhrimann *et al*. 2015) yet wetlands are the main source of raw materials for *Emumbwa*. Henceforth, this study aimed to assess the levels of heavy metals in geophagic products, and the health risks via the oral route of exposure on the health of consumers in Kampala city.

1.4 Objectives of the study

1.4.1 Major objective

To assess the health risks posed by heavy metals consumed in *Emumbwa* (geophagic products) by pregnant women in Kampala city.

1.4.2 Specific objectives

The specific objectives of the study were to:

1. Determine the Pb, Cd, As, Hg, Cu, Cr, Ni, Mn and Zn content in different samples of geophagic products obtained from various sources in Kampala city.
2. Determine the soil ingestion rate among pregnant women in Kampala city Kampala city and the surrounding areas.
3. Determine the relationship between the health risks of the heavy metals Pb, As, Cr and Ni posed to pregnant women in Kampala city, and soil consumption.

1.5 Hypotheses

1. There is no difference in the concentration of Pb, Cd, As, Hg, Cu, Cr, Ni, Mn and Zn in the geophagic products obtained from different sources.
2. There is no difference in soil ingestion rates among pregnant women in Kampala city.
3. There is no relationship between non-cancer risks of heavy metals posed to pregnant women in Kampala city and soil ingestion.

CHAPTER 2: LITERATURE REVIEW

2.1 Geophagia

2.1.1 History of geophagia

Geophagia is the practice of eating soil, ground-up rock, termite mound earth, clay, and dirt, which is common in mammals, birds, reptiles, and invertebrates and is nearly universal around the world (Williams, Haydel, & Ferrell-Jr., 2009). The practice provides a direct connection between human health and Earth's rocks and minerals. Geophagia is found among many contemporary indigenous peoples, including the Aboriginal people of Australia and the traditional peoples of East Africa and China (Abrahams, 1996). Although eating soil can also be found among children, geophagia is most commonly found among pregnant and breastfeeding women in sub-Saharan Africa with the highest prevalence during pregnancy (Abrahams & Parsons, 1996). According to Archaeology, geophagia is the earliest form of medicine and besides some soils being a source of minerals, the primary benefit of clay consumption is its effect of countering dietary toxins and the effects of parasites (Abrahams & Parsons, 1996). Clays can bind mycotoxins, endotoxins, toxic chemicals and bacteria in the body. They protect the gut lining from corrosion, act as antacids and curb diarrhea.

2.1.2 Soil consumption patterns across the globe

The practice of geophagia has been reported in several countries in Africa (South Africa, Namibia, Cameroon, Democratic Republic of Congo, Nigeria, Swaziland, Malawi, Tanzania Zambia, Zimbabwe, Kenya and Uganda), Asia (China, India, Philippines, and Thailand) and the Americas (Slabach, Corey, Aprille, Starks, & Dane, 2015; Netshitanini, Ubomba-Jaswa, Abia, Fosso-

Kankeu, & Waanders, 2016; Nkansah, Korankye, Darko, & Dodd, 2016). The amount of soil ingested varies between 5 and 219 g, and the frequency of consumption ranges from one to twenty times a day (Hueb, Leick, Guett, Akello & Kutalek, 2016). The soil is obtained from local sources such as riverbanks, termite mounds, house walls, or can be bought at local markets. Geophagic soil is mostly air dried, but can also be baked, smoked, salted, or mixed with herbs or water. Geophagia is observed across all ages, irrespective of education level and socio-economic status (Bisi-Johnson, Obi, & Ekosse, 2010). According to Abrahams (1997) different geophagic products are available for purchase from street and market vendors in Kampala and geophagic products are particularly consumed as traditional medicine commonly known as *emumbwa*.

2.1.3 Reasons for soil consumption

Geophagia is practiced during gestation largely for pregnancy-related cravings, enjoyment of the taste, texture and or smell of the substance consumed (Slabach, Corey, Aprille, Starks, & Dane, 2015; Seim *et al.,* 2013). In South Africa geophagia is widely spread among young girls, pregnant as well as non-pregnant women and the reasons include craving due to smell and texture, the soils' ability to reduce the symptoms of morning sickness and the belief that soils can provide some micronutrients important for health (George & Ndip, 2011). Soil is thought to provide a means to increase the intake of essential elements especially calcium (Ca), magnesium (Mg), Zinc (Zn), Iron (Fe), Copper (Cu), Manganese (Mn) and selenium (Se) during pregnancy. It is also thought to boost fertility, lead to delivery of beautiful babies, reduce stress, and prevent morning sickness, nausea and vomiting, cravings and appetite suppression (Bisi-Johnson, Obi & Ekosse, 2010; Ishmael, Fred, & Anthony, 2015). Geophagia is also practiced for religious, cultural, medicinal reasons, famine and perceived enhancement of personal appearance. Probably it is for such reasons that some men also eat soil (Seim *et al*., 2013).

The consumption of soil also provides benefits including the absorption of certain heavy metals, free radicals and pesticides from the gastrointestinal tract, and the ability to alleviate diarrhea by retaining water in the human digestive tract (Diko & Siewe épse, 2014). Certain soils have also been reported to have special constituents valuable as oral and topical antimicrobials (Ndze, 2013). For example, various varieties of the astringent soil, terra sisillata, have been used for the treatment of bites and stings of venomous animals, malignant ulcers, nose bleeds, gout, dysentery and poisoning in Europe. Specific mineral products such as $CsAgO_2$ that is present in some soils, demonstrated bactericidal activity against pathogenic *Escherichia coli*, extended-spectrum β-lactamase (ESBL) E.coli, *Salmonella enterica*, *Pseudomonas aeruginosa* and *Mycobacterium smegmatis* (Gedikoglu, Gedikoglu, Berkin, Ceyhan, & Altinoz, 2012).

2.1.4 Health hazards associated with soil consumption

Soil has a considerable effect on human health, whether those effects are positive or negative, direct or indirect (Steffan, Brevik, Burgess, & Cerdà, 2018). According to Nkansah, Korankye, Darko & Dodd (2016), soil may constitute a health risk for human beings because the soil is mostly contaminated with parasitic worms, microorganisms and toxic heavy metals.

2.1.4.1 Biological hazards

In general, infectious organisms that can cause disease come from the five major phylogenetic groups: viruses, bacteria, fungi, protozoa and helminths (Vaumourin, Vourc’h, Gasqui, &

Vayssier-Taussat, 2015). Generally, soil microbial populations are more abundant in surface horizons than in deeper horizons (Vermeire *et al*., 2018). Specifically, the distribution of helminths in soils fluctuates greatly with season, climate, and the amount of organic matter in the soil. Helminths are normally more abundant in warm, moist soils with plentiful organic material. They may move vertically in the soil profile in response to seasonal weather changes. Maximum survival times of viruses, bacteria, protozoa and helminths in soil and on plant surfaces are given in Table 1.

Table 1: Maximum survival times of pathogens in soils and on plant surfaces

Pathogen	Soil		Plants	
	Abs. max.	Com. max.	Abs. max.	Com. max.
Viruses	6 months	3 months	2 months	1 month
Bacteria	1 year	2 months	6 months	1 month
Protozoa	10 days	2 days	5 days	2 days
Helminths	7 years	2 years	5 months	1 month

Abs.-absolute; Com. –common; max. -maximum
Source: Gerba & Smith (2005).

Based on the usual habitat of soil-borne pathogens, Jeffery & van der Putten (2011) classified soil pathogens into euedaphic pathogenic organisms (EPO) and soil-transmitted pathogens (STP). Euedaphic pathogenic organisms are true soil organisms (soil inhabitants) with soil as their usual habitat and include most bacteria and fungi). Soil-transmitted pathogens on the other hand, are pathogens for which soil is not the right habitat but can be maintained in the ground for a more extended period (Jeffery & van der Putten, 2011). Unlike EPO, STP must infect the host to complete the life cycle. *Strongyloides stercoralis* is part of the EPO group, whereas *Echinococcus multilocularis, Ascaris lumbricoides, Ancylostoma duodenale, Enterobius vermicularis, Trichuris* *trichiura*, *Entamoeba histolytica*, *Balantidium coli*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Giardia duodenalis*, *Isospora belli*, *Toxoplasma gondii* and others are part of the STP group. Also, the STP group includes important species such as Taeniidae (*Taenia* spp., *Echinococcus* spp.), Ascaridae (*Ascaris* spp.), Toxocaridae (*Toxocara* spp.), Rhabditidae (*Strongyloides* spp.), Ancylostomatidae (*Ancylostoma* spp., *Uncinaria stenocephala*), Trichuridae (*Trichuris* spp.), Capillaridae (*Capillaria* spp.), for which animals are the final hosts (Jeffery & van der Putten, 2011).

Highly toxigenic and parasitic organisms such as *Clostridium perfringens, C.tetani, C.botulinum* have been reported in geophagic materials (Bisi-Johnson, Oyelade, Adediran & Akinola, 2013). Microbiological hazards also include worms such as *Ascaris lumbricoides*, *Trichuris trichiura* and or their eggs which have dire health related consequences (Bisi-Johnson, Obi, & Ekosse, 2010). Geophagic materials in South Africa have been reported to be contaminated with either of the following: *geohelminths, Ascaris lumbricoides, Trichuris trichuriasis, Nectar Americans, Ancylostoma duodenal* and *Strongyloides stercoralis* (Fosso-Kankeu, Netshitanini, Abia, Ubomba-Jaswa & Waanders, 2015). Considerable contamination with both bacteria and fungi has also been reported in Nigeria (Bisi-Johnson, Oyelade, Adediran, & Akinola, 2013). Geophagia is therefore considered as a medical condition by the World Health Organization (Fosso-Kankeu, Netshitanini, Abia, Ubomba-Jaswa & Waanders, 2015).

2.1.4.2 Chemical hazards

According to Agene, & Umar (2015), the ingestion of geophagic materials by pregnant women and children when it contains HM like Pb, As, Cd, Se, and antimony (Sb) poses the risk of some medical disorders. Ngole-Jeme, Ekosse, & Songca (2018) analyzed 57 samples of commonly ingested geophagic products for As, Cd, Co, Cr, Cu, Pb, Mn, Ni, and Zn and their bio-accessibility in the human gastrointestinal tract. The mean concentrations of the elements in the samples were 7.2, 83.3, 77.1, 15.4, 28.6, 24.9, 56.1, 2.8, and 26.5 mg/kg for As, Cr, Mn, Co, Ni, Cu, Zn, Cd, and Pb, respectively. The authors observed higher bio-accessibility in respect of Pb (13 to 49%) and Zn (38 to 56%) among elements studied. Most of the samples had chronic hazard index (HI) values between 0.5 and 1.0. Nyanza *et al.* (2014) reported that geophagic substances consumed by humans contain Pb, Hg, As and Cd. The determination of both essential and toxic elements in soils commonly consumed by pregnant women has been carried out in Tanzania, Ghana and Nigeria (Gomes, 2017). Nkansah *et al*. (2016) noted high levels of As, Pb, Hg, Cd and cobalt (Co) in soils sold in major markets in Ghana. The latter metals have no known beneficial biological functions to the body and are toxic at low concentrations (Morais, Costa & Pereira, 2012). Levels of Fe as high as 81696 mg/kg and Pb as high as 145 mg/kg in geophagic products in Uganda, have previously been reported (Table 2).

Table 2: Data of heavy metal contamination (mg/kg) in geophagic products reported from different countries

Country	Element										Source
	Pb	Cd	As	Hg	Ni	Mn	Cr	Cu	Fe	Zn	
Ghana	5.3×10^{-4}	-	1.63×10^{-3}	-	1.85×10^{-3}	4.72×10^{-3}	-	2.40	1.38	7.74	1
S. Africa	26.5	2.8	7.2	-	28.6	77.1	83.3	24.9	-	56.1	2
Kenya	9-10	0.2-0.4	1-4	-	-	-	-	-	11500-14200	-	3
S. Africa	13.0-64.0	2.1-2.9	-	-	7.9-67.0	-	49.0-181.0	8.9-106.0	-	28.0-88.0	4
Ghana	549.34-622.92	-	8.11-14.18	5.71-9.75	-	-	-	-	-	-	5
India	_	_	_	_	_	24.04	-	0.78	38.16	7.62	6
Nigeria	0.57	1.93	3.92	-	89.34	-	0.17	40.69	151.92	0.83	7
Nigeria	22.62-187.0	-	ND-105.4	-	-	-	6.00-16.26	1.13-23.90	1787-5781	ND-82.99	8
Tanzania	ND-11.3	ND-0.22	0.19-19.7	ND-0.075	2.3-128	2.9-2515	16.0-287	3.9-169	442-89756	13.5-112	9
Ghana	10.7-18.3	0.7-1.6	0.6-10.6	0.3-4.2	7.2-26.2	-	-	0.9-26.6	-	-	10
Uganda	23-145	-	-	-	-	41-2247	-	14-45	16643-81696	35-71	11

Source: 1. Kortei *et al*. (2020); 2. Ngole-Jeme, Ekosse, & Songca (2018); 3. Miller *et al*. (2018); 4. Ekosse, Ngole-Jeme, & Diko (2017); 5. Nkansah *et al*. (2016); 6. Mishra & Roy (2015); 7. Ogah & Ikelle (2015); 8. Lar, Agene, & Umar (2014); 9. Nyanza, Joseph, Premji, Thomas, & Mannion (2014); 10. Woode & Hackman-Duncan (2014); 11. Abrahams (1997).

2.2 Heavy metals in the environment

Heavy metals are considered to be the most important environment problem worldwide (Singh, Gautam, Mishra, & Gupta, 2011). Metals such as Cr, Cu, Fe and Zn play important biochemical roles in the life processes of many organisms, and their intake in trace amounts is essential for human health (Bamuwamye, Ogwok, & Tumuhairwe, 2015). However, beyond certain threshold, these metals can have damaging effects on human beings. In contrast, Pb, Cd, Hg, and As are non-biodegradable and cumulative poisons with no beneficial effects to humans, and for which there is no known homeostasis mechanism (Vieira, Morais, Ramos, Delerue-Matos, & Oliveira, 2011). They are generally considered the most toxic to humans and animals with adverse effects even at low concentrations (Castro-González & Méndez-Armenta, 2008). Their actions may lead to adverse developmental, reproductive, neurological, immunological, teratogenic and carcinogenic effects in mammals (Singh, Gautam, Mishra, & Gupta, 2011; Tchounwou, Yedjou, Patlolla, & Sutton, 2012; Zhuang, Li, McBride, Zou, & Wang, 2013). A relationship between antibiotic resistance and Cr(VI), Hg and Pb concentrations in the environment has also been reported (Barifaijo, Musisi, Muwanga, Nakavuma, & Opuda-Asibo, 2007; Ţugui, Szekeres, & Baricz, 2017).

The health effects of heavy metals and their notable presence in the environment have attracted attention from researchers and policymakers in Uganda. Urbanization and industrialization have caused soils to become progressively contaminated with heavy metals which have in turn caused threats to human health (Shakoor, Farid, Ali, & Farooq, 2013). Risk assessment of heavy metals in street meats, drinking water and black tea in Kampala showed non-cancer and cancer risks in excess of management levels (Bamuwamye, Ogwok, & Tumuhairwe, 2015; Bamuwamye *et al.*, 2017). Mwesigye, Young, Bailey, & Tumwebaze (2016) reported Zn and Pb levels in excess of WHO/FAO thresholds in *Amaranthus* vegetable. Twenty percent of bananas also contained Pb concentrations in exceedance of the WHO/FAO recommended threshold of 0.3 mg/kg. High levels of Pb and Cd have been reported in milk from dairy farms in Wakiso District (Nyakairu, Muhwezi, & Biryomumaisho, 2011). Concentrations of Cd higher than permissible have also been reported for silver fish from Lake Kyoga (Mbabazi & Wasswa, 2010). Airborne particulate matter mass concentrations in Kampala were observed to be four times higher than the WHO air quality guideline (Schwander *et al*., 2014).

2.2.1 Sources of heavy metals in the environment

Heavy metals in the environment originate from geogenic, industrial, agricultural, pharmaceutical, domestic effluents and atmospheric sources (He, Yang, & Stoffella, 2005; Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Sources of heavy metals in the environment include air which contains fumes from mining and smelting places, refining of fossil fuels, production and use of metallic commercial products, vehicle exhaust, water having domestic sewage, industrial effluents, thermal power plants, atmospheric fallout, soil like-agricultural and animal wastes, municipal and industrial sewage, coal ashes, fertilizers and discarded manufactured goods (Chopra, Pathak & Prasad, 2009). Natural phenomena such as weathering and volcanic eruptions have also been reported to significantly contribute to heavy metal pollution (Jozef & Winchester, 1990). The heavy metals are leached out in sloppy areas and carried by acid water downstream or run-off to the sea. Environmental pollution with heavy metals in Greater Kampala Metropolitan Area derives from emissions from industries, industrial effluents, use of leaded gasoline and paints, agricultural activities, indiscriminate disposal of municipal wastes and incineration of toxic substances (Muwanga & Barifaijo, 2006).

2.2.2 Effects of heavy metals on human health

2.2.2.1 Essential (trace) elements

The word “trace element” is used for elements existing in natural and perturbed environments in small amounts, with excess bioavailability having a toxic effect on the living organism (Bhattacharya, Misra, & Hussain, 2016; Preeti, Satya, & Mohsina, 2016). Essential elements considered in this study include; Cr(III), Fe, Cu, Mn and Zn. Chromium(III)) is essential both in animal feeding and human nutrition (Bogdan *et al.*, 2014). It is essential for normal carbohydrate metabolism. The biological function of chromium is closely associated with that of insulin and most chromium-stimulated reactions are also insulin dependent. Chromium(III) is an integral part of the Glucose Tolerance Factor (GTF), and assists insulin in reducing blood glucose, by stimulating glucose uptake by the muscles and tissues in the body (Mehri & Marjan, 2013). Iron is the most abundant trace element in the human body (Bhattacharya, Misra, & Hussain, 2016). It is essential for blood production, a component of hemoglobin, essential for respiration and energy metabolism, and as a component of enzymes involved in the synthesis of collagen and some neurotransmitters. Iron is also needed for proper immune function. Copper is present throughout the brain and is most prominent in the basal ganglia, hippocampus, cerebellum, numerous synaptic membranes, and in the cell bodies of cortical pyramidal and cerebellar granular neurons (Desai & Kaler, 2008). Enzymes in the central nervous system that depend on copper for their function include tyrosinase, peptidylglycine α-amidating monooxygenase, Cu/Zn superoxide dismutase, ceruloplasmin, hephaestin, dopamine-β-hydoxylase, and cytochrome ***c*** oxidase. According to Chen, Bornhorst, & Aschner (2018), Mn is essential for intracellular activities. It functions as a cofactor for a variety of enzymes, including arginase, glutamine synthetase (GS), pyruvate carboxylase and Mn superoxide dismutase (Mn-SOD). Through these metalloproteins, Mn plays critically important roles in development, digestion, reproduction, antioxidant defense, energy production, immune response and regulation of neuronal activities. Zinc is the second most abundant trace mineral in the body (Vidyavati, Sneha, & Katti, 2016). It is ubiquitous within cells and its role in biology can be grouped into three general functions, namely catalytic, structural and regulatory functions (Roohani, Hurrell, Kelishadi, & Schulin, 2013). It is essential for the functioning of more than 300 enzymes involved in the metabolism of carbohydrates, lipids, proteins as well as in the metabolism of certain micronutrients. It is a constituent of both DNA and RNA polymerases and influences the activity of thymidine kinase and ribonuclease (Bhattacharya, Misra, & Hussain, 2016). Zinc is also a component of insulin, the growth- and sex-hormones, and plays a role in the structure of proteins and cell membranes.

2.2.2.2 Toxic effects of Cr(III), Fe, Cu, Mn and Zn

Chromium(III) is poorly absorbed by any route, and consequently, the toxicity of chromium is mainly attributable to the Cr(VI) form. In as far as Fe is concerned, the focus surrounding Fe-intake by the Public Health sector was related to iron deficiency (Fonseca-Nunes, Jakszyn, & Agudo, 2013). However, Fe has been suggested as a risk factor for different types of cancers mainly due to its prooxidant activity, which can lead to oxidative DNA damage (Davoodi, Jamshidi-Naeini, Esmaeili, Sohrabvandi, & Mortazavian, 2016). In addition, heme iron, can be involved in carcinogenesis by acting as a nitrosating agent, forming N-nitroso compounds (Fonseca-Nunes, Jakszyn, & Agudo, 2013). Iron overload generally occurs when total body Fe exceeds 5 g. Chronic Cu poisoning leading to liver failure was reported in a young adult male with no known genetic susceptibility who consumed 30 to 60 mg/d of copper as a mineral supplement for 3 yr. Excessive Cu intake also induces toxicity indirectly by interacting with other nutrients; for example, it may lead to anemia by interfering with Fe transport and/or metabolism. In humans, the liver is the primary organ of copper-induced toxicity. Other target organs include bone and the central nervous and immune systems. Manganese toxicity represents a serious health hazard, resulting in severe pathologies of the central nervous system in humans. The element has been reported in miners, workers in dry-cell battery factories, smelters, and steel manufacturing workers or welders (O'Neal & Zheng, 2015). It accumulates substantially in bone, with a half-life of about 8 to 9 years in human bones. Manganese is associated with dopaminergic dysfunction by recent neurochemical analyses and synchrotron X-ray fluorescent imaging studies (O'Neal & Zheng, 2015). Rather than being toxic, Zn is an essential trace element (Plum, Rink, & Haase, 2010). However, whereas intoxication by excessive exposure is rare, Zn deficiency is widespread and has a detrimental impact on growth, neuronal development, and immunity, and in severe cases its consequences are lethal. Zinc deficiency caused by malnutrition and foods with low bioavailability, aging, certain diseases, or deregulated homeostasis is a far more common risk to human health than intoxication (Plum, Rink, & Haase, 2010).

2.2.2.3 Toxic elements

Lead, Cd, Cr(VI), As and Hg are potentially toxic elements even at very low concentrations (Mehri & Marjan, 2013). Lead exposure causes neurological, neurobehavioral, immunotoxic and developmental effects in children; neurotoxic effects in adults; hematologic effects; renal toxicity; effects on cardiovascular system (Zhai, Narbad, & Chen, 2015). It also leads to anemia, psychological disorders, peripheral neuropathy, nephropathy, and abdominal colic. Furthermore, effects on the female reproductive system (alterations in pregnancy) and male reproductive system (morphological alterations in spermatozoa and the sperm count) have been linked to Pb exposure (Vigeh, Smith, & Hsu, 2011).

Cadmium exposure is linked to increased incidence of renal pathologies, osteoporosis and hypertension (Satoh, Koyama, Kaji, Kito, & Tohyama, 2002; Zhai, Narbad, & Chen, 2015). In general, women have higher blood Cd levels than men, due to increased oral Cd absorption because of relatively low iron stores in women of childbearing age (Liu, Lu, Wu, Goyer & Waalkes, 2008). Cadmium absorption can be increased by dietary deficiencies of Ca or Fe and by diets low in protein (Järup, 2003; Asagba, 2009). Once absorbed, Cd is very poorly excreted and only about 0.001% of the body burden is excreted per day (Liu, Lu, Wu, Goyer & Waalkes, 2008). The biological half-life of Cd in humans is in the range of 10 to 30 years (Järup, 2003). In men, Cd has adverse effects on prostate impairment and serum testosterone levels.

Soil can be a source of hexavalent Cr; Cr(VI), but little is known about the health effects of Cr(VI) from environmental exposures (Costa & Klein, 2006). Absorption of Cr(VI) compounds is higher (2 to 10%) than that of trivalent Cr compounds (0.5 to 2%). Once in the blood, hexavalent Cr is taken up by erythrocytes, whereas trivalent Cr is only loosely associated with erythrocytes (Denil, Fui, & Ransangan, 2017). Chromium compounds are distributed to all organs of the body, with high levels in the liver, spleen, and kidney. Chromium(VI) has been implicated in cancers of the respiratory system, buccal cavity and pharynx, prostate, and stomach in humans, and it is related to increased risk of overall mortality owing to lung, larynx, bladder, kidney, testicular, bone, and thyroid cancer (Deng *et al*., 2019). Exposure to Cr can also be a cause of asthma (García-Esquinas *et al*., 2014; Schneider, Constant, Patierno, Jurjus, & Ceryak, 2012).

Arsenic exposure is a risk factor for cancer and cardiovascular disease (Silbergeld & Nachman, 2008). An association between ingestion of inorganic As and cardiovascular disease has been shown (Navas-Acien *et al.,* 2005). Chronic As exposure is also linked to; peripheral neuropathy, gastrointestinal symptoms, non-insulin-dependent diabetes mellitus (NIDDM or Type 2 DM), adverse effects on the male reproductive system, enlarged liver, bone marrow depression, destruction of erythrocytes and high blood pressure (Fernández-Luqueño *et al.,* 2013).

The major route of human exposure to Hg is through eating contaminated fish, seafood, and wildlife which have been exposed to Hg through ingestion of contaminated lower organisms, and dental amalgam (Rice Jr., Wu, Gillette & Blough, 2014). However, there is easy access of this element to man through multiple pathways including air, water, soil and cosmetic products (Zahir, Rizwi, Haq, & Khan, 2005). Fetuses and children are more susceptible towards mercury toxicity because mothers consuming diets containing mercury pass it to fetuses and to infants through breast milk (Zahir, Rizwi, Haq & Khan, 2005). Infants whose mothers are intoxicated with Hg can develop mental retardation, peripheral neuropathy, cerebral palsy, and blindness (Azevedo *et al*., 2012). Mercury is also a causative agent of various other disorders, including nephrological, immunological, cardiac, motor, reproductive and even genetic disorders (Zahir, Rizwi, Haq, & Khan, 2005; Pizent, Tariba, & Živković, 2012). In the cardiovascular system, Hg induces hypertension in humans and animals with wide-ranging consequences, including alterations in endothelial function (Azevedo *et al.,* 2012). Diabetes may also be associated or caused by excessive exposure to Hg because the metal binds the sulphur binding sites on the insulin molecule thereby causing dysregulation of blood glucose levels (Rice Jr., Wu, Gillette, & Blough, 2014).

2.2.3 Determination of heavy metals in biological materials

Many analytical instruments may be employed to measure the concentration level of HM in various media. The most predominant techniques are Atomic Absorption Spectrophotometry (AAS); Atomic Emission/Fluorescence Spectrometry (AES/AFS); Inductively Coupled Plasma Mass Spectrometry (ICP-MS); Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES); Neutron Activation Analysis (NAA), X-ray Fluorescence (XRF); and Anodic Striping Voltammetry (AVS). In this study, heavy metal determination in the geophagic products was done using the ICP-OES.

Inductively Coupled Plasma/Optical Emission Spectrometry is a spectroscopic technique suitable for trace elements analysis in several types of media. It is a technique based on the unprompted emission of photons from atoms and ions that have been excited in a radiofrequency (RF) discharge. Samples are usually introduced into the plasma in liquid form; thus, solid samples require acid digestion prior to injection, while gas and liquid samples can be injected directly into the instrument. The sample solution is converted to an aerosol then sent into the center of the plasma which maintains an atomization temperature of about 9700°C. The excited ions may return to the ground state via emission of photons. Specific wavelength of the photons can be used to identify the elements and the number of photons is directly proportional to the concentration of the element in the sample.

2.3 Risk assessment of chemical hazards in food and the environment

Risk is the analysis and prioritization of the combined probability of a product contamination, consumer exposure and the size of the anticipated public health impact of specific chemical, microbiological and/or nutritional hazards (Van-der-Fels-Klerx *et al*., 2018). It is the combination of the probability that a hazard may occur and the effect of exposure to the hazard on human health. Health risk assessment (HRA) is the process used to evaluate the toxic properties of a chemical and the conditions of human exposure to chemicals by monitoring such exposure and characterizing the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media (Chen *et al*., 2014). Human health risk assessment is therefore used to estimate the health effects that might result from exposure to either cancerous or non-cancerous chemicals (Van-der-Fels-Klerx *et al*., 2018).

2.3.1 Risk determination process

The risk assessment process involves four steps, viz. hazard identification, dose-response assessments or hazard characterization, exposure estimates and risk characterization.

2.3.1.1 Hazard identification

Hazard identification, the first step of risk assessment involves the identification of biological, chemical and physical agents which may be present in a particular food or environmental media such as soil, that are capable of causing adverse health effects (Vinci *et al*., 2012). The main purpose of hazard identification as applied to chemicals is to evaluate the weight of evidence for adverse health effects, based on assessment of all the available data regarding toxicity and mode of action of the particular chemical. Exposure to HM may generate different adverse effects in humans: diseases, formation of tumors, reproductive defects, death and other effects (Vinci *et al*., 2012).

2.3.1.2 Hazard characterization or dose-response relationship

Hazard characterization or dose-response relationship describes how the likelihood and severity of an adverse health effect (the response) is related to the amount and condition of exposure to an agent; the dose provided (Anthony *et al*., 2017; Pratt & Barlow, 2009). At low doses there may be no response. However, as the dose increases, the measured response also increases. At some level of dose the responses begin to occur in a small fraction of the population or at a low probability rate (Vinci *et al*., 2012). Both the dose at which response begins to appear and the rate at which it increases given increasing dose can be variable between different pollutants, individuals, exposure routes, etc. The shape of the dose-response relationship depends on the agent, the kind of response (tumor, incidence of disease, death, etc.), and the subject (human or animal) in question. For example, there may be one relationship for a response such as 'weight loss' and a different relationship for another response such as 'death'.

2.3.1.3 Exposure assessment

Exposure assessment is the process of measuring or estimating the magnitude, frequency, and duration of human exposure to an agent in the environment or estimating future exposures for an agent that has not yet been released (Bamuwamye, Ogwok & Tumuhairwe, 2015). An exposure assessment includes some discussion of the size, nature, and types of human populations exposed to the agent, as well as discussion of the uncertainties in the above information. Exposure can be measured directly, but more commonly is estimated indirectly through consideration of measured concentrations in the environment, consideration of mode of chemical transport and fate in the environment, and estimates of human intake over time (Pratt & Barlow, 2009).

2.3.1.4 Risk characterization

Risk characterization involves the quantitative estimation of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment. It therefore conveys the risk assessor's judgment as to the nature and presence or absence of risks, along with information about how the risk was assessed, where assumptions and uncertainties still exist, and where policy choices will need to be made. Risk characterization integrates the hazard identification and characterization, leading to a health-based guidance value and the human exposure estimated from either a deterministic or probabilistic method to conclude on the likelihood of adverse effects for public health. For genotoxic carcinogens such as As, Ni, Cd and Cr(VI), the margin of exposure (MOE) is calculated by dividing the point of departure, often the human exposure by the benchmark dose lower limit (BMDL) of the respective hazard (Anthony *et al*., 2017).

2.3.2 Methods for the determination of health risks

Quantitative risk assessment is characterized by assigning a numerical value to the risk. A model is used to calculate the risk based on the exposure and the dose response. There are two main methods of risk assessment; deterministic and probabilistic approaches (Richardson, 2010).

2.3.2.1 Deterministic methods

Deterministic assessments are simple to carry out, often use readily available data, and produce results that are straightforward to interpret (US EPA, 2017). The methods use single point estimates to calculate the exposure and the outcome is a single risk estimate. It involves estimation of each variable within the model to determine the outcome. A fixed value for food consumption is multiplied by a fixed value for the concentration of the contaminant in that particular food to calculate the exposure. The intakes from all sources are added up to obtain the total exposure. The risk is determined by comparing the exposure with the BMDL for the different adverse health effects. Deterministic models are commonly used as a first step in exposure assessment because they are relatively quick, simple and inexpensive to carry out. Inherent, in the point estimates models are the assumptions that all individuals consume the specified foods at the same level, that the hazard is always present in the food and that it is always present at an average or high concentration. As a consequence, this approach does not provide an insight into the range of possible exposures that may occur within a population or the main factors influencing the results of the assessment. The approach therefore turns out to be very limited as a single value assignment clearly involves a stiffness, which often leads to overestimating the risk and, consequently, to conservative decisions, which are reflected in a higher remediation cost (Rivera-Velasquez *et al*., 2013). It provides limited information for risk managers and the public.

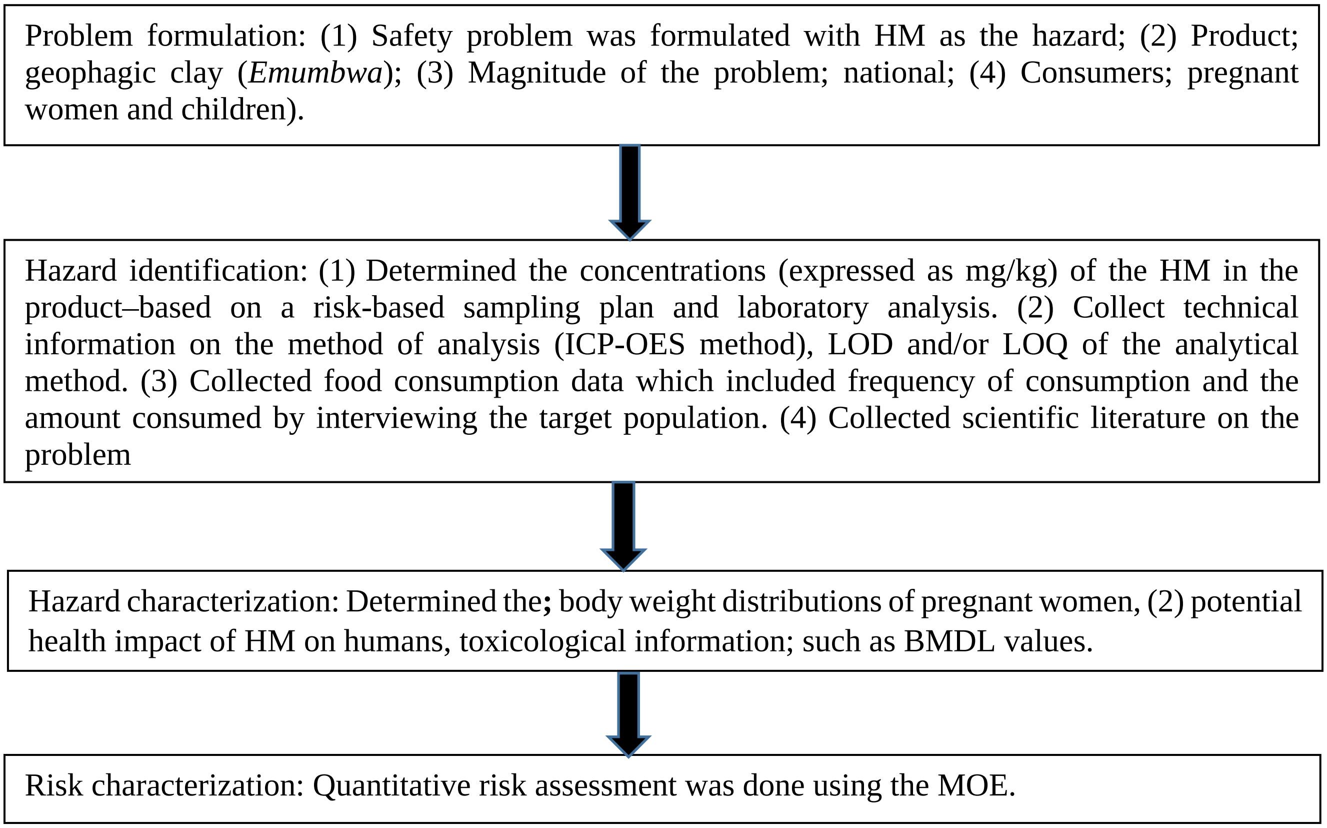
2.3.2.2 Probabilistic methods

Probabilistic assessments use more complicated models that rely on distributions of data as inputs in place of point values for key parameters (EPA, 2017). The approach has advantage over the deterministic approach in that it considers the whole range of exposure distributions, from minimum to maximum with all modes and percentiles. In this way, more meaningful information is provided to risk managers and the public. Probabilistic risk analysis considers the degree of variability and uncertainty of each parameter in the risk equation through an estimation based on stochastic methods such as the *Monte Carlo* simulation (Vinci *et al.*, 2012; Rivera-Velasquez *et al.*, 2013). The variables are described in terms of distributions instead of point estimates. This results in a distribution of possible exposure estimates and greater ability to characterize variability and uncertainty. Probabilistic risk assessments also cater for scenario analysis. Scenario analysis is the process of forecasting the expected value of a performance indicator, given a time period, occurrence of different situations, and related changes in the values of system parameters under an uncertain environment (Balaman, 2019). It is conducted to analyze the impacts of possible future events on the system performance by taking into account several alternative outcomes, and to present different options for future development paths resulting in varying outcomes and corresponding implications. Probabilistic models derive their data from three types of sources: first-hand data, expert knowledge, or pre-existing (probabilistic or deterministic) models (Uusitalo *et al.*, 2015). They employ three scenarios namely; lower, medium and upper bounds (Vinci *et al.*, 2012). The latter scenarios correspond to non-detected concentrations in the samples analyzed (ND), half of the method limit of detection (LOD) and the LOD, respectively. Probabilistic analysis is a hard to use method and time consuming because it requires considerable amounts of data. However, due to its ability to reduce data variations and uncertainties, the approach is most preferred in risk assessment (Pratt & Barlow, 2009).

CHAPTER 3: MATERIALS AND METHODS

3.1 Scope of the study

The HM safety of “*Kumenya*” and “*Mukalakasa*” geophagic products used during pregnancy was investigated. According to the market vendors, the major source of these products is Nakuwadde, Nsangi and Namagoma in Wakiso district, Kasawo in Mukono district, and Butambala and Kiboga districts of Uganda. The major events of the risk assessment of the HM exposure in the geophagic products are summarized in Figure 1.



Problem formulation: (1) Safety problem was formulated with HM as the hazard; (2) Product; geophagic clay (*Emumbwa*); (3) Magnitude of the problem; national; (4) Consumers; pregnant women and children).

Hazard identification: (1) Determined the concentrations (expressed as mg/kg) of the HM in the product–based on a risk-based sampling plan and laboratory analysis. (2) Collect technical information on the method of analysis (ICP-OES method), LOD and/or LOQ of the analytical method. (3) Collected food consumption data which included frequency of consumption and the amount consumed by interviewing the target population. (4) Collected scientific literature on the problem

Hazard characterization: Determined the**;** body weight distributions of pregnant women, (2) potential health impact of HM on humans, toxicological information; such as BMDL values.

Risk characterization: Quantitative risk assessment was done using the MOE.

Figure 1: Schematic overview of the risk assessment of heavy metal exposures in *Emumbwa*

3.2 Materials

Kumenya and *Mukalakasa Emumbwa* products used in the study were obtained from Katwe, Kibuye, Nakawa, Bugolobi and Nakasero markets in Kampala city. All chemicals used were of analytical grade and were obtained from Perlin suppliers located in Kampala, Uganda. Hydrochloric acid (HCl; 35-38%) and Nitric acid (HNO_3; 65-68%) was used for sample digestion. Lithium carbonate (Li_2CO_3; 99%) was used for preparing the blank. Double distilled water was used for preparing solutions and washing glassware. Standard solutions of the different elements were prepared from stock solutions obtained from Sigma-Aldrich, Germany (Nkansah *et al*., 2016).

3.3 Equipment and tools

A set of open-ended semi-structured food frequency questionnaires (FFQ) were used to determine the geophagic products ingestion rate. The questionnaires assessed practices such as the source of products and mode of utilization (Appendix 1). Both soil consumers, and street and market vendors were interviewed. The questionnaires were pretested once with 10% of the respondents.

Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Model-FMS26) manufactured by Spectro Analytical Instruments GmbH (Germany), was used to determine heavy metal concentrations in the geophagic products (Retka, Maksymowicz & Karmasz, 2010). This equipment is found at the Uganda National Bureau of Standards (UNBS) chemistry laboratory. Sample weight was taken on a Mettler-Toledo (AA-200DS, Germany) analytical balance.

3.4 Methods

3.4.1 Sampling

3.4.1.1 Determination of the population to be considered in the geophagic clay intake study

Chronbach’s alpha (equation 1) was used to correlate the scores of the responses.

$$Cronbach's\ Alpha = \left(\frac{n}{n-1}\right)\left(\frac{SD^2 - \sum Variance}{SD^2}\right) \tag{1}$$

Where: *n = Number of items on the test*

SD = The Standard Deviation for the set of test scores, and ΣVariance = Summation of the variances of the scores for each of individual item on the test.

A Cronbach’s Alpha value > 0.7 indicates that the tool is reliable (Amin, 2005). According to the Uganda Bureau of Statistics (UBOS, 2016), Kampala has an estimated resident population of 1,936,080 (0.78 male: 1.0 female) of whom 16% are children 5 to 9 years of age (Bamuwamye *et al.*, 2017). Based on the adult population size, the sample size of 500 respondents was determined using Krejcie & Morgan’s 1970 Table (Amin, 2005). Considering the geophagic products consumption prevalence of 50% for the 500 respondents, 250 respondents were considered. However, another 30 respondents were added in order to cater for errors, making a total of 280 respondents. All participants in the survey were females aged 18 years and above.

3.4.1.2 Determination of *Emumbwa* ingestion rate

The dietary history method described by Patterson & Pietinen (2007) was used to determine the soil ingestion rate (IR). This method involves use of face-to-face interview to reconstruct a pattern of intake typical of the previous week. The method has been used to ascertain nutrient intake in the national dietary survey in many countries (Patterson & Pietinen, 2007).

3.4.1.3 Risk based sampling plan to determine the number of analytical samples

The contribution of geophagic products to the disease burden in Uganda is not documented mainly due to lack of data on geophagic soil contaminants and the lack of consumption data. The risk-based sampling approach described previously by Delcour *et al.* (2015) was used. Based on the Lahoe *et al* (2014) Table, the number of samples needed to detect a total positive fraction of 5% at a confidence interval of 95% was determined as 60 (Table 3). These samples were evenly divided between "*Kumenya*" and "*Mukalakasa*" products. According to the vendors, the actions of these products involve the modulation of uterine contractions at labor, resulting from the stimulation of smooth muscle. *Kumenya* and *Mukalakasa* products were obtained from; Kiboga, Nsangi, Nakuwade, Butambala, Kamengo, Namagoma and Kasawo. The samples were analyzed for Pb, Cd, As, Hg, Cu, Cr, Ni, Fe, Mn and Zn. The choice of these HM is based on their public health risk, and evidence of occurrence in the soil documented in the literature (Kortei *et al.*, 2020; Ekosse, Ngole-Jeme, & Diko, 2017; Ogah & Ikelle, 2015; Lar, Agene, & Umar, 2014).

Table 3: Risk based sampling plan

Hazard	Risk profile of the product (R=P*E)	Major/minor consumption	Risk profile of region of origin	Risk profile of supplier	Major or minor volume	Total score	Risk level	Samples /year
HM	6	3	1	5	1	16	Medium	60

HM: heavy metal; R: risk; P: probability; E: effect

3.4.2 Sample treatment

The samples were packed in clean plastic sample bags, labelled according to type and place of origin, and transported to UNBS chemistry laboratory for analysis. The samples were dried in a hot air oven (Memmer1t UFE-600; Germany) at 105°C for 5 hours. The dried sample material were ground using porcelain mortar and pestle and passed through a 250 µm sieve in order to obtain a fine homogeneous sample. The mortar was cleaned after each sample preparation to avoid contamination. The fine samples were packed in labelled plastic sample bottles and kept under cool dry conditions till analysis.

3.4.3 Determination of heavy metals in geophagic products

Geophagic products samples were digested following the method described by Usman, Al-Ghouti, & Abu-Dieyeh (2019). The digests were analyzed by direct injection into the ICP-OES (Nkansah *et al.*, 2016; Usman *et al.*, 2019). Calibration standards were prepared from multi-element standard stock solutions (1000 ppm) supplied by Spex®. Standard solutions of the metals were prepared at 5 different concentrations; 0.05, 0.5, 1.0, 2.0 and 4.0 ppm. A calibration curve in each case was generated from the curve, the unknown concentrations of the analytes in the different samples were determined. Metal concentrations were recorded as mg/kg.

3.5 Exposure assessment

Exposure assessment was done using the probabilistic approach. Probabilistic exposure assessment employs three scenarios namely; lower, medium and upper bounds. The latter scenarios correspond to non-detects (ND) concentrations in the analyzed samples, half of the limit of detection (LOD) and LOD for the lower (LB), medium (MB) and upper bound (UB) respectively

(Vinci *et al*., 2012; Papastergiadis *et al*., 2014). Estimated heavy metal intakes (mean, max and 50, 75, 90, 95 and 99 percentiles) were determined for each scenario. The average consumption data per day was calculated as described by the European Food Safety Agency; EFSA (Papastergiadis *et al*., 2014). A distribution for consumption, body weight and concentration data was fitted for consumers and the total population while catering for the ND. For the risk output calculations, the fractions of < LOD was utilized using an “if” logical function of the MS excel for the LB, MB and UB, respectively. Best fit distributions were determined for the different scenarios of heavy metal concentration data using the Chi-square statistics. Probability/probability (P/P) and quantile/quantile (Q/Q) plots were used to determine the best fit distribution for both consumption and hazard concentration data (Vinci *et al.*, 2012). The best fit distribution selected for the LB was also applied to the MB and UB data. First order *Monte Carlo* simulation was performed with 5,000 to 10,000 iterations.

3.6 Margin of exposure (MOE)

The MOE was determined by dividing the BMDL toxicological values by the exposure values for each heavy metal. The value obtained was compared with the safety value for each heavy metal as described earlier by EFSA and Codex (Papastergiadis *et al*., 2014).

$$\text{MOE} = \frac{\text{Reference value (BMDL)}}{\text{Exposure}} \qquad (2)$$

Table 4: BMDL toxicological values of the metals and their associated health effects

Metal	BMDL value	Associated health effects
Pb	BMDL10=0.63	chronic kidney disease
	BMDL01=1.5	cardiovascular disease (CVD)
As	BMDL$_{0.5}$=3.0	lung cancer
Cr	BMDL$_{0.5}$=0.011	lesions in liver
	BMDL$_{0.5}$=0.11	lesions in duodenum
	BMDL$_{0.5}$=0.2	heamostolic effects
	BMDL$_{0.5}$=0.27	lesions in pancrease
	BMDL$_{10}$=1.0	combined adenomas and carcinomas in rats
Ni	BMDL$_{10}$=0.28	post implantation fetal loss
	BMDL$_{10}$=1.1	eczematous flare up skin reaction in rats

3.7 Data analysis

Means were separated by two-way analysis of variance (ANOVA) using XLSTAT, version 2020. Statistical significance was considered at $P < 0.05$. Chi square statistics of the Microsoft Excel in built @risk software (Version 8.1, Palisade Corporation, USA) was used to determine the level of risk and the relationship between non-carcinogenic effects of heavy metals and the soil consumption.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Heavy metal content of geophagic products

A total of 10 heavy metals; Pb, Cd, As, Hg, Mn, Cr, Cu, Ni, Fe and Zn were determined as the metal concentrations in two types of geophagic products (*Kumenya and Mukalakasa*). The concentration of heavy metals in the samples varied as indicated in Table 5. Iron had the highest concentration (1500 to 22900 mg/kg) in the geophagic products, while Hg and As were not detected in any of the samples analyzed. In general, *Kumenya* products contained higher amount of iron than *Mukalakasa* products. The concentration of Fe in *Kumenya* products that originated from Kasawo was significantly ($P < 0.05$) different from that of the products from Kiboga and Nsangi and Butambala. There was a pronounced difference between Fe concentrations of geophagic products that originated from Nakuwade, Butambala, Kamengo and Namagoma. Manganese (0.68 to 8.99 mg/kg) was the second most abundant element followed by Zn (0.48 to 2.44 mg/kg), Cr (0.51 to 1.57 mg/kg), Cu (0.14 to 1.20 mg/kg), Pb (0.29 to 0.66 mg/kg), Ni (0.08 to 0.39 mg/kg) and Cd (0.02 to 0.06 mg/kg) in that order. The *Mukalakasa* products contained higher amount of Mn than *Kumenya* products from Kamengo. There was a difference ($P < 0.05$) in the content of HM among different products obtained from different geographical sources.

High levels of HM have been reported in geophagic products from countries such as Tanzania, South Africa, Nigeria and Ghana. Studies on geophagic products have concentrated on the quantification of Pb, Cd, As, Cu, Fe and Zn (Kortei *et al*., 2020; Ekosse, Ngole-Jeme, & Diko, 2017; Ogah & Ikelle, 2015; Lar, Agene, & Umar, 2014). Arsenic and Hg content was below the LOD of 0.05 µg/kg for both elements. Lead and Cd content was lower than that reported in previous studies (Ngole-Jeme, Ekosse, & Songca, 2018).

Table 5: Mean concentrations (mg/kg) of heavy metals in *Kumenya* and *Mukalakasa* geophagic products obtained different sources

Source	Type	Pb	Cd	As	Hg	Ni	Mn	Cr	Cu	Fe*	Zn
Nakuwadde	*Kumenya*	0.66±0.11^{a}	0.06±0.02^{a}	<LOD	<LOD	0.13±0.00^{a}	3.68±0.23^{b}	1.57±0.25^{b}	0.44±0.02a	1.58±1.22^{b}	1.66±0.52^{a}
	Mukalakasa	0.46±0.11^{a}	0.04±0.01^{a}	<LOD	<LOD	0.15±0.03^{a}	1.51±0.02^{a}	1.35±0.30^{b}	0.46±0.01^{a}	0.54±0.00^{a}	0.56±0.05^{a}
Kasawo	*Kumenya*	0.48±0.27^{a}	0.05±0.02^{a}	<LOD	<LOD	0.13±0.06^{a}	1.92±2.33^{a}	0.76±0.64^{a}	0.25±0.02^{a}	2.29±1.1^{c}	1.46±0.93^{a}
	Mukalakasa	0.29±0.01^{a}	0.02±0.00^{a}	<LOD	<LOD	0.08±0.01^{a}	1.75±1.84^{a}	0.67±0.04^{a}	0.27±0.01^{a}	0.23±0.01^{ab}	0.48±0.01^{a}
Kiboga	*Kumenya*	0.45±0.07^{a}	0.03±0.02^{a}	<LOD	<LOD	0.33±0.46^{a}	3.45±1.93^{b}	0.63±0.25^{a}	0.16±0.01^{a}	0.15±0.05^{a}	0.84±0.66^{a}
	Mukalakasa	0.35±0.06^{a}	0.02±0.02^{a}	<LOD	<LOD	0.09±0.04^{a}	3.14±3.04^{b}	0.75±0.31^{a}	0.15±0.02^{a}	0.88±0.70^{a}	1.10±1.05^{a}
Butambala	*Kumenya*	0.33±0.09^{a}	0.03±0.02^{a}	<LOD	<LOD	0.16±0.03^{a}	4.85±3.78^{c}	0.51±0.10^{a}	0.34±0.02^{a}	0.99±0.59^{a}	2.44±0.04^{b}
	Mukalakasa	0.55±0.31^{a}	0.03±0.01^{a}	<LOD	<LOD	0.15±0.02^{a}	1.38±0.49^{a}	1.21±0.11^{b}	0.33±0.02^{a}	0.37±0.20^{a}	0.49±0.11^{a}
Nsangi	*Kumenya*	0.562±00^{a}	0.06±0.01^{a}	<LOD	<LOD	0.12±0.01^{a}	0.53±0.25^{a}	0.69±0.07^{a}	1.20±0.03^{a}	0.58±0.06^{a}	0.77±0.10^{a}
	Mukalakasa	0.57±0.07^{a}	0.05±0.01^{a}	<LOD	<LOD	0.14±0.02^{a}	0.68±0.08^{a}	0.63±0.09^{a}	1.44±0.47^{a}	0.58±0.11^{a}	0.83±0.04^{a}
Kamengo	*Kumenya*	0.57±0.05^{a}	0.06±0.04^{a}	<LOD	<LOD	0.32±0.18^{a}	2.60±2.12^{b}	1.13±0.16^{a}	0.72±0.00^{a}	1.70±0.46^{b}	0.77±0.06^{a}
	Mukalakasa	0.58±0.01^{a}	0.06±0.00^{a}	<LOD	<LOD	0.39±0.06^{a}	8.99±0.07^{d}	0.91±0.05^{a}	0.73±0.02^{a}	1.42±0.02^{b}	0.75±0.05^{a}
Namagoma	*Kumenya*	0.68±0.04^{a}	0.05±0.02^{a}	<LOD	<LOD	0.25±0.14^{a}	3.21±2.70^{bc}	1.33±0.42^{a}	0.62±0.01^{a}	1.11±0.84^{b}	0.83±0.13^{a}
	Mukalakasa	0.57±0.18^{a}	0.06±0.01^{a}	<LOD	<LOD	0.17±0.01^{a}	1.59±0.11^{b}	1.40±0.18^{a}	0.58±0.06^{a}	1.00±0.75^{b}	0.91±0.07^{a}

* Concentration (10^{4}); LOD: Limit of detection (LOD for As & Hg is 0.05 µg/g); Means are the average of 3 values: Values with different :superscripts are significantly different ($p < 0.05$): N=60.

The Fe content of geophagic products of this study was lower than reported by Abrahams (1997) but higher than reported elsewhere in Africa (Nyanza *et al.*, 2014; Lar *et al.*, 2014; Miller *et al.*, 2018). In general, the content of Fe in geophagic products obtained from different African countries is high. Iron deficiency is a leading cause of anemia and affects many children younger than 5 years of age and pregnant women globally. According to Nankinga & Aguta (2019), the prevalence of anemia in Uganda is 53% in children age 6-59 months, up from 49% in 2011, and is 32% in women of reproductive age, up from 23% in 2011. Another 17.3% of the population is at risk for zinc deficiency due to dietary inadequacy (Nankinga & Aguta, 2019). The content of Cr, Ni, Mn, Cu and Zn was lower than reported in previous studies. Manganese is a powerful neurotoxin (Crossgrove & Zheng, 2004). Contact with Ni compounds can cause a variety of adverse health effects including Ni allergy, lung fibrosis, cardiovascular and kidney diseases. Copper, Fe and Zn are essential elements, with functions indispensable for various biological processes also driving the entire human metabolism (Nankinga & Aguta, 2019).

4.2 Distribution and consumption practices of *Emumbwa*

A total of 280 people participated in the survey. All participants in the survey were females the majority (87.5%) of whom practiced geophagia (Table 6). The age range was between 18 and 60 years. Most (70%) women had 35 to 54 yrs, followed by 18 to 34 (25%) year category and the old-age (> 55) group (5%). The mean body weight was 69.94±13.96 kg. A bigger (52%) fraction of the women had body weight between 60 and 80 kg while the number of women in the 40 to 60 and 80 to 100 kg body weight categories was evenly divided at 24%. About 8% of the women had primary school education, 19.02% secondary school, 66.80% tertiary education, and the remaining 6.07% had non-formal education. The results also reveal that the majority (83.81%) of the clay consumers earn less than one million Uganda shillings per month. The findings of this study are in agreement with those of Lar, Agene, & Umar (2015) who stated that geophagia is a practice restricted mainly to the poor and unemployed, ignorant of any dangers caused by it.

Table 6: Socio-demographic characteristics of consumers of *Emumbwa*

Profile of consumer	Frequency	Percent (%)
Geophagic products consumers	247	87.5
Consumer age: 18 to 34 years	62	25
35 to 54 years	173	70
> 55	12	5
Body weight: 69.94±13.96 kg	34	68
40 to 60 kg	59	24
60 to 80 kg	128	52
80 to 100 kg	59	24
Education**:** No formal schooling	15	6.07
Primary school	20	8.1
Secondary school	47	19.03
Tertiary education	165	66.8
Monthly income: Below 1,000,000 UGX	207	83.81
> 1,000,000 UGX	40	16.19

N=280

Most (95.55%) women reported consuming *emumbwa* 1 to 3 times a day (Table 7). The remaining 4.45% consumed it more than 4 times each day. The mean geophagic product ingestion rate was found to be 17.08±7.32 g per day. Nearly 98% of the respondents consume soil in the evening and or in between meals. The majority (87.85%) of women consumed a combination of "*Mukalakasa* and *Kumenya*" geophagic products. The fraction of women who consumed other types of geophagic products was 43.72%. Only 4.86% reported consuming one type of soil (*Mukalakasa*). Most (95.14%) pregnant women obtained their *Emumbwa* from the markets while 4.05% obtained theirs from herbalists. Only 0.8% of consumers obtained their soil from traditional birth attendants (TBA). The major reasons reported for eating geophagic products was pregnancy related cravings. About 97.2% of the women consumed soil as a source of iron, while about 81% of them consumed it because it softens the pelvic muscles (locally referred to as *Kumenya*). Another 68.83% believe eating soil helps in stopping morning sickness.

Table 7: Consumption practices of *Emumbwa* consumers

Consumption practice	Frequency	Percent
Amount of geophagic products consumed/day	7 to 40 g (mean=17.08±7.32 g)	-
Consumption: 1 to 3 times	236	95.55
4 to 6 times	11	4.45
Consumption time: Morning	2	0.81
Afternoon	4	1.62
Evening and or in between meals	241	97.57
Clay type consumed: *Mukalakasa*	12	4.86
Mukalakasa and *Kumenya*	217	87.85
Others geophagic products	108	43.72
Source of the clay**:** Herbalists	10	4.05
Traditional birth attendants	2	0.81
Market	235	95.14
Purpose for consumption: Softening bones	200	80.97
Morning sickness	170	68.83
Prevention of child abnormalities	2	0.81
Source of iron	240	97.17
General wellbeing of the pregnancy	18	7.29
Period of use till now: Less than 2 years	30	12.15
2 to 4 years	150	60.73
More than 10 years	67	27.13
Consumption after giving birth	47	19.03

N=280

About 7% of the pregnant women believe it is good for the general wellbeing of the pregnancy. Only 0.8% of consumers believe it prevents the fetus from developing abnormalities. Hueb *et al*. (2016) reported the prevalence of geophagy among pregnant women in Africa to range between 28 and 84%. In a study involving 431 pregnant women at various stages of pregnancy in Kenya, the point and period prevalence rates of geophagia were determined to be 35% and 58% pregnant women, respectively (Odongo, Moturi, & Wangari, 2016). Similarly, in a cross-sectional study involving 299 pregnant or postpartum women, 81 women (27.1%) engaged in pica in the previous 24 h, with 56.8% reporting geophagy, charcoal, and/or ash consumption (Chung, *et al.*, 2019). In South Africa, a study of 200 male and female participants between 18 and 65 years of age, geophagia was reported in 86% of the respondents (Ekosse & Odiyo, 2019). Ivoke *et al*. (2017) reported the proportion of pregnant women who consumed soil during gestation in tropical Nigeria to be 46.4%. In a similar study, Nyanza *et al*. (2014), reported that 65% of pregnant women in Tanzania ate soil 2 to 3 times a day while 13% ate the soil more than 3 times a day. The authors also reported the mean quantity of soil consumed to be 62.5 grams/day. Globally, the available literature shows that the prevalence rate of geophagia varies from place to place, depending on the characteristics of the population studied (Odongo, Moturi, & Wangari, 2016). The findings of this study are in agreement with Unarine *et al. (*2021), Kutalek *et al*. (2016) & Nkansah *et al*. (2016), who reported that the reasons for practicing geophagia include cravings, nutrient supplementation, gastrointestinal disorders, relief from morning sickness and as part of cultural beliefs.

4.3 Non-carcinogenic effects of heavy metals in geophagic products

Risk assessment was done for the potentially toxic elements Pb, As, Cr and Ni. These elements are systemic toxicants and have a strong correlation with human health (Jan *et al*., 2015; Kortei *et al*., 2020).

4.3.1 Lead

In the risk assessment of Pb in geophagic products, the Risk Pert Monte Carlo Distribution (Appendix 2 & 3) was preferred because it gave the best fit after simulating the data. The mean exposure for the consumers and total population was 0.12 and 0.09 µg/KgBw/day, respectively. Because there were no non-detects for Pb, there was no observable difference between the LB, MB and UB for the mean, 50, 75, 90, and 95 percentiles. Therefore, only the UB scenario was considered in this case. The mean MOE at $BMDL_{10}$ of 0.63 µg/BW/day were correspondingly 7.10 and 7.11 µg/KgBw/day for the consumers and total population. The $BMDL_{10}$ of 0.63 µg/KgBw/day is an indicator for the risk of chronic kidney disease in humans (EFSA, 2010). Similarly, at the $BMDL_{01}$ (1.5 µg/KgBw/day), which is an indicator of the risk of cardiovascular disease in humans, the mean MOE was 16.90 and 16.93 µg/KgBw/day for the consumers and total population, respectively. The results showed no pronounced difference between the LB, MB, and the UB scenarios for the mean and max at the 50, 75, 90, 95 and 99 percentile (Table 8). Based on the $BMDL_{01}$ of 1.5 µg/KgBw/day, the European Food Safety Authority (EFSA) CONTAM Panel concluded that a MOE ≥ 10 should be sufficient to ensure no appreciable risk of a clinically significant effect on intelligence quotient; IQ (EFSA, 2010). A MOE between 1.0 and 9.9 means that the population will experience appreciable health risk (EFSA, 2010). At 75th percentile, both consumers and total population had a MOE between 8.79 and 8.88 at $BMDL_{10}=0.63$ µg/KgBw/day, implying that at that percentile, both the consumers and total population are likely to experience risks due to lead exposure in geophagic products.

Table 8: Estimated daily exposure to lead due to consumption of *Emumbwa* at the Upper Boundary (UB) scenario and Margin of exposure at BMLD10=0.63 and BMDL01=1.5 µg/KgBw/day

parameter	Calc. BMDL	Simulated values													
		Mean		Max		P-50		P-75		P-90		P-95		P-99	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2
Exposure	0.12	0.12	0.09	0.47	0.47	0.10	0.08	0.15	0.13	0.21	0.19	0.25	0.24	0.32	0.31
^{a}MOE (BMDL$_{10}$)=0.63	5.26	7.10	7.11	49.22	49.22	6.22	6.22	8.88	8.79	12.35	12.43	15.03	15.25	21.20	21.39
^{b}MOE (BMDL$_{01}$)=1.5	12.52	16.90	16.93	117.20	117.20	14.86	14.82	21.14	20.92	29.40	29.60	35.77	36.31	50.47	50.92

1< ^{a}MOE<9 at BMDL$_{10}$=0.63 µg/KgBw/day is an indicator of potential risk of chronic kidney disease in human.

1< ^{b}MOE<9 at BMDL$_{10}$=1.5 µg/KgBw/day is an indicator of potential risk of cardiovascular disease (CVD).

1. Consumers of geophagic products
2. Total population

Kortei *et al*. (2020) observed a likelihood of adverse health risk in an individual’s lifetime due to consumption of geophagic products. Similarly, Nkansah, Korankye, Darko, & Dodd (2016) observed potential health threat to both children and adults through the consumption of geophagic products over a long time. Chronic exposure to Pb has been associated with neurological, neurobehavioral, immunotoxic and developmental effects in children; neurotoxic effects in adults; hematologic effects; renal toxicity; effects on cardiovascular system (Zhai, Narbad, & Chen, 2015; Olaonipekun, Ekosse, & John, 2020). Lead has also been implicated in having negative effects on the female reproductive system by impairing menstruations, reducing fertility potential, delaying conception time, altering the hormonal production, circulation, and affecting pregnancy and its outcome (Vigeh, Smith, & Hsu, 2011).

4.3.2 Arsenic

In the risk assessment of As, the Risk Triangle Monte Carlo Distribution gave the best fit after simulating the data and was therefore preferred. The mean MOE for the consumers and total population were 503.0 and 501.8, and 251.5 and 250.9 for both MB and UB, respectively. The LB mean values for exposure assessment and MOE were infinitely large. According to EFSA (2020), the Scientific Contam Panel on As concluded that a benchmark dose lower confidence limit ($BMDL_{01}$) between 0.3 µg/KgBw/day and 8 µg/KgBw/day is sufficient to cause cancer of the lungs, skin lesions and cancer of the bladder (Codex, 2019). The results in Table 9 imply that up to the 99 percentile, consumers of geophagic products, and the total population will not experience adverse health effects due to long term exposure to inorganic As. In spite of this, the possibility of a risk to some consumers cannot be excluded (EFSA, 2009).

Table 9: Estimated daily exposure to As and Margin of exposure at BMDL$_{0.5}$=3.0 (µg/KgBw/day) due to consumption of *Emumbwa* at the Lower (LB), Medium (MB) and Upper (UB) Boundary

Parameter	Calc. values	Simulated values													
		Mean		Max		P-50		P-75		P-90		P-95		P-99	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2
Exposure	^{LB}-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	^{MB}0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
	^{UB}0.01	0.01	0.01	0.031	0.03	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.02
MOE(BMDL$_{0.5}$=3.0)	^{LB}-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	^{MB}416.5	503.0	501.8	1050.9	1050.9	448.9	444.6	651.8	655.7	851.7	851.7	942.3	948.8	1026.7	1034.7
	^{UB}208.2	251.5	250.9	525.5	525.5	224.4	222.3	325.9	327.9	425.9	428.4	471.1	474.4	513.4	517.3

A MOE between 0.3 to 8 µg/KgBw/day at BMDL$_{0.5}$=3.0 µg/KgBw/day is an indicator of increased risk of lung cancer, skin lesions and cancer of the bladder (Codex, 2019 &EFSA., 2021).

1. Consumers of geophagic products
2. Total population

4.3.3 Chromium (VI)

According to EFSA (2014), in risk assessment, all Cr is considered to be Cr(VI). The Risk Triangle distribution (Appendix 2 & 3), which gave the best fit, was preferred in the Cr(VI) risk assessment. Just like in the case for Pb, there were no non-detects hence only the UB scenario was considered in this case. The MOE at $BMDL_{0.5}$=0.011, 0.11, 0.2, 0.27 and at $BMDL_{10}$=1.0 for consumers was 0.07, 0.74, 1.35, 1.82 and 6.75, respectively. Similarly, at $BMDL_{0.5}$=0.011, 0.11, 0.2, 0.27 and at $BMDL_{10}$=1.0, the MOE for the total population was correspondingly 0.08, 0.76, 1.37, 1.85 and 6.87. Chromium level at $BMDL_{0.5}$=0.011, 0.11, 0.2, 0.27 µg/KgBw/day signifies neoplastic lesions in the liver, lesions in the duodenum, heamostolic effects and lesions in the pancrease (EFSA, 2014). On the other hand, a $BMDL_{10}$=1.0 µg/KgBw/day is an indicator of combined adenomas and carcinomas (EFSA, 2014). At the $BMDL_{05}$ of 0.2 mg BW per day, EFSA considers that for the critical threshold effects, a MOE larger than 1 would indicate a no concern for public human health hematological effects and non-neoplastic lesions in the pancrease due to Cr(VI). On the other hand, at $BMDL_{10}$=1.0 µg/KgBw/day, a MOE less than100 indicates concern for public human health combined adenomas & carcinomas effects due to Cr(VI) (EFSA, 2014). The results in Table 10 suggest that at 99th percentile, the consumers and their fetus are at a risk of combined adenomas & carcinomas effects due to Cr toxicity as a result of consuming geophagic products. (EFSA, 2014)

Table 10: Estimated daily exposure to chromium (µg/KgBw/day) due to consumption of *Emumbwa* at the Upper Boundary (UB) scenario and Margin of exposure at BMLD$_{0.5}$=0.011, 0.11, 0.2, 0.27 and BMDL$_{10}$= 1.0 µg/KgBw/day

Parameter	Calc. BMDL	Simulated values													
		Mean		Max		P-50		P-75		P-90		P-95		P-99	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2
Exposure	0.23	0.23	0.19	0.98	0.98	0.20	0.16	0.30	0.28	0.43	0.39	0.51	0.49	0.70	0.70
MOE (BMDL$_{0.5}$=0.011)	0.01	0.07	0.08	0.66	0.66	0.06	0.06	0.09	0.09	0.14	0.15	0.19	0.19	0.32	0.33
MOE (BMDL$_{0.5}$=0.11)	0.47	0.74	0.76	6.63	6.63	0.56	0.56	0.87	0.88	1.43	1.46	1.86	1.93	3.25	3.25
MOE (BMDL$_{0.5}$=0.2)	0.86	1.35	1.37	12.05	12.05	1.0	1.02	1.58	1.61	2.59	2.65	3.37	3.51	5.90	5.90
MOE (BMDL$_{0.5}$=0.27)	1.15	1.82	1.85	16.27	16.27	1.34	1.38	2.13	2.17	3.50	3.58	4.55	4.74	7.97	7.97
MOE (BMDL$_{10}$=1.0)	4.28	6.75	6.87	60.25	60.25	4.98	5.11	7.89	8.04	12.98	13.26	16.87	17.55	29.52	29.52

MOE < 1 at BMDL$_{0.5}$ = 0.2 and 0.27 µg/KgBw/day is an indicator of neoplastic heamostolic effects and lesions in the pancrease in rats, respectively. MOE < 100 at BMDL$_{1.0}$ =1.0 µg/KgBw/day is an indicator of combined adenomas and carcinomas in rats. Safety factor =100 to cater for humans.

1. Consumers of geophagic products
2. Total population

4.3.4 Nickel

The Risk Extra value distribution was the preferred fit for the Ni risk assessment. Only the UB scenario was considered in the case of Ni for reasons similar to those outlined for Pb and Ni. The mean MOE at $BMDL_{10}$=0.28 µg/KgBw/day was 11.56 and 11.27 µg/KgBw/day for the consumers and total population, respectively. At $BMDL_{10}$=1.1 µg/KgBw/day the MOE was 30.79 for the consumers and 46.71 µg/KgBw/day for the total population (Table 11). The lowest $BMDL_{10}$ for post-implantation fetal loss in rats has been identified as 0.28 mg/kg BW (EFSA, 2015). On the other hand, for the systemic contact dermatitis (SCD) elicited in Ni-sensitive humans after oral exposure was identified as $BMDL_{10}$ of 1.1 µg Ni/Kg Bw. According to EFSA, a MOE less than 10 is indicative of health risk concern (EFSA, 2015). Therefore, the results in table 11 indicate that while the population may not be at risk of systemic contact dermatitis, at 50th percentile, pregnant women and their fetus who consume geophagic products are at a risk of post-implantation fetal loss.

Table 11: Estimated daily exposure to nickel (µg/KgBw/day) due to consumption of *Emumbwa* at the Upper Boundary (UB) scenario and Margin of exposure at BMLD$_{10}$=0.28 and BMDL$_{10}$=1.1 µg/KgBw/day

Parameter	Calc. BMDL	Simulated values													
		Mean		Max		P-50		P-75		P-90		P-95		P-99	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2
Exposure	0.40	0.04	0.03	0.27	0.23	0.03	0.03	0.05	0.05	0.08	0.07	0.10	0.09	0.15	0.14
MOE (BMDL$_{10}$=0.28)	6.97	11.56	11.27	196.93	196.93	8.62	8.56	13.72	13.56	21.20	21.07	28.91	28.79	61.72	56.41
MOE (BMDL$_{10}$=1.1)	27.38	30.79	46.71	773.64	773.64	33.88	33.61	53.90	53.29	83.27	82.79	113.56	113.11	242.46	221.61

MOE < 10 at BMDL$_{10}$=0.28 µg/KgBw/day is an indicator of risk of post implantation fetal loss.

MOE < 10 at BMDL$_{10}$=1.1 µg/KgBw/day is an indicator of risk of systemic contact dermatitis in rats. Safety factor to cater for humans = 100.

1. Consumers of geophagic products
2. Total population

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Human chronic exposure to toxic metals can lead to their bio-accumulation in the body thereby causing hyper-toxic effects. Determination of the health risks due to heavy metal exposure in geophagic products is therefore an important contribution towards strategies to improve public health in Uganda. From the results of the study, it can be concluded that geophagic products vended and consumed in Kampala city are not safe for human consumption according to population risk distributions. Lead, arsenic, chromium and nickel were major heavy metals and showed potential risks due to consumption of geophagic products. Presence of heavy metals in soil is an indicator of a contaminated environment. There is no pronounced difference in terms of HM concentration among the different products, irrespective of the geographical source. Chronic exposure to heavy metals in geophagic products may lead to adverse health effects and should be given priority as a public health concern. Iron is the predominant element and is in a big excess in geophagic products, irrespective of source and type. Although iron deficiency is a leading cause of nutritional anemia worldwide, too much iron in the clays could be a health hazard.

5.2 Recommendations

A vital and productive society with a prosperous and sustainable future is built on a foundation of healthy child development. Health in the earliest years, beginning with the mother's health before and during pregnant, lays the groundwork for a lifetime of well-being. It is thus recommended that:

1. If geophagic practice must be encouraged, there is need to find a safe method of reducing the heavy metal content and consequently the associated health risks.

2. Sensitization of the target population by health workers about the potential health risks of heavy metals in geophagic products be done.
3. Attention should be paid to the discharge of pollutants into the environment, regular chemical residue monitoring especially in water and soil.
4. Further studies be conducted in respect of the in-vitro bioaccessibility and bioavailability of iron in geophagic products.

REFERENCES

Abrahams, P. W. (1997). Geophagy (soil consumption) and iron supplemenstation in Uganda. *Tropical medicine and International health, 2*(7), 617-623.

Abrahams, P. W., & Parsons, J. A. (1996). Geophagy in the Tropics: A Literature Review. *The Geographical Journal, 162*(1), 63-72.

Amin, M. E. (2005). *Social science research: conception, methodology and analysis.* Kampala: Makerere University.

Anthony, H., Diane, B., Thorhallur, H., Michael, J. J., Katrine, H. K., Simon, M., . . . Josef, R. S. (2017). Use of the Benchmark Dose Approach in Risk Assessment. *Journal European Food Safety Authority, 15*(1), e04658.

Asagba, S. O. (2009). Role of Diet in Absorption and Toxicity of Oral Cadmium-A Review of Literature. *African Journal of Biotechnology, 8*(25), 7428-7436.

Azevedo, B. F., Furieri, L. B., Peçanha, F. M., Wiggers, G. A., Vassallo, P. F., Simões, M. R., . . . Vassallo, D. V. (2012). Toxic Effects of Mercury on the Cardiovascular and Central Nervous Systems. *Journal of Biomedicine and Biotechnology, 2012,* 949048.

Balaman, Ş. Y. (2019). *Uncertainty Issues in Biomass-Based Production Chins.* Elsevier Inc. Pp 113-142. https://doi.org/10.1016/B978-0-12-814278-3.00005-4

Bamuwamye, M., Ogwok, P., & Tumuhairwe, V. (2015). Cancer and Non-cancer Risks Associated With Heavy Metal Exposures from Street Foods: Evaluation of Roasted Meats in an Urban Setting. *Journal of Environment Pollution and Human Health, 3*(2), 24-30 .

Barifaijo, E., Musisi, L. N., Muwanga, A., Nakavuma, J. L., & Opuda-Asibo, J. (2009). Heavy metal loading of wetlands around Lake Victoria in Uganda and its implications to antibiotic resistance in environmental E.coli. *African Journal of Science and Technology,* 10, 64–78.

Bhattacharya, P. T., Misra, S. R., & Hussain, M. (2016). Nutritional Aspects of Essential Trace Elements in Oral Health and Disease: An Extensive Review. *Scientifica, 2016*, 5464373.

Bisi-Johnson, M. A., Obi, C. L., & Ekosse, G. E. (2010). Microbiological and health related perspectives of geophagia. *African Journal of Biotechnology , 9*(19), 5784-5791.

Bisi-Johnson, M. A., Oyelade, H. A., Adediran, K. A., & Akinola, S. A. (2013). Microbial Evaluation of geophagic and cosmetic clays from southern and Western Nigeria, Potential Natural Nanomaterials. *International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering, 7*(12), 832-835.

Bogdan, D., Aneta, L., Marcin, N., Małgorzata, K., Robert, Z., Sławomir, L., & Mariusz, G. (2014). The role of Chromium III in the organism and its possible use in diabetes and obesity treatment. *Annals of Agricultural and Environmental Medicine, 21*(2), 331–335.

Castro-González, M. I., & Méndez-Armenta, M. (2008). Heavy metals: Implications associated to fish consumption. Environmental Toxicology & Pharmacology. *Environmental Toxicology and Pharmacology, 26*(3), 263-271.

Chen, P., Bornhorst, J., & Aschner, M. (2018). Manganese metabolism in humans. *Frontiers In Bioscience, Landmark, 23*, 1655-1679.

Chen, Z., Myers, R., Wei, T., Bind, E., Kassim, P., Wang, G., . . . Wang, X. (2014). Placental transfer and concentrations of cadmium, mercury, lead, and selenium in mothers, newborns, and young children. *Journal of Exposure Science & Environmental Epidemiology, 24*(5), 537–544.

Chopra, A. K., Pathak, C., & Prasad, G. (2009). Scenario of heavy metal contamination in agricultural soil and its management. *Journal of Applied and Natural Science, 1*(1), 99-108.

Chung, E. O., Mattah, B., Hickey, M. D., Salmen, C. R., Milner, E. M., Bukusi, E. A., . . . Fiorella, K. J. (2019). Characteristics of Pica Behavior among Mothers around Lake Victoria, Kenya: A Cross-Sectional Study. *International Journal of Environmental Research and Public Health, 16*(14), 2510.

Costa, M., & Klein, C. B. (2006). Toxicity and carcinogenicity of chromium compounds in humans. *Critical Reviews in Toxicology , 36*(2), 155-163.

Davoodi, S. H., Jamshidi-Naeini, Y., Esmaeili, S., Sohrabvandi, S., & Mortazavian, A. M. (2016). The Dual Nature of Iron in Relation to Cancer: A Review. *Iranian Journal of Cancer Prevention, 9*(6), e5494.

Delcour, I., Rademaker, M., Jacxsens, L., Win, J. D., Baets, B. D., & Spanoghe, P. (2015). A risk-based pesticide residue monitoring tool to prioritize the sampling of fresh produce. *Food Control, 50*, 690-698.

Deng, Y., Wang, M., Tian, T., Lin, S., Xu, P., Zhou, L., . . . Dai, Z. (2019). The Effect of Hexavalent Chromium on the Incidence and Mortality of Human Cancers: A Meta-Analysis Based on Published Epidemiological Cohort Studies. *Frontiers in Oncology, 9*: 24.

Denil, D. J., Fui, C. F., & Ransangan, J. (2017). Health Risk Assessment Due to Heavy Metal Exposure via Consumption of Bivalves Harvested from Marudu Bay, Malaysia. *Open Journal of Marine Science, 7*, 494-510.

Desai, V., & Kaler, S. G. (2008). Role of copper in human neurological disorders. *The American Journal of Clinical Nutrition, 88*(3), 855S–858S.

Diko, M. L., & Siewe épse, D. C. (2014). Physico-chemistry of geophagic soils ingested to relief nausea and vomiting during pregnancy African Journal of Traditional, Complementary and Alternative Medicines, 11(3), 21-24.

EFSA (European Food Safety Agency). (2005). Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *The EFSA Journal*, 282, 1-31.

EFSA (European Food Safety Agency). (2014). Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water. *EFSA Journal*, 12(3):3595

EFSA. (2015). Scientific Opinion on the risks to public health related to the presence of nickel in food and drinking water.

Egwurugwu, J. N., Ufearo, C. S., Abanobi, O. C., Nwokocha, C. R., Duruibe, J. O., Adeleye, G. S., . . . Onwufuji, O. (2007). Effects of ginger (Zingiber officinale) on cadmium toxicity. *African Journal of Biotechnology, 6*(18), 2078-2082.

Ekosse, G.-I. E., Ngole-Jeme, V. M., & Diko, M. L. (2017). Environmental Geochemistry of Geophagic Materials from Free State Province in South Africa. *Open Geosciences, 9*, 114–125.

Ekosse, M. V., & Odiyo, J. O. (2019). The prevalence of geophagic practices and causative reasons for geophagia in Sekhukhune area, Limpopo Province, South Africa. *Transactions of the Royal Society of South Africa , 74*(1), 19-26.

Fernández-Luqueño, F., López-Valdez, F., Gamero-Melo, P., Luna-Suárez, S., Aguilera-González, E. N., Martínez, A. I., . . . Pérez-Velázquez, I. R. (2013). Heavy metal pollution in drinking water - a global risk for human health: A review. *African Journal of Environmental Science and Technology, 7*(7), 567-584.

Fonseca-Nunes, A., Jakszyn, P., & Agudo, A. (2013). Iron and Cancer Risk—A Systematic Review and Meta-analysis of the Epidemiological Evidence. *Cancer Epidemiol Biomarkers Prevention, 23*(1), 12–31.

Fosso-Kankeu. E, Netshitanini. T, L., Abia. A. L. K, Ubomba-Jaswa.E, & Waanders. F. B. (2015). *Application of solar treatment for the disinfection of geophagic products from markets and mining sites. African Journal of Biotechnology, 14*(50), 3313-3324.

Fuhrimann, S., Stalder, M., Winkler, M. S., Niwagaba, C. B., Babu, M., Masaba, G., & Cissé, G. (2015). Microbial and Chemical Contamination of Water, sediment and Soil in the Nakivubo Wetland Area in Kampala, Uganda. *Environmental Monitoring and Assessment, 187*(7), 475.

García-Esquinas, E., Pollan, M., Tellez-Plaza, M., A, K., Francesconi, Goessler, W., . . . Navas-Acien, A. (2014). Cadmium Exposure and Cancer Mortality in a Prospective Cohort: the strong heart study. *Environmental Health Perspectives, 122*(4), 363-370.

Gedikoglu, Y., Gedikoglu, G., Berkin, G., Ceyhan, T., & Altinoz, M. A. (2012). Employing volcanic tuff minerals in interior architecture design to reduce microbial contaminants and airborne fungal carcinogens of indoor environments. *Toxicology and Industrial Health, 28*(8), 708-719.

George, G., & Ndip, E. (2011). Prevalence of Geophagia and its possible implications to health – A study in rural South Africa. *2nd International Conference on Environmental Science and Development. 4*, pp. 166-169. IACSIT Press, Singapore.

Gerba, C. P., & Smith, J. E. (2005). Sources of Pathogenic Microorganisms and Their Fate during Land Application of Wastes. *Journal of Environmental Quality, 34*(1), 42-48.

Gomes, C. D. (2017). Healing and edible clays: A review of basic concepts, benefits and risks. *Environmental Geochemistry and Health, 40*(5), 1739-1765.

He, Z., Yang, X. E., & Stoffella, P. J. (2005). Trace Eelements in Agroecosystems and Impacts on the Environment. *Journal of Trace Elements in Medicine and Biology, 19*(2-3), 25-140.

Hueb, L., Leick, S., Guettl, L., Akello, G., & Kutalek, R. (2016). Geophagy in Northern Uganda: Perspectives from Consumers and Clinicians. *American Society of Tropical Medicine and Hygiene, 95*(6), 1440–1449.

Ishmael, D. N., Fred, N. B., & Anthony, H. G. (2015). Geophagia: A cultural-nutrition health seeking behaviour with no redeeming with no redeeming psycho-social qualities. South Eastern European Journal of Public Health (SEEJPH), 2015, 1-13.

Ivoke, N., Ikpor, N., Ivoke, O., Ekeh, F., Ezenwaji, N., Odo, G., . . . Eyo, J. (2017). Geophagy as risk behaviour for gastrointestinal nematode infections among pregnant women attending antenatal clinics in a humid tropical zone of Nigeria. *African Health Sciences, 17*(1), 24-31.

Järup.L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin, 68,* 167-182.

Jeffery, S., & van-der-Putten, W. (2011). *Soil Borne Diseases of Humans.* Luxembourg: European Union.

Kambunga, S. N., Candeias, C., Hasheela, I., & Mouri, H. (2019). Review of the nature of some geophagic materials and their potential health effects on pregnant women: some examples from Africa. *Environmental Geochemistry and Health , 41*, 2949–2975.

Kawai, K., Saathoff, E., Antelman, G., Msamanga, G., & Fawzi, W. W. (2009). Geophagy (Soil-eating) in Relation to Anemia and Helminth Infection Among HIV-Infected Pregnant Women in Tanzania. *American Society of Tropical Medicine and Hygiene, 80*(1), 36–43.

Kortei, N. K., Koryo-Dabrah, A., Akonor, P. T., Manaphraim, N. Y., Ayim-Akonor, M., Boadi, N. O., & Tettey, E. K. (2020). Potential health risk assessment of toxic metals contamination in clay eaten as pica (geophagia) among pregnant women of Ho in the Volta Region of Ghana. *BMC Pregnancy and Childbirth* 20:160.

Kutalek, R., Wewalka, G., Gundacker, C., Auer, H., Wilson, J., Haluza, D., . . . Prinz, A. (2010). Geophagy and Potential Health Implications: Geohelminths, Microbes and Heavy Metals. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 104*(12), 787-795.

Lar, U. A., Agene, J. I., & Umar, A. I. (2015). Geophagic clay materials from Nigeria: a potential source of heavy metals and human health implications in mostlywomen and children who practice it. *Environmental Geochemistry and Health*, 37, 363–375.

Lavicoli, I., Fontana, L., & Bergamaschi, A. (2009). The Effects of Metals as Endocrine Disruptors. *Journal of Toxicology and Environmental Health, 12*(3), 206-223.

Liu, J., Lu, Y., Wu, Q., Goyer, R., & Waalkes, M. (2008). Mineral arsenicals in traditional medicines: orpiment, realgar, and arsenolite. *Journal of Pharmacology and Experimental Therapeutics, 326*(2), 363-368.

Mbabazi, J., & Wasswa, J. (2010). Contamination by heavy metals in silver fish (Rastreneobola argentea) caught from Lakes Kyoga and Victoria, Uganda. *International Journal of Environmental Studies*, *67*(4), 543-556.

Mehri, A., & Marjan, R. F. (2013). Trace Elements in Human Nutrition. *International journal of medical investigation, 2*(3), 115-128.

Miller, J. D., Collins, S. M., Omotayo, M., Martin, S. L., Dickin, K. L., & Young, S. L. (2018). Geophagic earths consumed by women in western Kenya contain dangerous levels of lead, arsenic, and iron. *American Journal of Human Biology, 30,* e23130.

Mishra, B. B., & Roy, R. (2015). Soil Science Vs Science for Medicine. *EC Agriculture, 2*(5), 454-461.

Mohammadi, A. A., Zarei, A., Majidi, S., Ghaderpoury, A., Hashempour, Y., Saghi, M. H., . . . Ghaderpoori, M. (2019). Carcinogenic and non-carcinogenic healthrisk assessment of heavy metals in drinking water of Khorramabad, Iran. *MethodsX, 6*, 1642-1651.

Morais, S., Costa, F. G., & De Lourdes Pereira, M. (2012). Heavy metals and human health. In Environmental health-emerging issues and practice. 228-246.

Mudassir, I. Z., Asrar, A., Arsala, M., & AFarooqui, M. (2005). The Heavy Metal Concentration along Roadside Trees of Quetta and its Effects on Public Health. *Journal of Applied Sciences, 5*(4), 708-711.

Muwanga, A., & Barifaijo, E. (2006). Impact of Industrial Activities on Heavy metal Loading and Their Physio-Chemical Effects on Wetlands of Lake Victoria Basin (Uganda). *African Journal of Science and Technology, 7*(1), 51-63.

Mwesigye, A. R., Young, D. S., Bailey, H. E., & Tumwebaze, S. B. (2016). Population Exposure to Trace Elements in the Kilembe Copper Mine Area, Western Uganda: A pilot Study. *Science of The Total Environment, 573*(15), 366-375.

Nabulo, G., Oryem-Origa, H., Nasinyama, G. W., & Cole, D. (2008). Assessment of Zn, Cu, Pb and Ni Contamination in Wetland Soils and Plants in the Lake Victoria Basin. *International Journal of Environmental Science & Technology, 5*(1), 65-74.

Nankinga, O., & Aguta, D. (2019). Determinants of Anemia among women inUganda: further analysis of the Ugandademographic and health surveys. *BMC Public Health*, 1757.

Navas-Acien, A., Sharrett, A. R., Silbergeld, E. K., Schwartz, B. S., Nachman, K. E., Burke, T. A., & Guallar, E. (2005). Arsenic Exposure and Cardiovascular Disease: A Systematic

Review of the Epidemiologic Evidence. *American Journal of Epidemiology, 162*(11), 1037–1049.

Ndze, D. J. (2013). Geophagic materials: the possible effects of their chemical composition on human health. *Transactions of the Royal Society of South Africa*, *68*(3), 177-182.

Ngole-Jeme, V. M., Ekosse, G.-I. E., & Songca, S. P. (2018). An analysis of human exposure to trace elements from deliberate soil ingestion and associated health risks. *Journal of Exposure Science & Environmental Epidemiology, 28*, 55–63.

Nkansah, M. A., Korankye, M., Darko, G., & Dodd, M. (2016). Heavy Metal Content and Potential Health Risk of Geophagic White Clay From Kumasi Metropolis in Ghana. *Toxicology Reports*, 3, 644–651;

Nyakairu, G. W., Muhwezi, G., & Biryomumaisho, S. (2011). Assesment of Heavy Metals in Milk From Selected Dairy Farms and Shops in Wakiso District. *Suza Journal of Naturaal and Social Science, 1*(1), 36-52.

Nyanza, E. C., Joseph, M., Premji, S. S., Thomas, C., & Mannion, D. S. (2014). Geophagy Practices and the Content of Chemical Elements in the Soil Eaten by Pregnant Women in Artisanal and Small Scale Gold Mining Communities in Tanzania. *BMC Pregnancy and Childbirth*, *14*:144.

O’Neal, S. L., & Zheng, W. (2015). Manganese Toxicity Upon Overexposure: a Decade in Review. *Current Environmental Health Reports*, *2*(3), 315–328.

Odewumi, S. C. (2013). Mineralogy and geochemistry of Geophagic products from the Share Area Northern Bida .Sedimentary Basin, Nigeria. *2*(1), 1-6.

Odongo, A. O., Moturi, W. N., & Wangari, S. N. (2016). Geophagic behaviour and factors influencing it among pregnant women: a case study of Nakuru Municipality, Kenya. *International Journal of Behavioural and Healthcare Research, 6*(1), 28–41.

Ogah, S. P., & Ikelle, I. I. (2015). The determination of the amount of some heavy metals in edible clay of Enyigba village in Abakaliki Ebonyi State, Nigeria. *Der Pharma Chemica, 7*(11), 264-267.

Oribhabor, B. J., & Ogbeibu, A. E. (2010). Concentration of Heavy Metals in Niger Delta Mangrove Creek, Nigeria. *Global Journal of Environmental Sciences, 8*(2), 1-10.

Papastergiadis, A., Jacxsens, L., Fatouh, A., Lachat, C., Shrestha, K., Daelman, J., & De Meulenaer, B. (2014). Exposure assesment of Malondialdehyde, 4-Hydroxy-2-(E)-Nonenal and 4-Hydroxy-2-(E)-Hexenal through specific foods available in Belgium. *73*, 51-58.

Patterson, R. E., & Pietinen, P. (2007). Assessment of Nutritional Status in Individuals and Populations. Dans M. J. Gibney, B. M. Margetts, J. M. Kearney, & L. Arab (Éds.), *Public Health Nutrition* (pp. 66-82). Kent: Blackwell Science.

Pizent, A., Tariba, B., & Živković, T. (2012). Reproductive toxicity of metals in men. *Archives of Industrial Hygiene and Toxicology, 63*(1), 35-46.

Plum, L. M., Rink, L., & Haase, H. (2010). The Essential Toxin: Impact of Zinc on Human Health. *International Journal Environment Research Public Health, 7*, 1342-1365.

Pratt, I., & Barlow, S. (2009). The Influence of Thresholds on the Risk Assessment of Carcinogens in Food. *Mutation Research, 678*(2), 113-117.

Preeti, T. B., Satya, R. M., & Mohsina, H. (2016). Nutritional Aspects of Essential Trace Elements in Oral Health and Disease: An Extensive Review. *Scientifica*, 2016:5464373.

Rice Jr., K. M., Walker, W. E., Wu, M., Gillette, C., & Blough, E. R. (2014). Environmental Mercury and Its Toxic Effects. *Journal of Preventive Medicine and Public Health, 47*(2), 74–83.

Richardson, G. M. (2010). Deterministic Versus Probabilistic Risk Assessment: Strengths and Weaknesses in a Regulatory Context. *Human and Ecological Risk Assessment: An International Journal , 2*(1), 44-54.

Rivera-Velasquez, Fallico, C., Guerra, L., & Straface, S. (2013). A Comparison of deterministic and probabilistic approaches for assessing risks from contaminated aquifers: An Italian case study. *Waste Management & Research, 31*(12), 1245-1254.

Rodríguez, J., & Mandalunis, P. M. (2018). A Review of Metal Exposure and Its Effects on Bone Health. *Journal of Toxicology, 2018:*4854152.

Roohani, N., Hurrell, R., Kelishadi, R., & Schulin, R. (2013). Zinc and its importance for human health: An integrative review. *Journal of Research in Medical Sciences, 18*(2), 144–157.

Satoh, M., Koyama, H., Kaji, T., Kito, H., & Tohyama, C. (2002). Perspectives on Cadmium Toxicity Research. *The Tohoku Journal of Experimental Medicine, 196*(1), 23-32.

Schneider, B., Constant, S. L., Patierno, S., Jurjus, R., & Ceryak, S. (2012). Exposure to Particulate Hexavalent Chromium Exacerbates Allergic Asthma Pathology. *Toxicology and Applied Pharmacology, 1,* 38–44.

Schwander, S., Okello, C. D., Freers, J., Chow, J. C., Watson, J. G., Corry, M., & Meng, Q. (2014). Ambient Particulate Matter Air Pollution in Mpererwe District, Kampala, Uganda: A Pilot Study. *Journal of Environmental and Public Health, 2014*: 763934.

Schümann, K., Borch-Iohnsen, B., Hentze, M. W., & Marx, J. J. (2002). Tolerable upper intakes for dietary iron set by the US Food and Nutrition Board. *The American Journal of Clinical Nutrition*, *76*(3), 499–500.

Seim, I., Fang, X., Xiong, Z., Lobanov, A. V., Huang, Z., Ma, S., . . . Gladyshev, V. N. (2013). Genome Analysis Reveals Insights into Physiology and Longevity of the Brandt's Bat Myotis Brandtii. *Nature Communications,* 4:221.

Shakoor, M. B., Farid, M., Ali, S., & Farooq, M. A. (2013). Heavy Metal Pollution, A global Problem and its Remediation by Chemically Enhanced Phytoremediation. *Journal of Biodiversity and Environmental Sciences, 3*(3), 12-20.

Silbergeld, E. K., & Nachman, K. (2008). The Environmental and Public Health Risks Associated with Arsenical Use in Animal Feeds. *Annals of the New York Academy of Sciences*, *1140*, 346–357.

Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy Metals and Living Systems: An Overview. *Indian Journal of Pharmacology, 43*(3), 246-53.

Steffan, J. J., Brevik, E. C., Burgess, L. C., & Cerdà, A. (2018). The effect of soil on human health: an overview. *European Journal of Soil Science*, *69*(1), 159–171.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Clinical and Environmental Toxicology, 101*, 133-164.

Țugui, C., Szekeres, E., & Baricz, A. (2017). Sources and Mechanisms of Combined Heavy-Metal and Antibiotic Resistance Traits in Bacteria. Studia Universitatis Babeş-Bolyai Biologia*, LXII*(1), 101-114.

USEPA. (2001). *Toxics Release Inventory: Public Data Release Report. USEPA: Washington, DC, USA.*

Usman, K., Al-Ghouti, M. A., & Abu-Dieyeh, M. H. (2019). The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant Tetraena qataranse. *Scientific Reports, 9*:5658.

Uusitalo, L., Lehikoinen, A., Helle, I., & Myrberg, K. (2015). An overview of methods to evaluate uncertainty of deterministic models in decision support. *Environmental Modelling & Software, 63*, 24-31.

Van-der-Fels-Klerx, H. J., Asselt, E. D., Raley, M., Poulsen, M., Korsgaard, H., Bredsdorff, L., . . . Frewer, L. J. (2018). Critical review of methods for risk ranking of food-related hazards, based on risks for human health. *Critical Reviews in Food Science and Nutrition, 58*(2), 178-193.

Vaumourin, E., Vourc'h, G., Gasqui, P., & Vayssier-Taussat, M. (2015). The importance of multiparasitism: examining the consequences of co-infections for human and animal health. *Parasites & Vectors , 8,* 545.

Vermeire, M.-L., Cornélis, J.-T., Ranst, E. V., Bonneville, S., Doetterl, S., & Delvaux, B. (2018). Soil Microbial Populations Shift as Processes Protecting Organic Matter Change During Podzolization. *Frontiers in Environmental Science,* 6:70.

Vidyavati, S. D., Sneha, A., & Katti, S. M. (2016). Zinc: The Importance in Human Life. *International J. of Healthcare and Biomedical Research, 4*(4), 18-20.

Vieira, C., Morais, S., Ramos, S., Delerue-Matos, C., & Oliveira, M. B. (2011). Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra- and inter-specific variability and human health risks for consumption. *Food & Chemical Toxicology, 49*(4), 923-932.

Vigeh, M., Smith, D. R., & Hsu, P.-C. (2011). How does lead induce male infertility? *Iranian Journal of Reproductive Medicine , 9*(1), 1-8.

Vinci, R. M., Jacxsens, L., Loco, J. V., Matsiko, E., Lachat, C., Schaetzen, T., . . . De Meulenaer, B. (2012). Assessment of human exposure to benzene through foods from the Belgian market. *Chemosphere, 88*, 1001-1007.

Williams, L. B., Haydel, S. E., & Ferrell-Jr., R. E. (2009). Bentonite, Bandaids and Borborygmi. *Elements, 5*, 99-104.

Woode, A., & Hackman-Duncan, S. F. (2014). Risks associated with geophagia in Ghana. *Canadian Journal of Pure and Applied Sciences, 8*(1), 2789-2794.

Zahir, F., Rizwi, S., Haq, S. K., & Khan, R. H. (2005). Low dose mercury toxicity and human health. *Environmental Toxicology and Pharmacology*, *20*(2), 351-60.

Zhai, Q., Narbad, A., & Chen, W. (2015). Dietary Strategies for the Treatment of Cadmium and Lead Toxicity. *Nutrients , 7*, 552-571.

Zhuang, P., Li, Z. A., McBride, M. B., & Zou, B. &. (2013). Health risk assessment for consumption of fish originating from ponds near Dabaoshan mine, South China. *Environmental Science and Pollution Research*, 20(8), 5844-5854.

Zolezzi, M., Cattaneo, C., & Tarazona, J. (2005). Probabilistic Ecological Risk Assessment of 1,2,4-Trichlorobenzene at a Former Industrial Contaminated Site. *Environtal Science and Technology, 39*(9), 2920-2926.

APPENDICES

Appendix 1: Survey questionnaire on geophagic products making, consumer choices, and consumption patterns in Kampala city (UGANDA)

Dear Sir/Madam

My name is Justine Nabuuma, a student of Kyambogo University pursuing a Master's Degree in Food Technology. I am conducting a study entitled **"Geophagic products choices, consumption and manufacturing in Kampala (Uganda)".** I kindly request you to fill in this questionnaire to best of your knowledge as your responses will provide important and useful information that will guide the study. The information provided will be treated with confidentiality and shall be used for academic purposes only. Thank you.

SECTION 1: IDENTIFICATION INFORMATION *Please fill in the spaces provided/*			
1.1	Participant ID	1.3	District
1.2	Interview date __/__/__ (dd/mm/yy)	1.4	Division
SECTION 2: DEMOGRAPHY *Please tick the right response*			
2.1	Gender of the respondent	1=Male 2=Female	
2.2	Age of the respondent	1= 16-34 2= 35-54 3= >55	
2.3	Body weight (kg)	1= 40-60 2=60-80 3=80-100	
2.4	Education level	1=Primary school	

		2=Secondary school 3= Tertiary Institutions 4= University degree 5= Master degree/PhD 6= Other (Specify)
2.5	Income status?	1 = <1,000,000 2 = 1,000,000-5,000,000 3 = > 5,000,000
SECTION 3: PURCHASING AND CONSUMPTION PATTERNS *Please tick the right response*		
3.1.	Do you consume geophagic products? (Emumbwa)?	0=No 1=Yes
3.2	If **Yes**, how often do you consume geophagic products (Emumbwa)?	1= 1 to 3 times 3= Daily 2= 4 to 6 times 99= Others (specify)……..
3.3	How much of geophagic products do you usually consume drink in a day?	1= Fewer than 1 2= 1 to 2 cups 3= 3 or more
3.4	At what times of the day do you usually drink geophagic product solution?	1= Breakfast 4= In between meals 2= Lunch 99 = Other (specify) 3= Dinner
3.5	How many types of geophagic products do you usually buy?	Please specify………………………………
3.6	Where do you normally buy the geophagic products (*emumbwa*)? ***Do not read options aloud, probe for as many options as the respondent can remember*** ***Record all given answers***	1= Herbalists 2. Traditional Birth attendants (TBA) 3= Supermarket 4= Market 5= Delivered 6= Others (Specify)

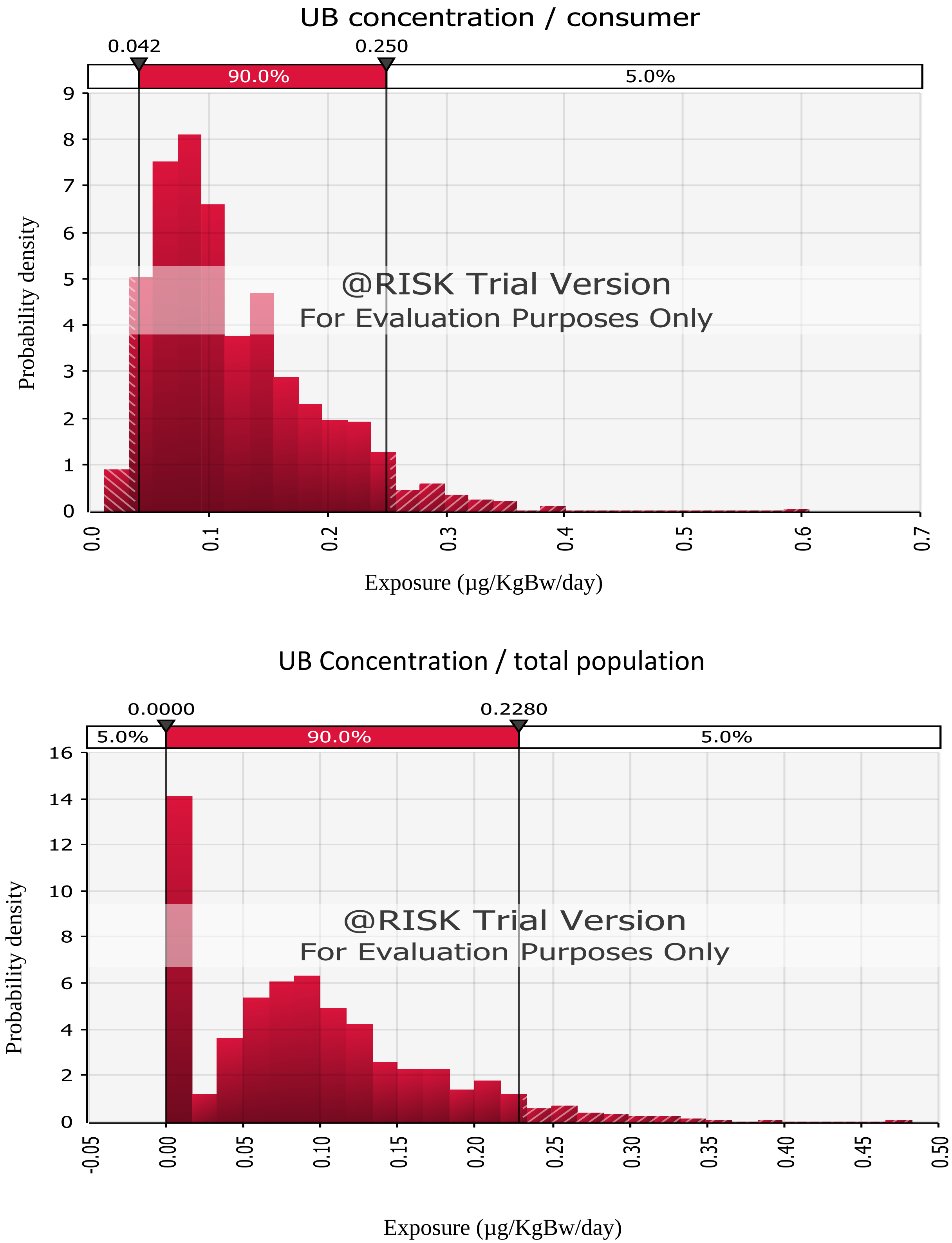
3.8	Why do you buy at that particular sell point?	1 = Close to home 2 = Do other shopping there 3 = Freshness 4 = Because it is cheap there 5 = Has the geophagia type I like 6 = Wide selection 7 = Enjoyment/social outing 8 = Other (specify)
3.9	For what purpose do you buy/consume geophagic products?	1 = Softening the bones (*okumenya*) 2= Prevention/treatment of morning sickness 3=prevention of delivery complications 4= prevention of child abnormalities 5=source of iron (prevention of anemia) 6= General well-being of the pregnancy 7 = Don't know 8 = Other (Specify)
3.10	What factors do you put into consideration when you're choosing geophagic products?	1 = The shape 2= Producer of geophagic products 3= Price 4= Ingredients 5= Size of the geophagia 6= It doesn't matter 7= I always buy the same geophagia 8= Origin 9 = The freshness 10 = Attractiveness

		11= Other (specify)………………………….
3.11	Do you always buy the same brand of geophagic products each time you are pregnant?	1= Always 2= Nearly always 3= Sometimes 4= No
3.13	What are the number of years during which the respondent has been eating products	1. Less than 2 years 2. 2-5 years 3. 3.More than 10 years
3.14	Are there other members of the house hold who consume these products	1. Yes 2. No
3.15	Do you consume other products	1. Yes 2. No If yes, please specify.
3.16	How many children do you have?	Please specify………………………………
3.17	Do you only consume geophagic products when pregnant or even after giving birth	1. Yes 2. No
3.18	What brand names can you name that are mostly consumed? **Record sequence of correct recall.**	
	SECTION 4: SELLERS/MANUFACTURERS *Please tick the right response and specify in the spaces provided*	
4.1	Do you make your own *Emumbwa*?	0 = No 1 = Yes
4.2	If *yes*, how often do you make a batch?	1 = Every few days 4 = Monthly 2 = Weekly 5 = 2 – 3 months 3 = Fortnightly 6 = Other (specify)
4.3	Do you ever use geophagic products yourself?	0 = No 1 = Yes
4.4	What are the sources of geophagic produucts that you use	Please specify.

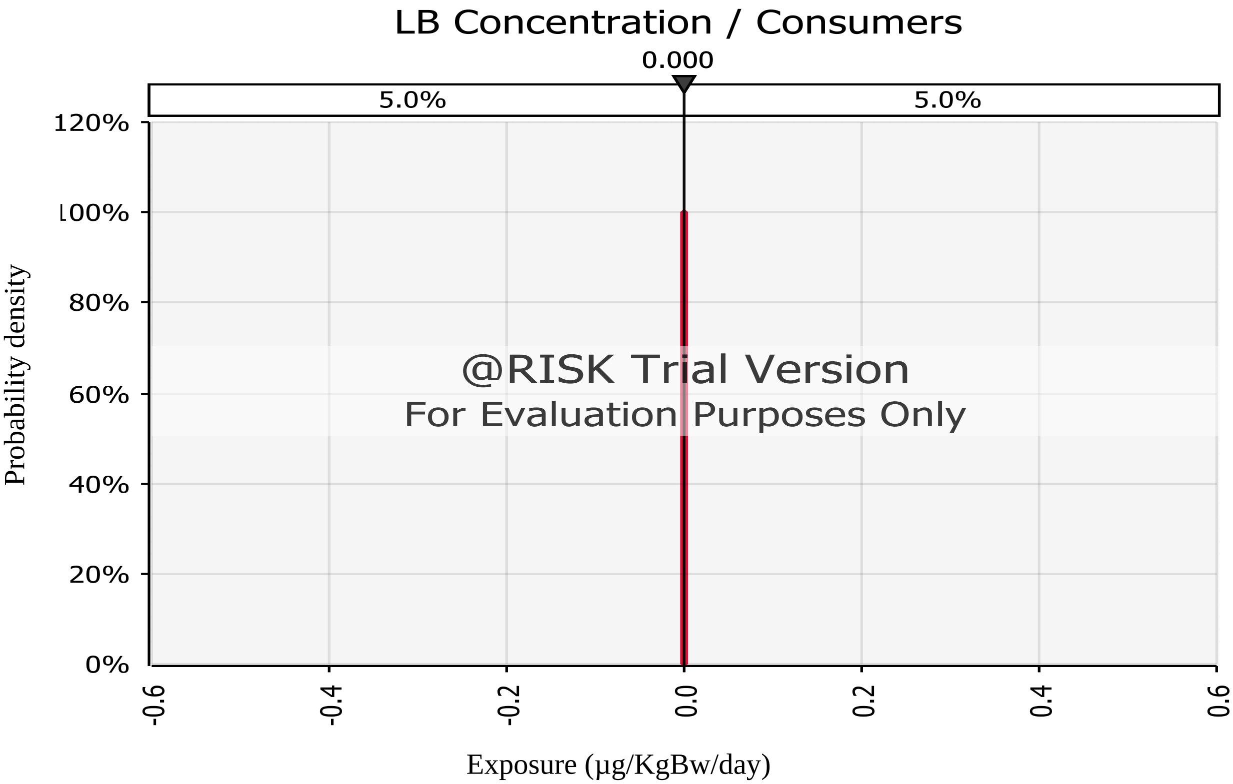
4.5	What are the sources of herbs that are used during the manufacture of geophagic products	1.forests 2. swamps 3. villages 4. herbalists
4.6	Could you be knowing some of the names of the local herbs that are being used during the manufacture of the geophagic products (emumbwa)	Please specify as many herbs as possible…………………………
4.7	Where do you sell the geophagic products; *Emumbwa*	1. Markets 2. herbalists 3. Traditional birth attendants 4. Health centers
4.8	What is your production capacity per month	Please specify……………………………….
4.9	What other ingredients you use during their manufacture?	
4.10	What kind/class of people buy the geophagic products	1. Educated 2. Semi educated 2. Not Educated

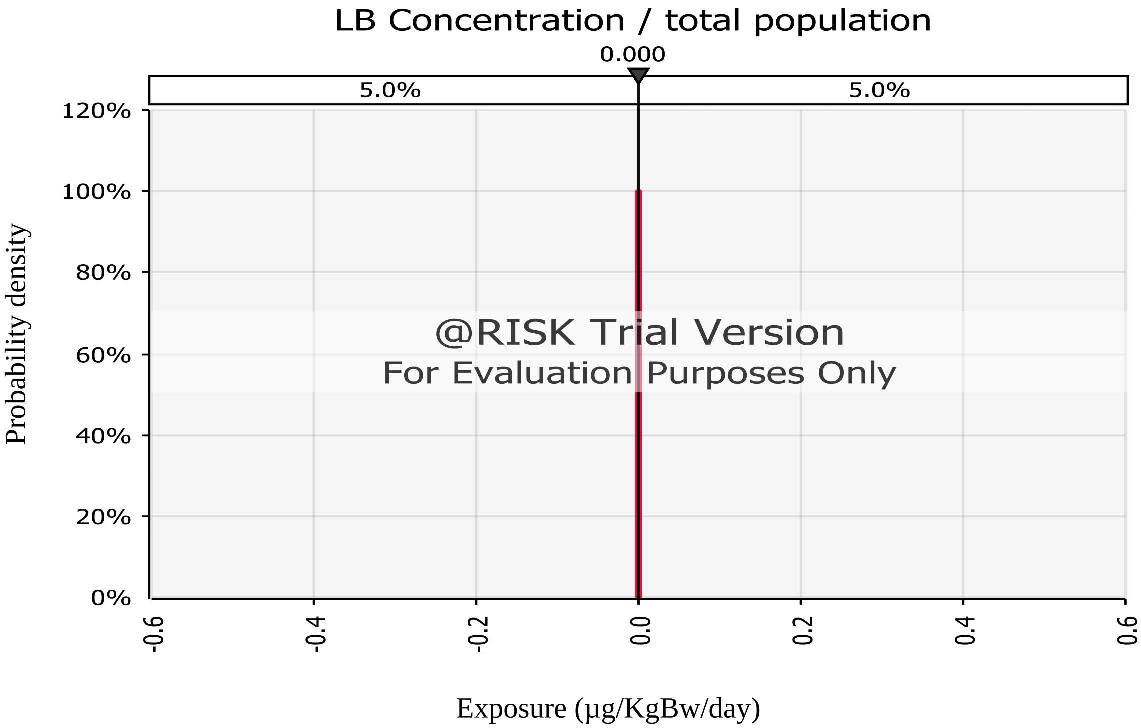
INTERVIEWER: This is the end of the interview. Thank you very much for your participation!

Appendix 2: Exposure assessment graphs

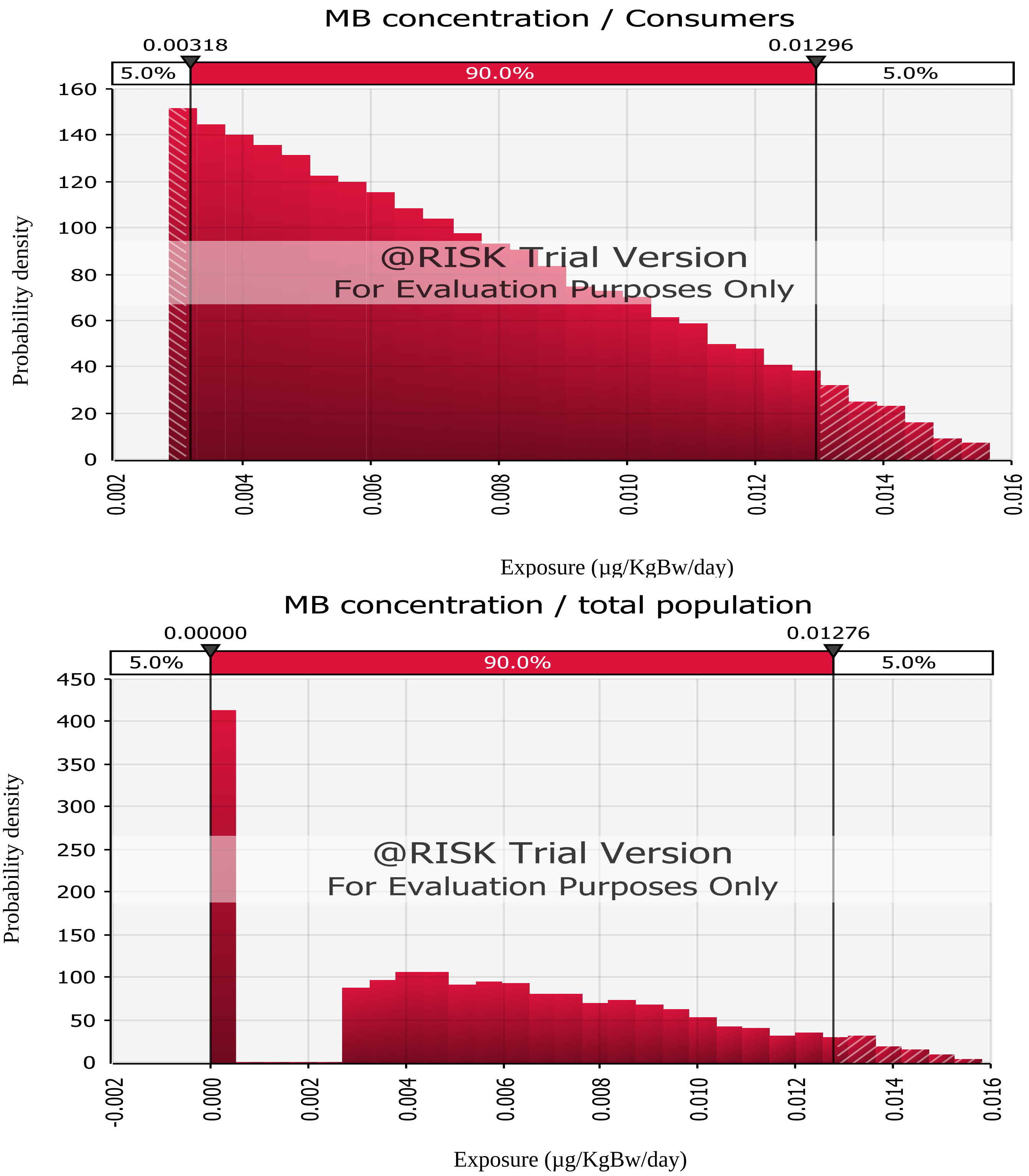


Exposure assessment graphs of lead at upper bound scenario for consumers and total population respectively.

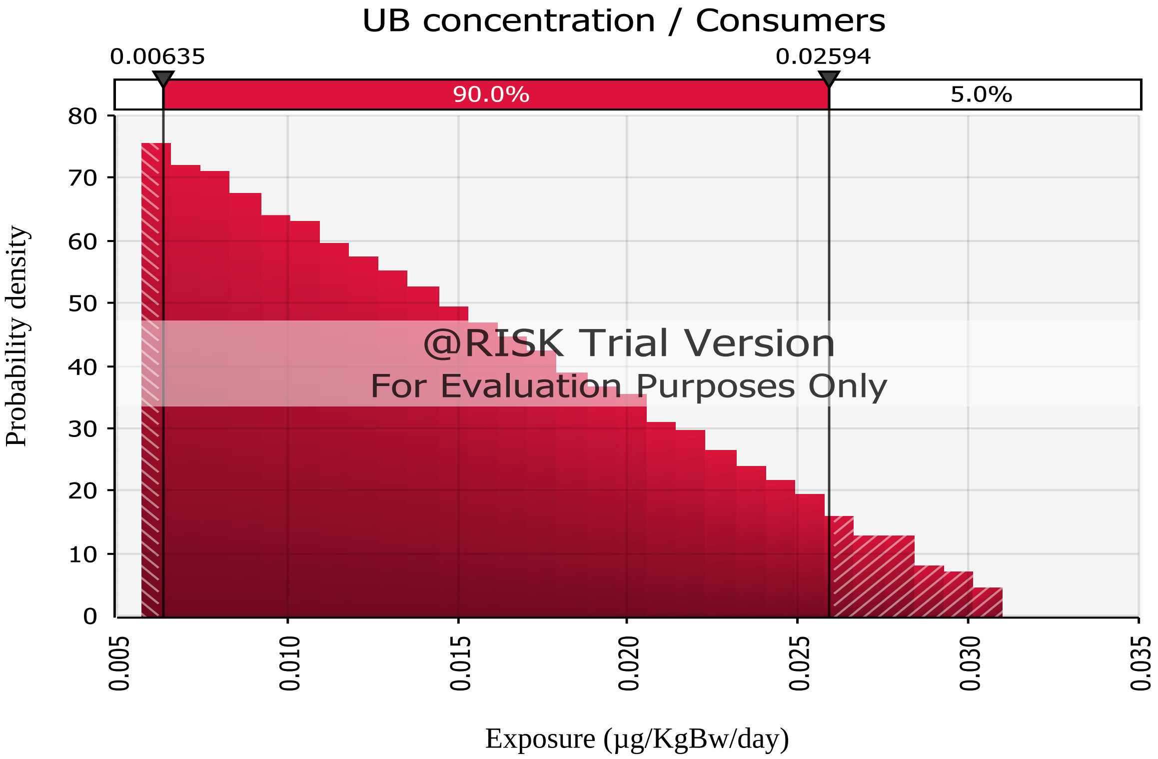


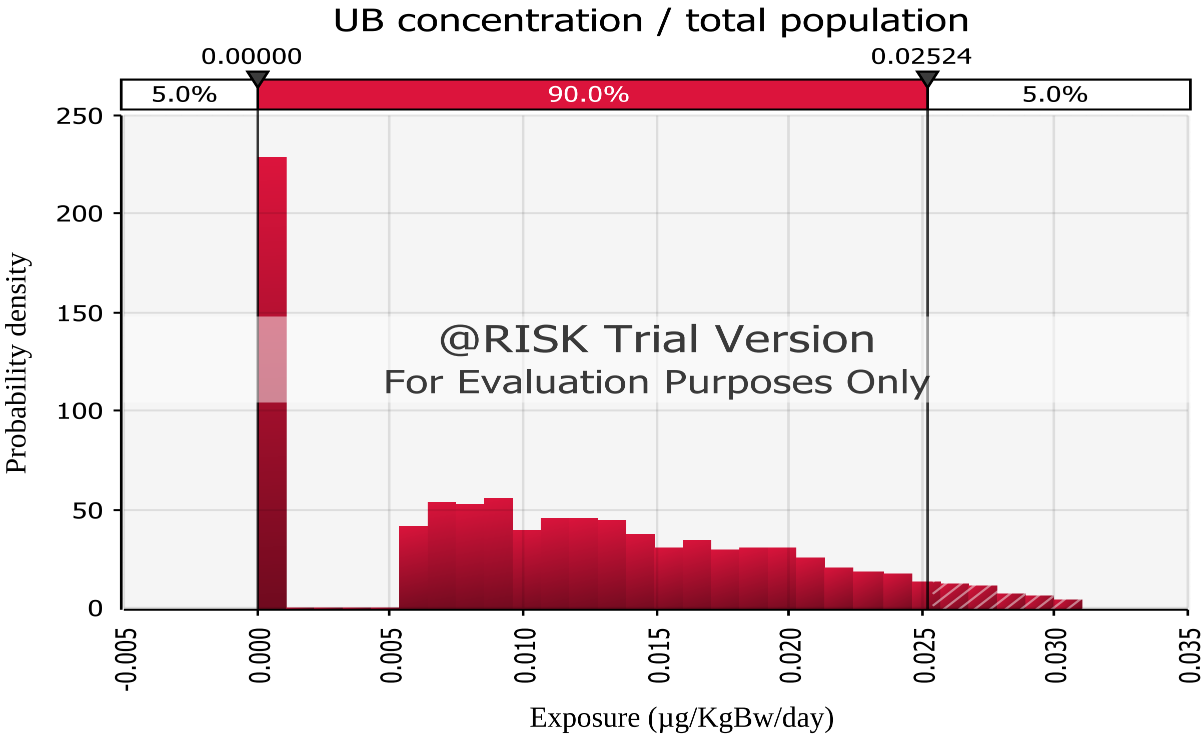


Exposure assessment graphs of arsenic at lower bound scenario for consumers and total population respectively.

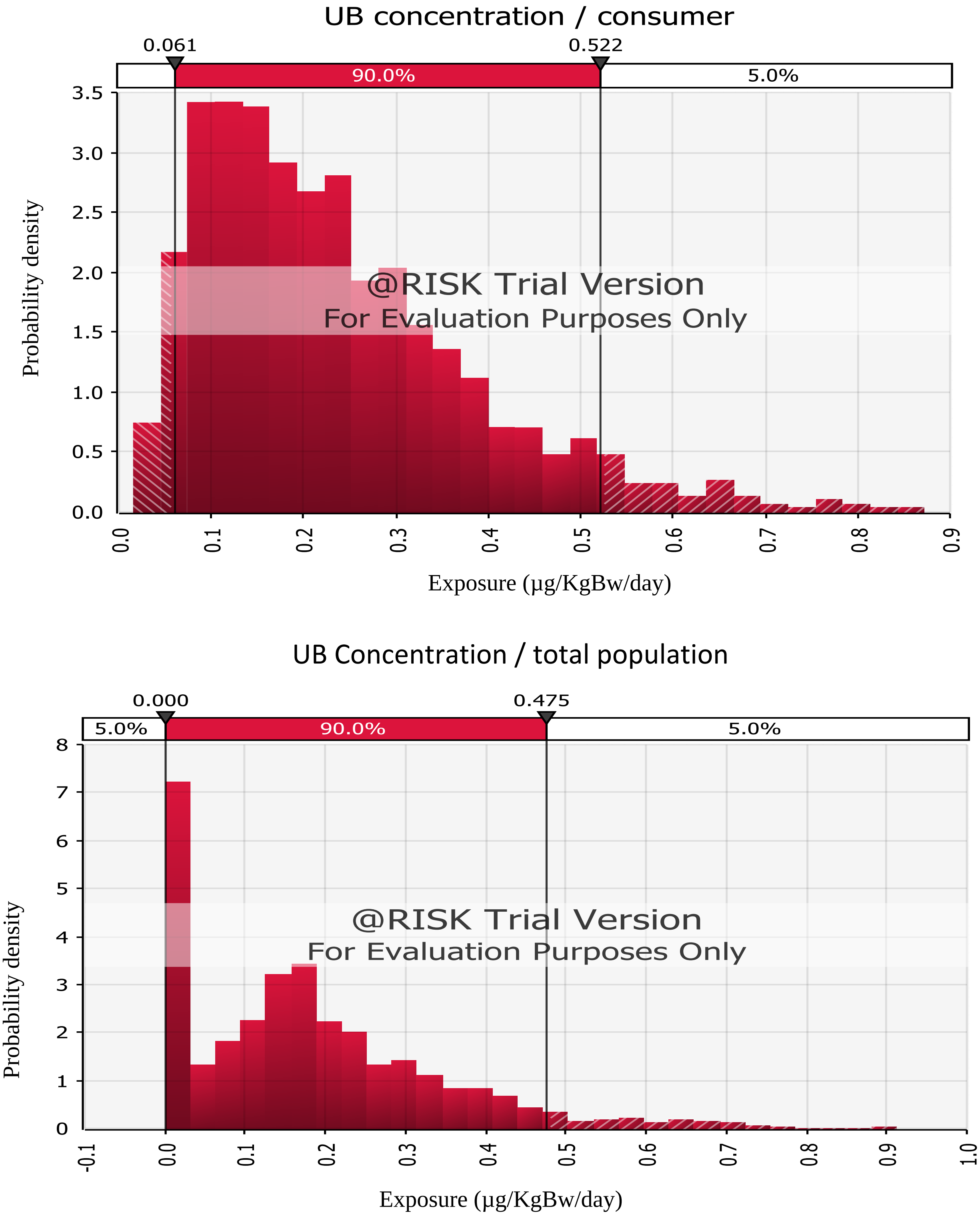


Exposure assessment graphs of arsenic at medium bound scenario for consumers and total population respectively.

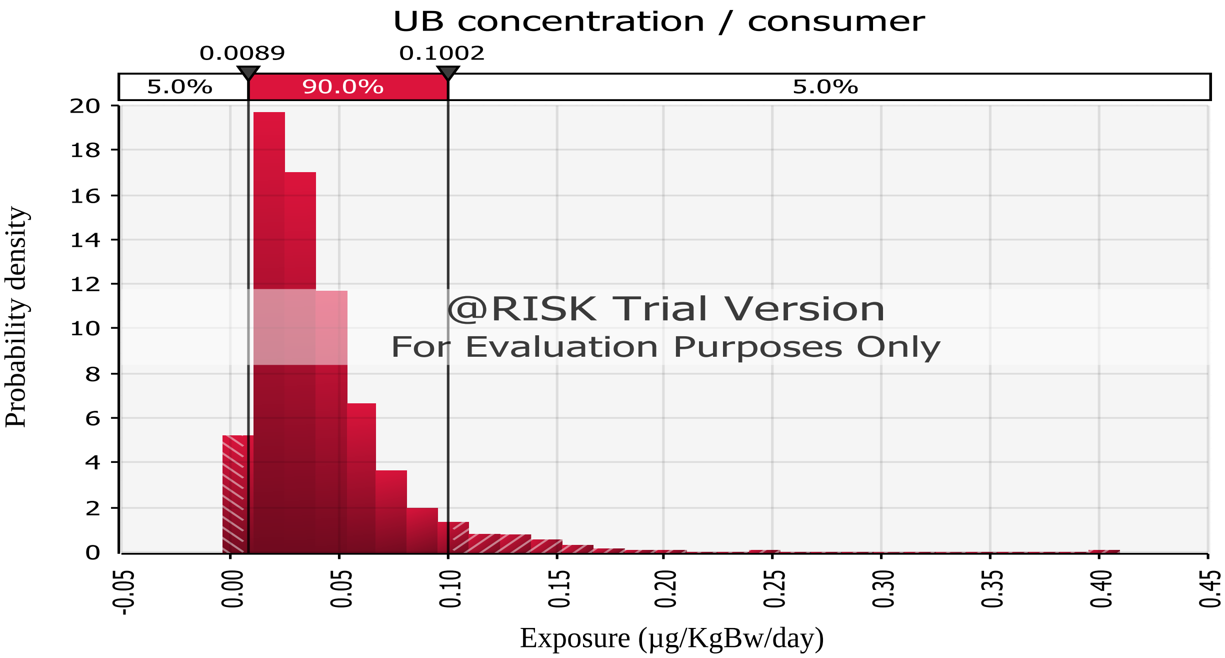




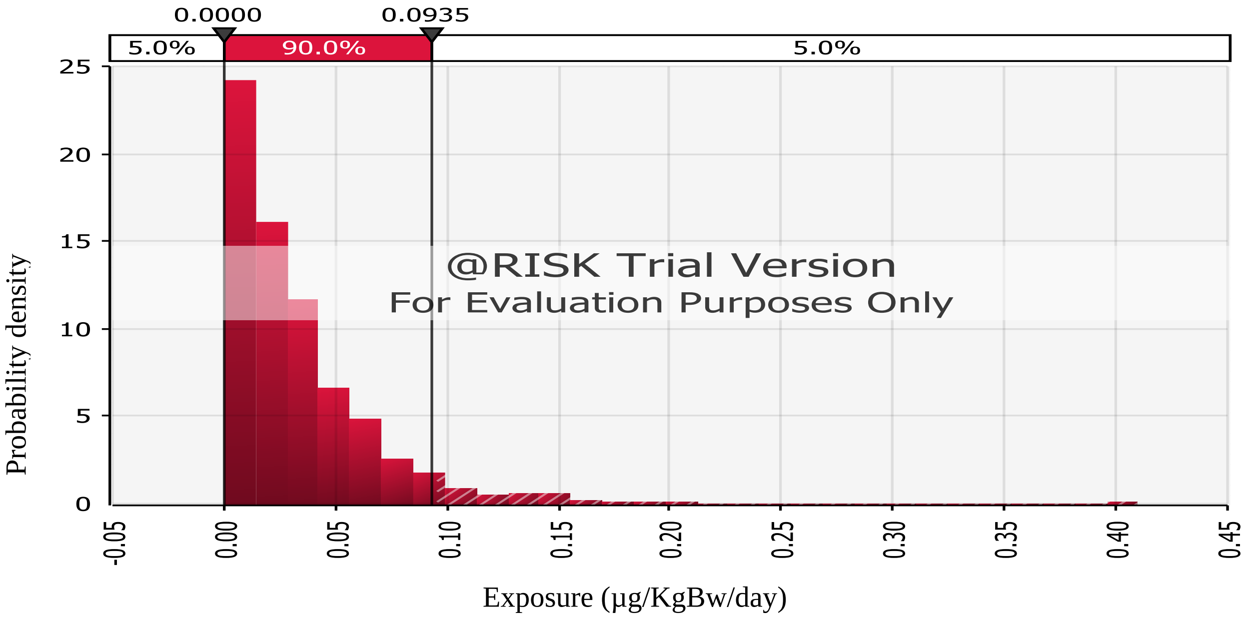
Exposure assessment graphs of arsenic at upper bound scenario for consumers and total population respectively.



Exposure assessment graphs of chromium at upper bound scenario for both consumers and total population respectively.


UB concentration / consumer

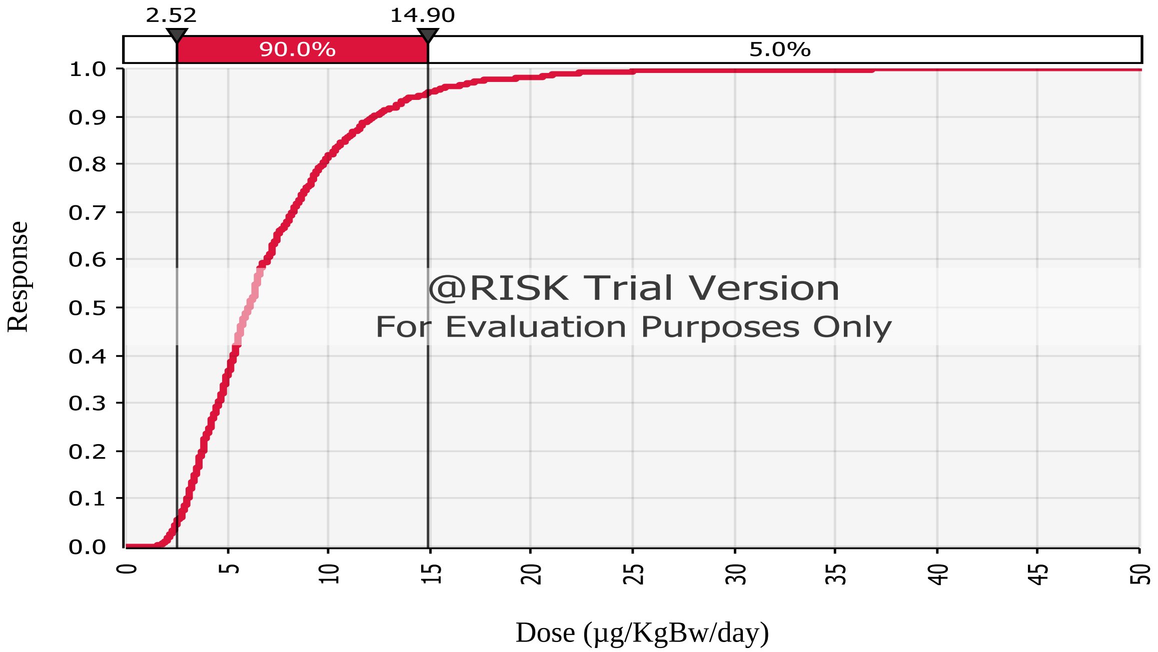
UB Concentration / total population



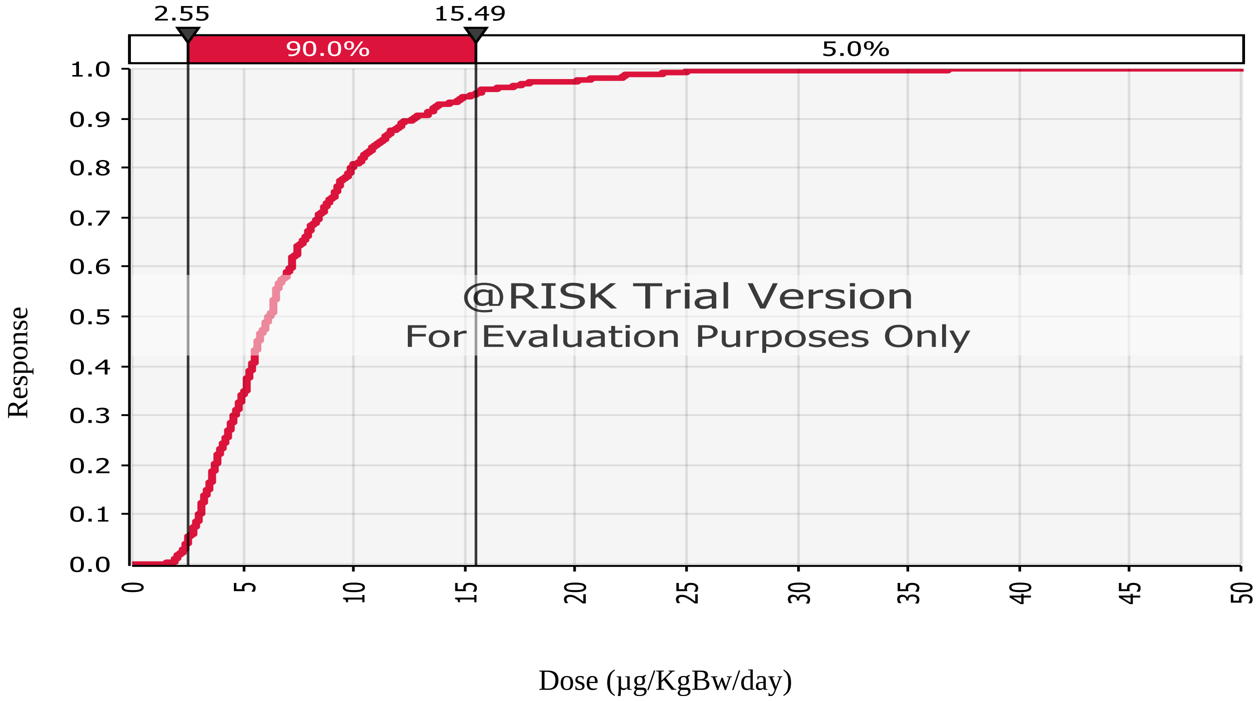
Exposure assessment graphs of nickel at upper bound scenario for consumers and total population respectively.

Appendix 3: Margin of exposure graphs

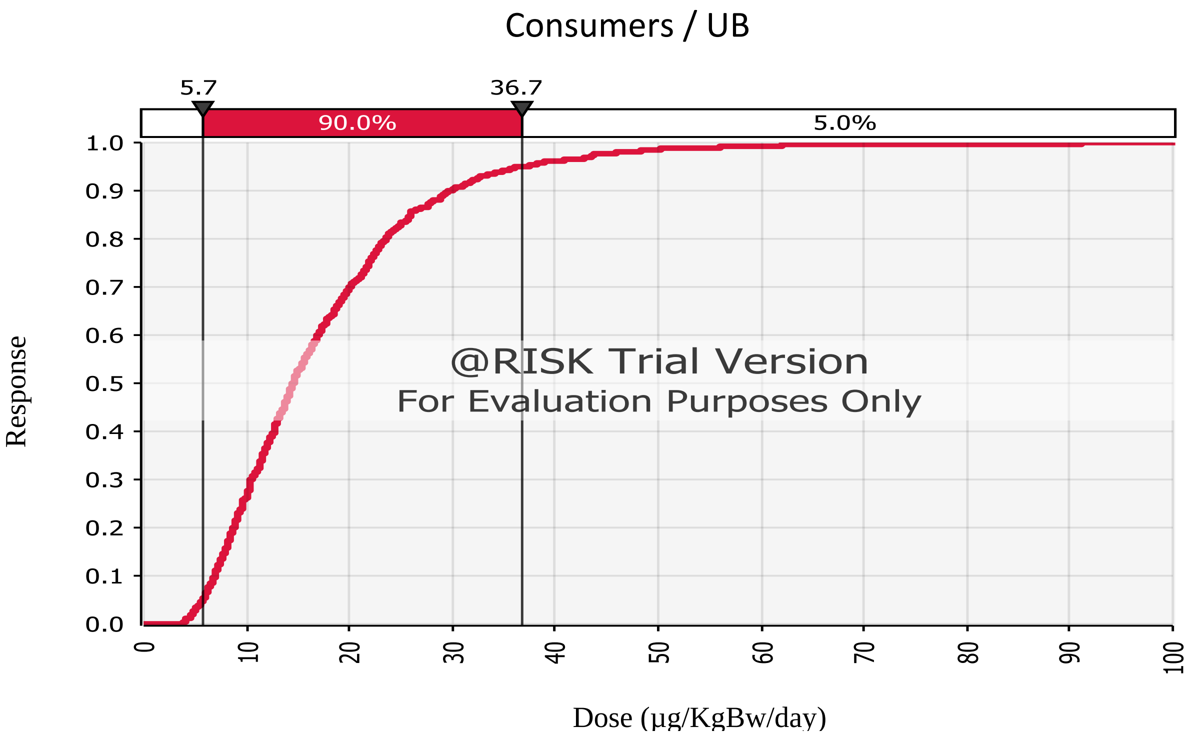
Consumers / UB

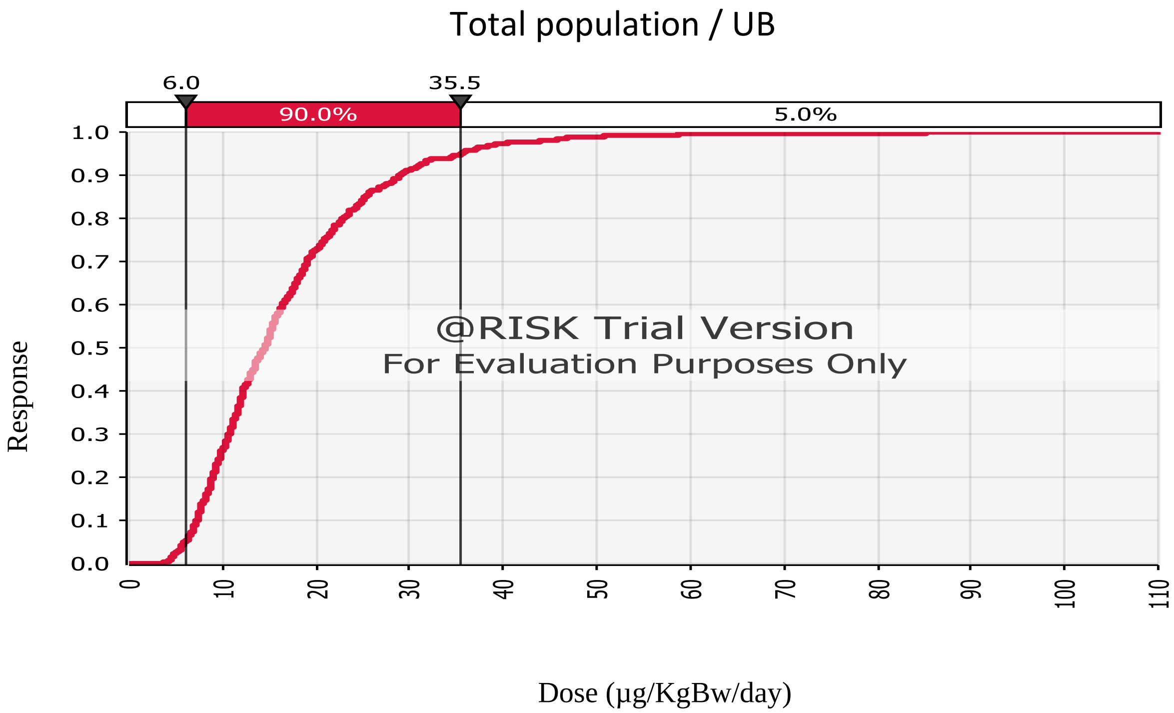


Total population / UB

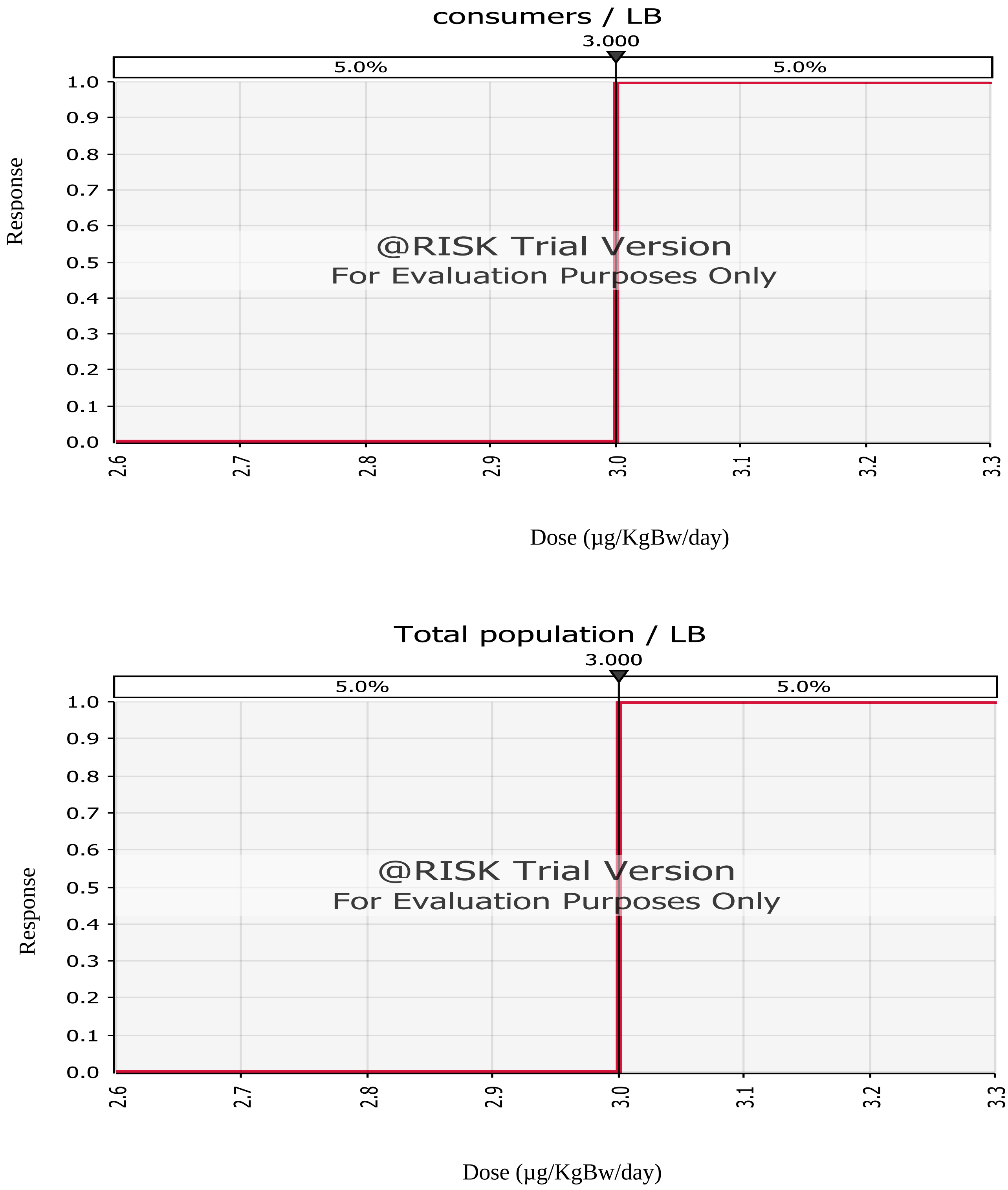


MOE graphs of lead for consumers and total population at $BMDL_{10}$ = 0.63µg/KgBw/day for human chronic kidney disease respectively.

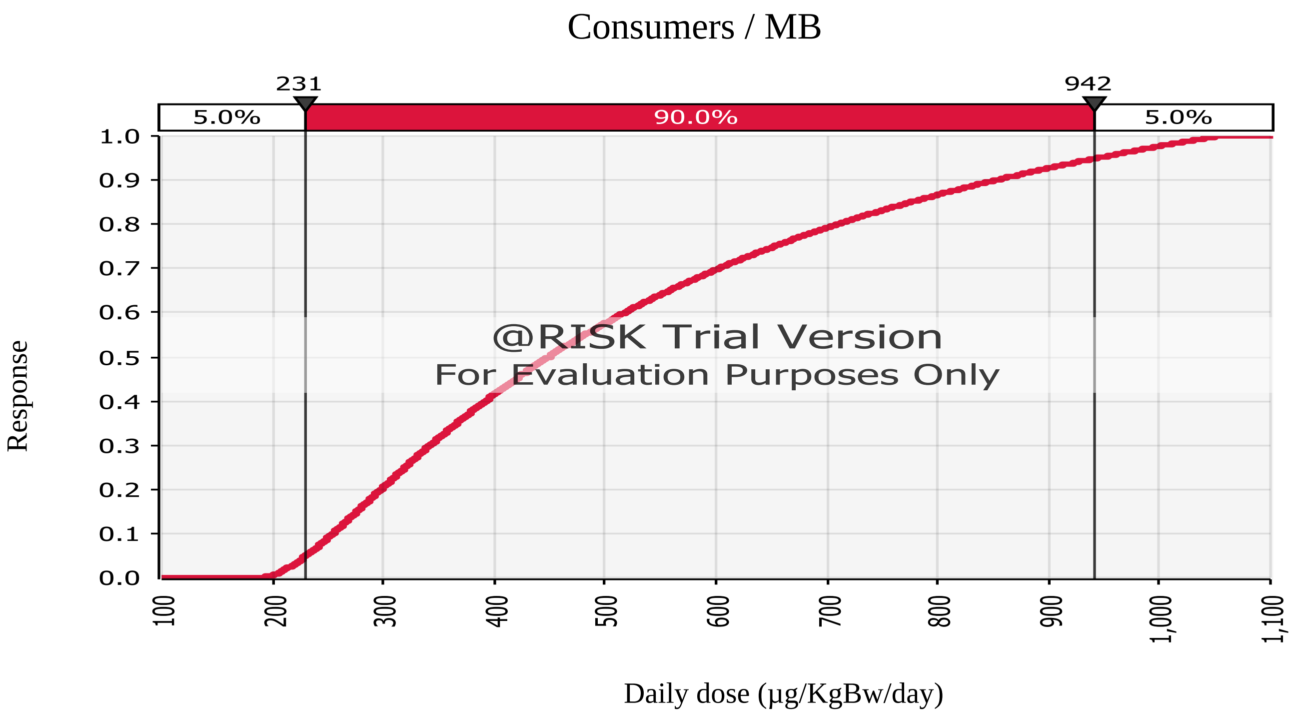


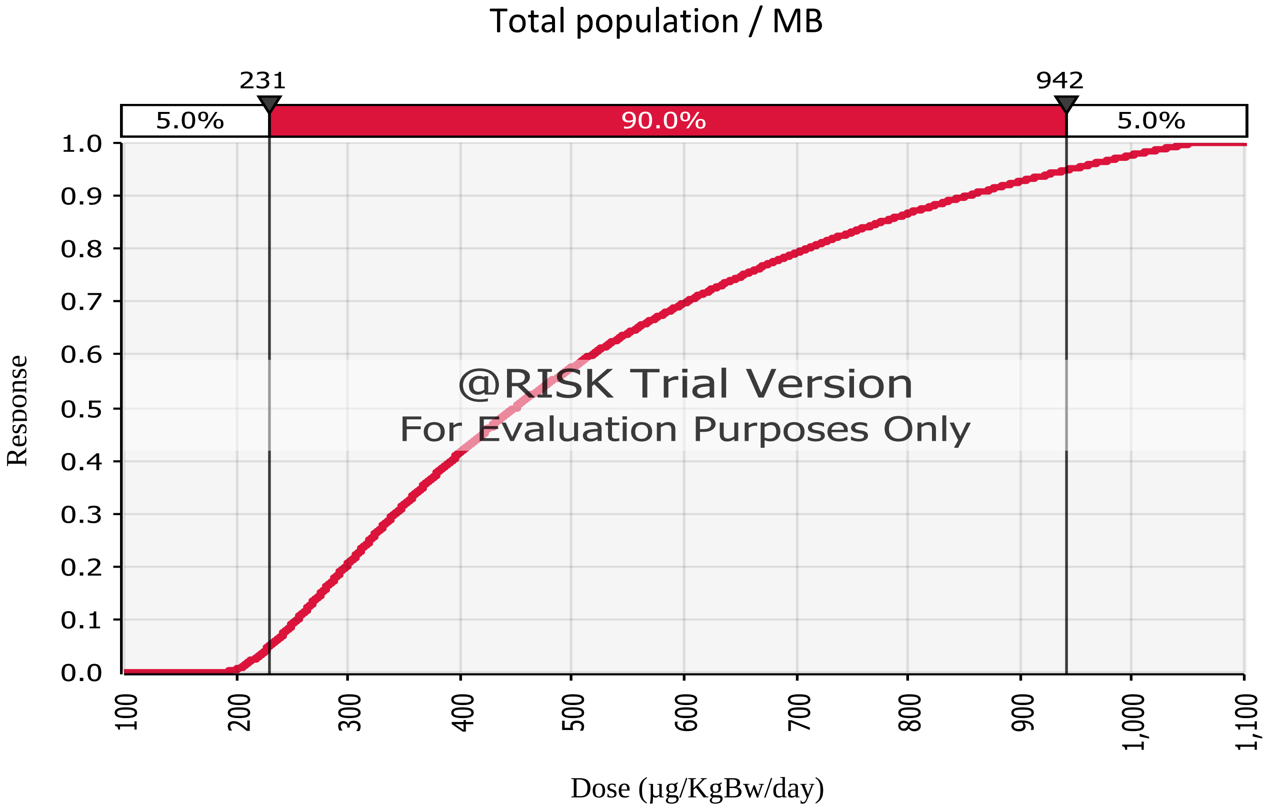


MOE lead graphs at $BMDL_{01}$ =1.5µg/KgBw/day responsible for humans with cardiovascular diseases for consumers and total population respectively

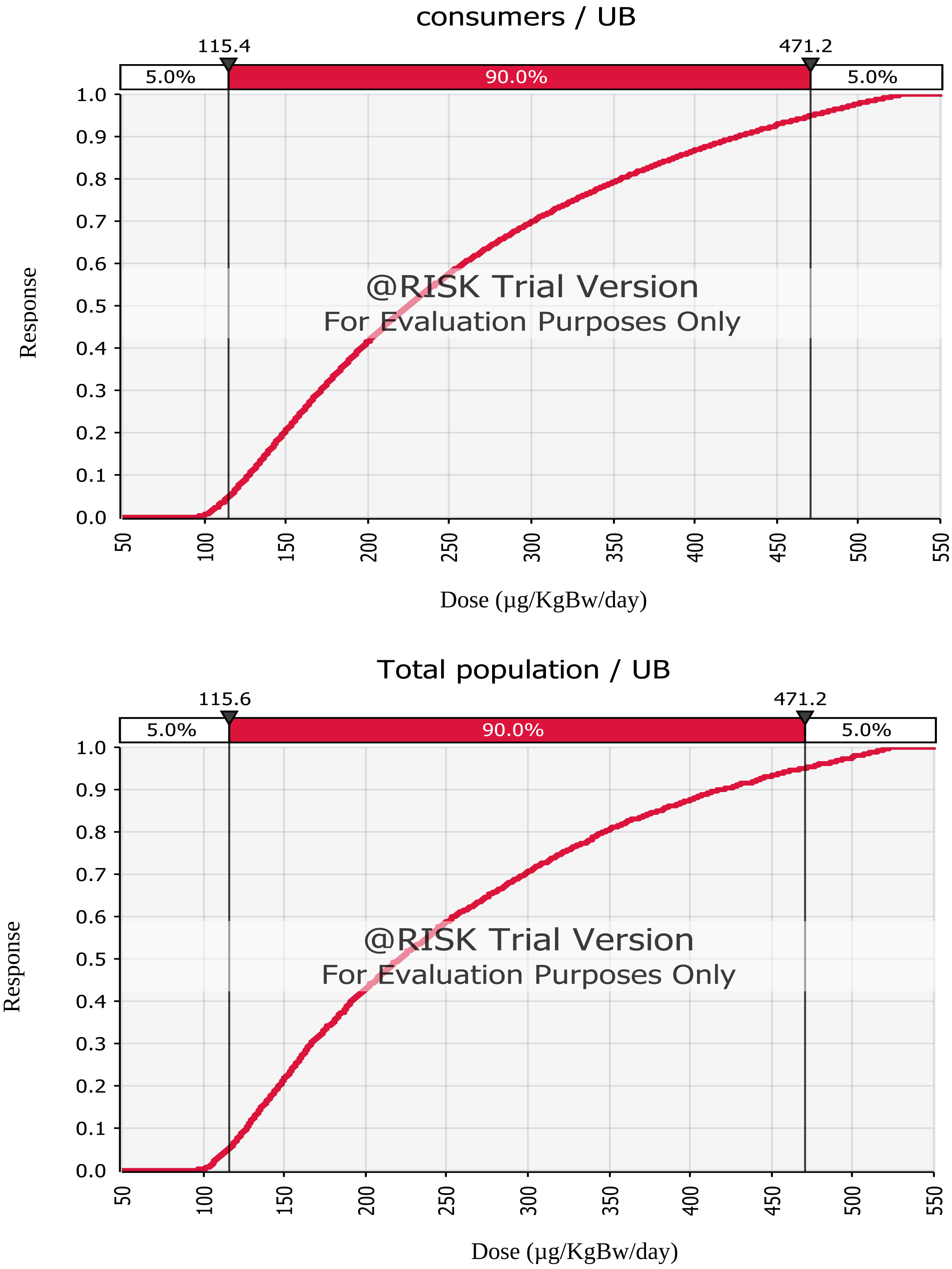


MOE graphs of arsenic at $BMDL_{0.5}$ =3.0 µg/KgBw/day for lower bound of increased incidence of lung cancer for consumers and total population respectively.

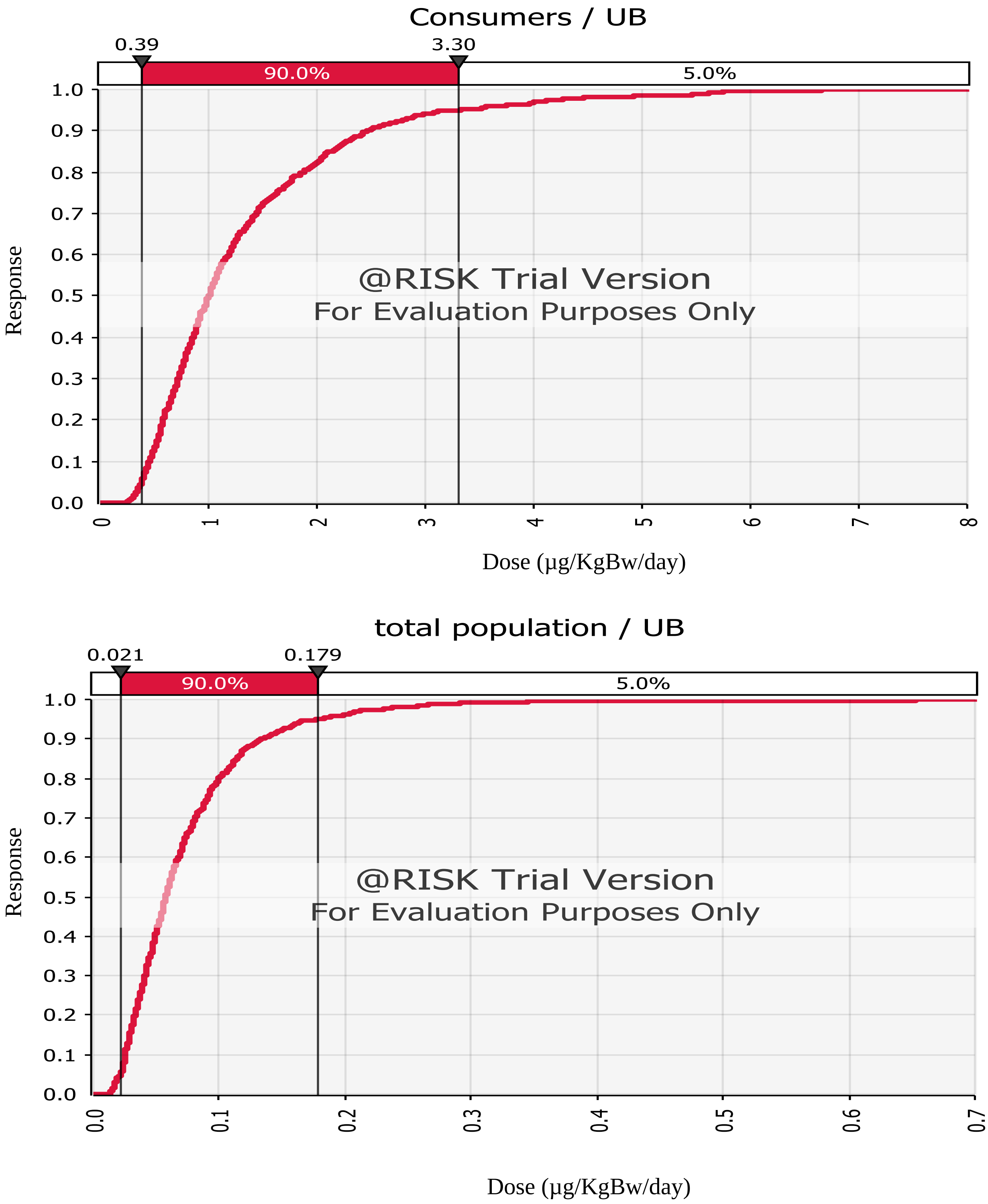




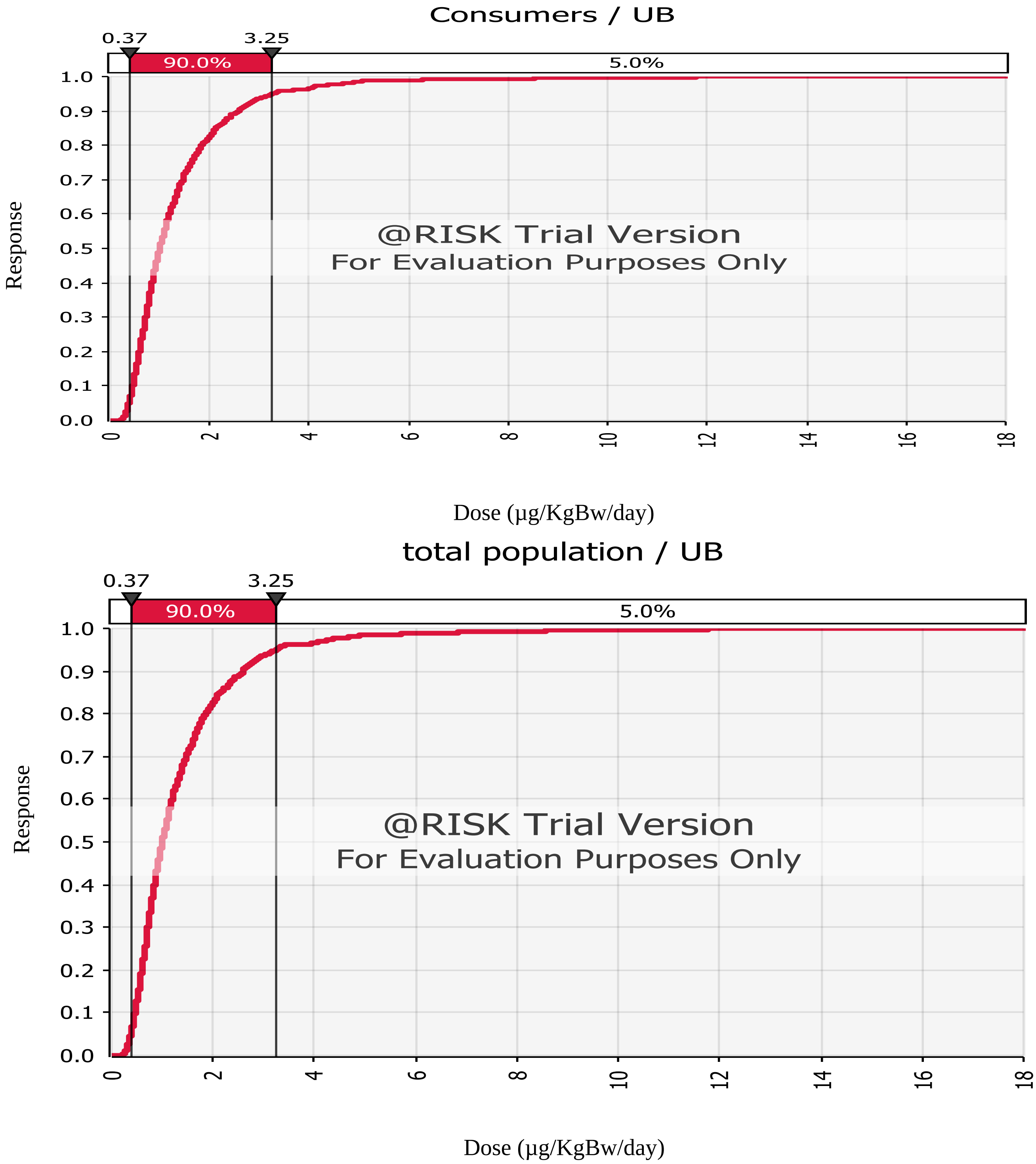
MOE graphs of arsenic at $BMDL_{0.5}$ =3.0 µg/KgBw/day at medium bound scenario of increased incidence of lung cancer for consumers and total population respectively



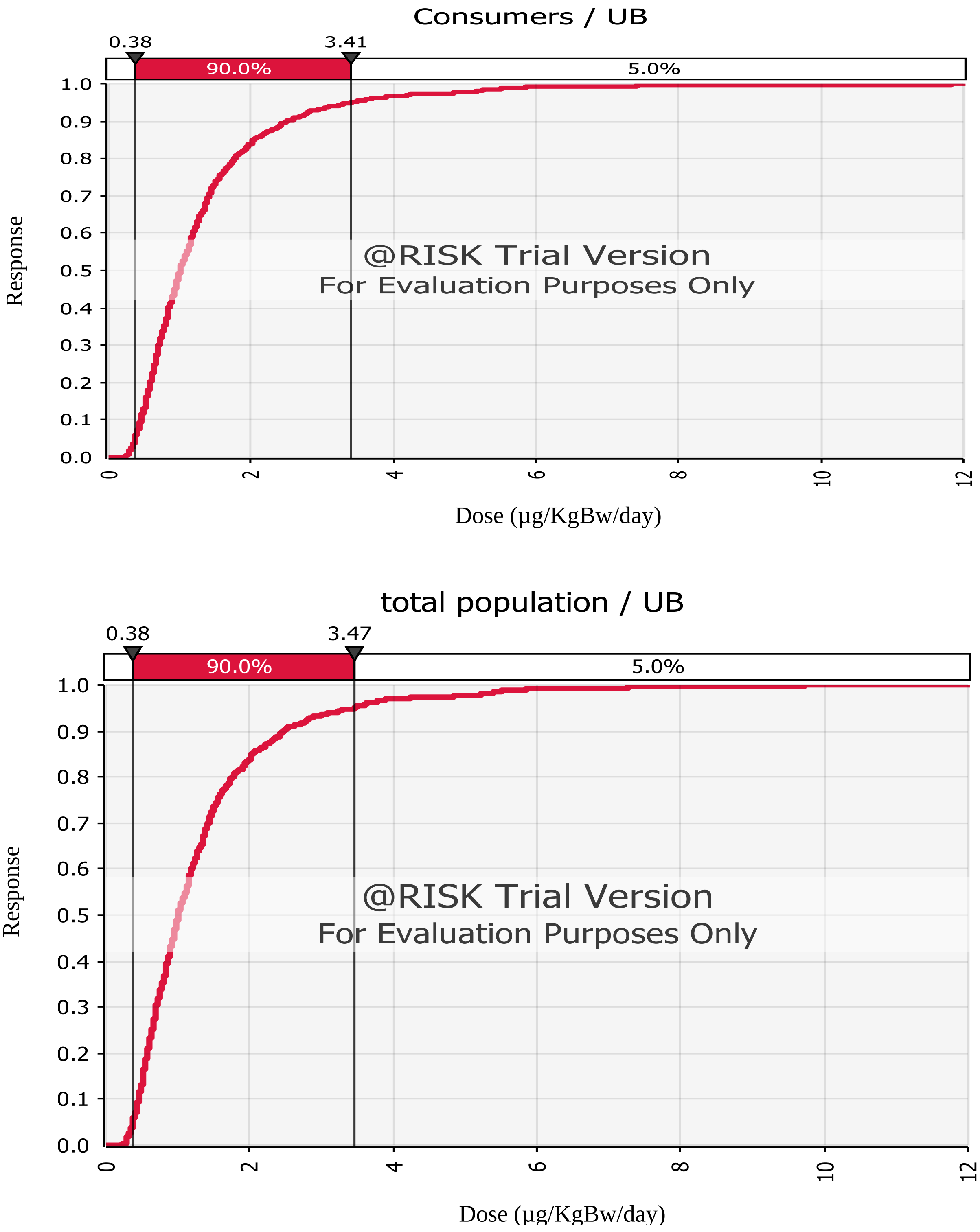
MOE graphs of arsenic at $BMDL_{0.5}$ =3.0 µg/KgBw/day at upper bound scenario of increased incidence of lung cancer for consumers and total population respectively.



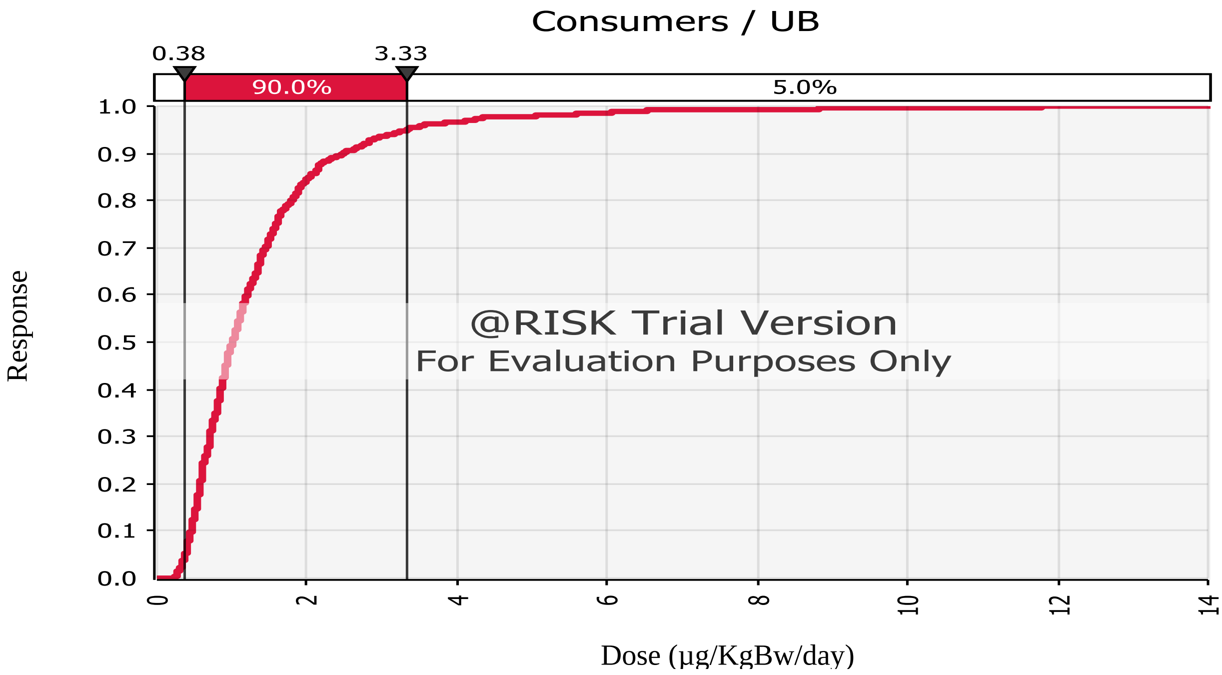
MOE graphs of chromium at $BMDL_{0.5}$ = 0.011 µg/KgBw/day, indicator of neoplastic lesions in liver in rats for consumers and total population respectively.

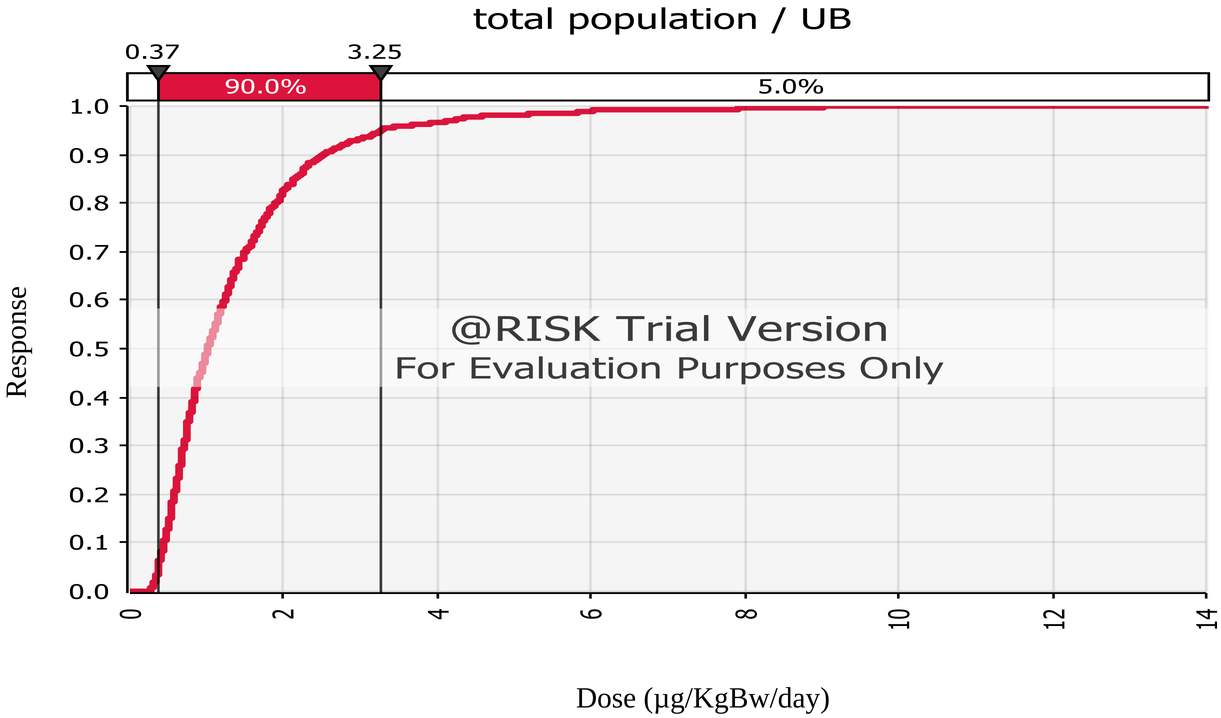


MOE graphs of chromium at $BMDL_{0.5}$=0.11 µg/KgBw/day an indicator of neoplastic lesions in duodenum in rats for consumers and total population respectively.

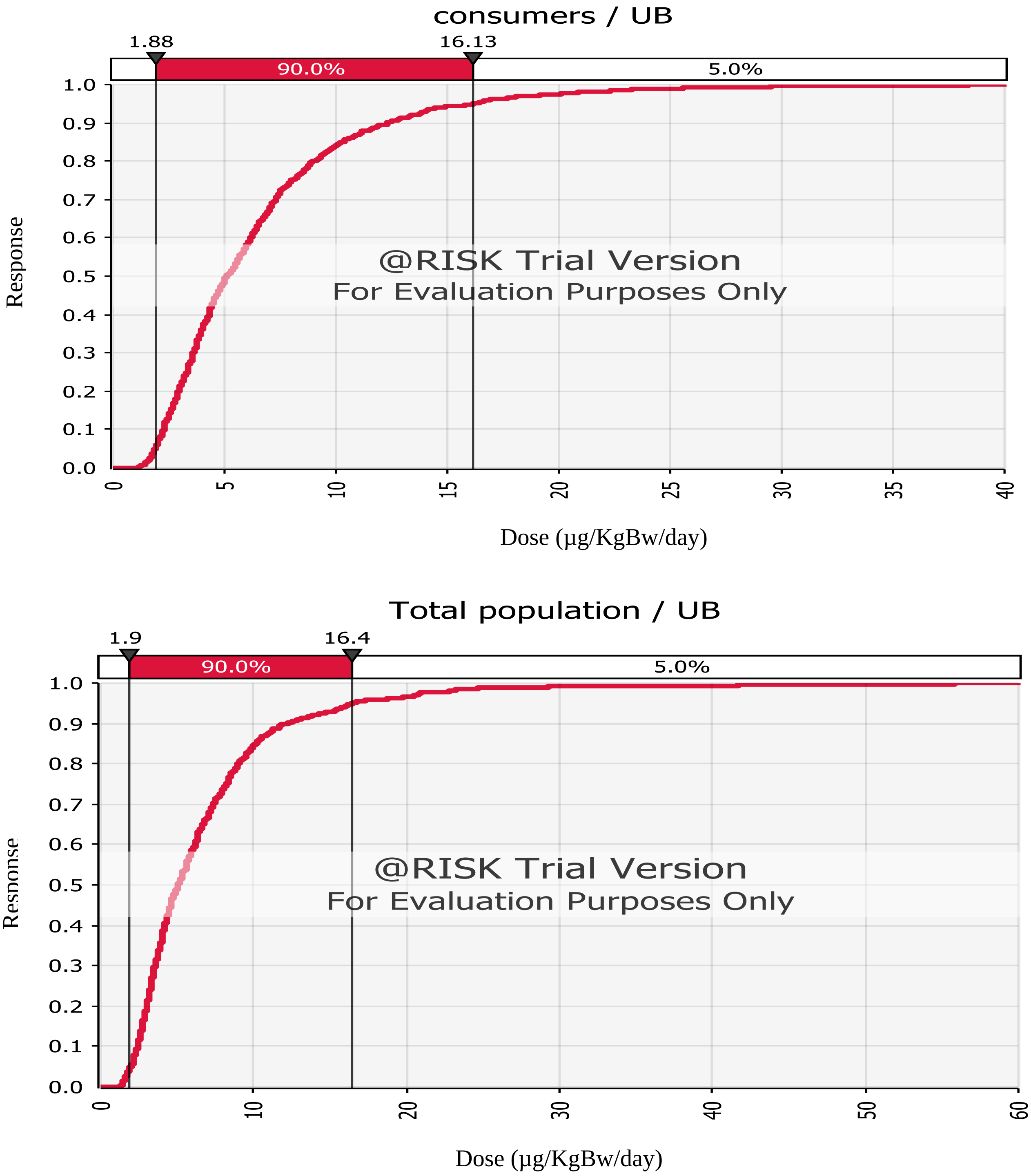


MOE graphs of chromium at $BMDL_{0.5}$ = 0.2 µg/KgBw/day responsible for heamostolic effects in rats for consumers and total population respectively.

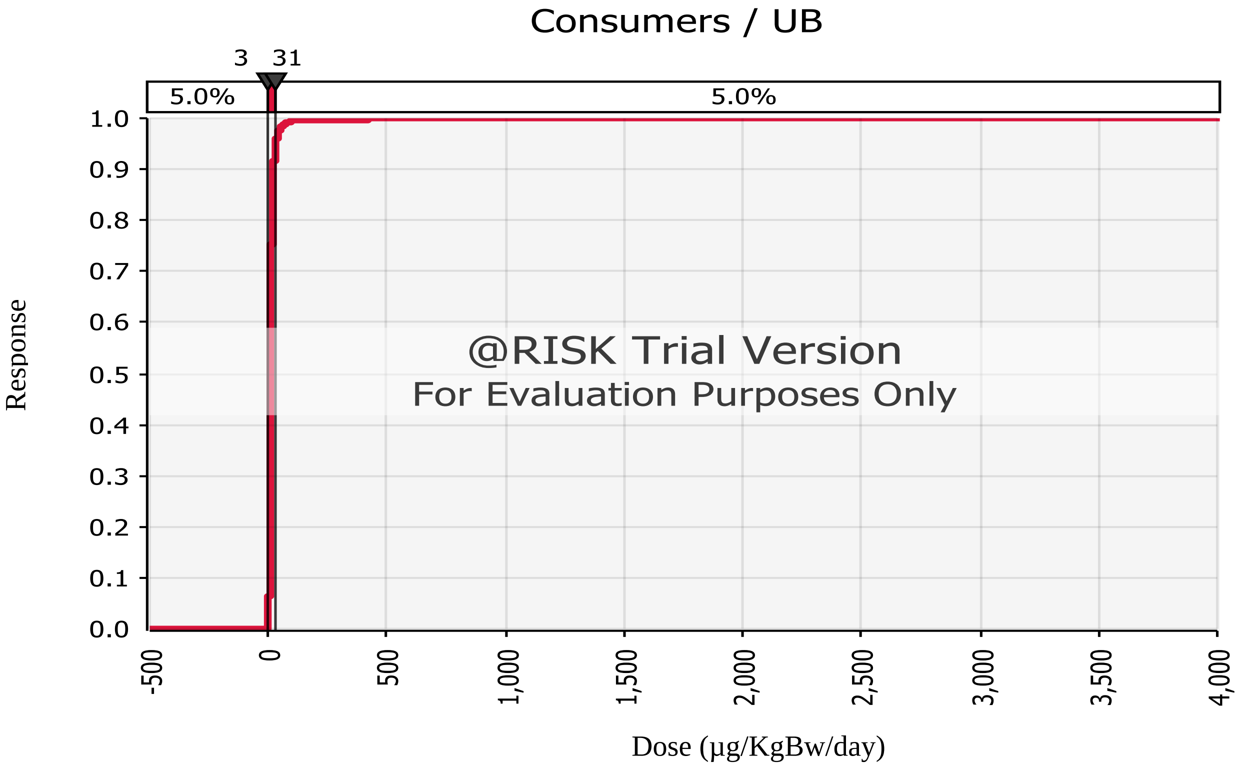


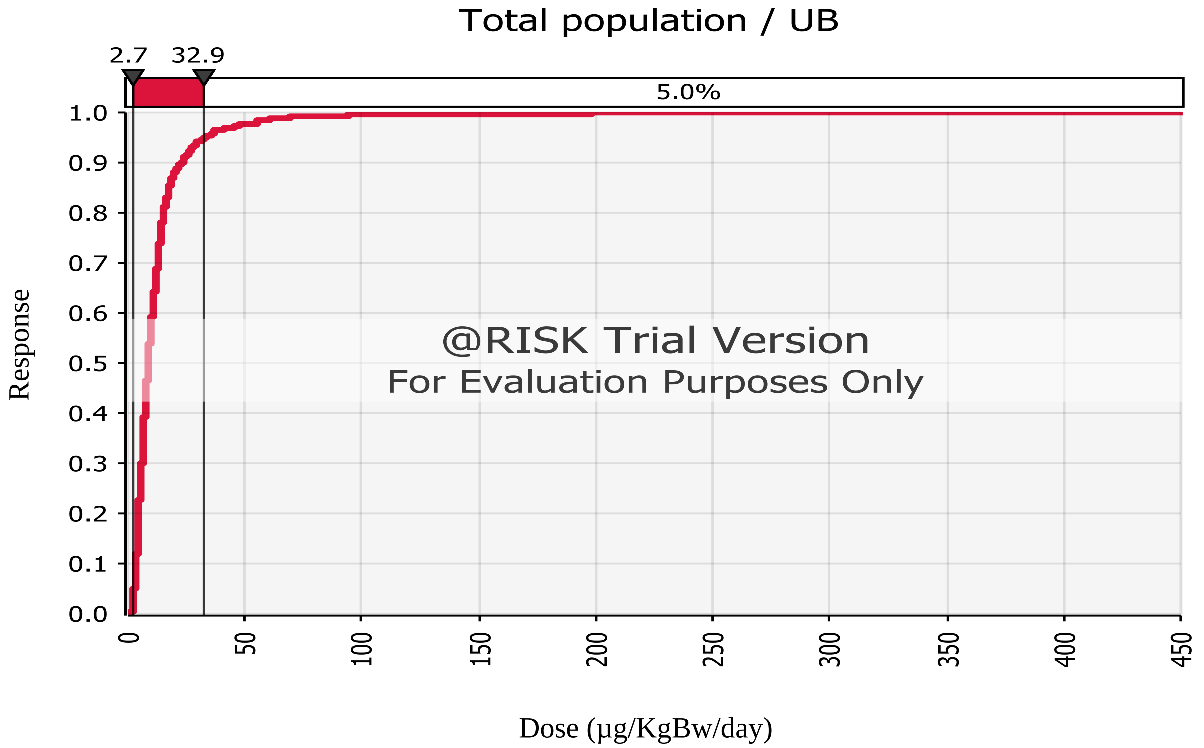


MOE graphs of chromium at $BMDL_{0.5}$ = 0.27 µg/KgBw/day indicator of neoplastic lesions in pancrease in rats for consumers and total population

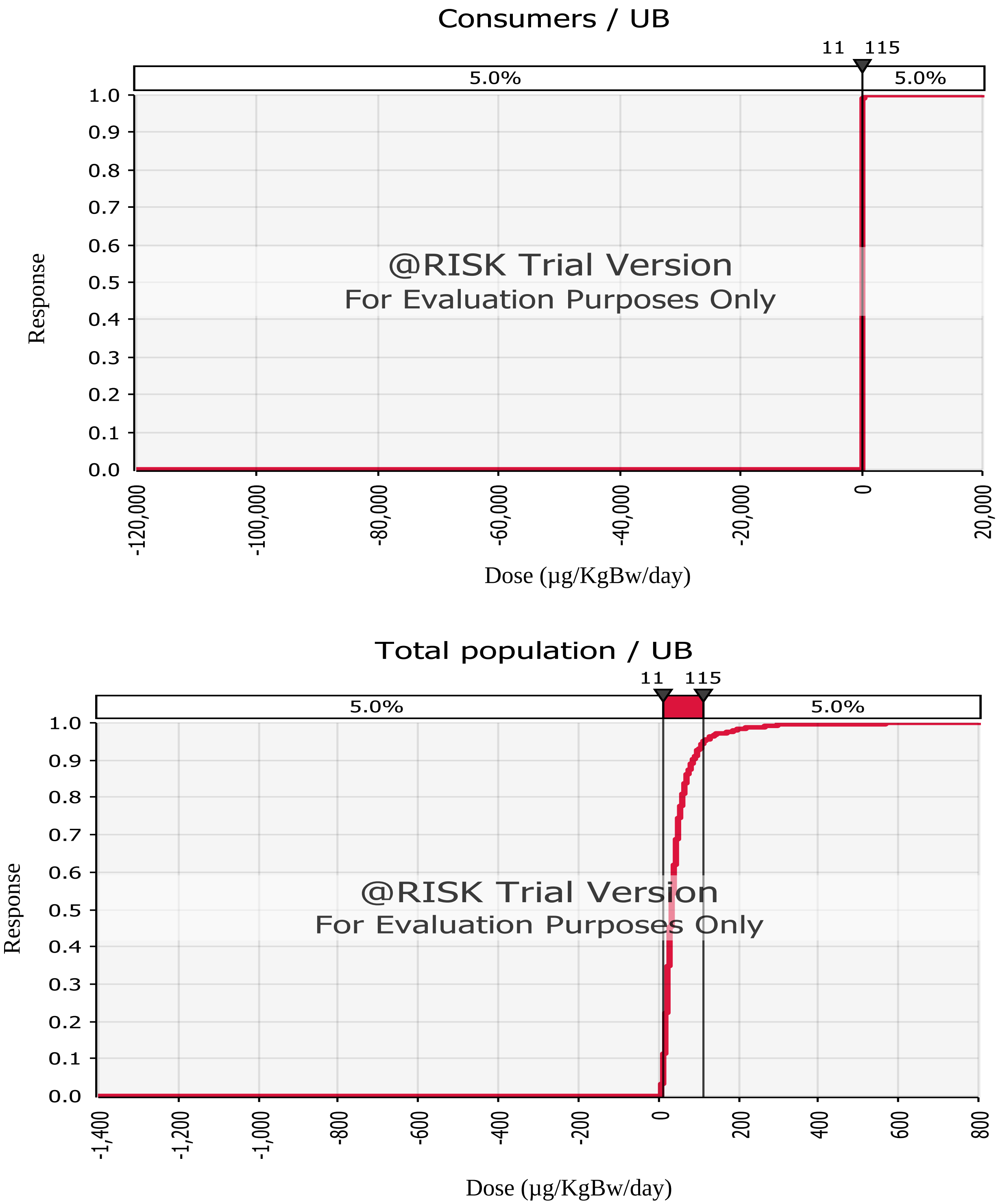


MOE graphs of Chromium at $BMDL_{10}$ =1.0 µg/KgBw/day responsible for increased incidence of lung cancer in rats for consumers and total population respectively.





MOE graphs of nickel at $BMDL_{10}$ =0.28 µg/KgBw/day responsible for post implantation fetal loss in rats in consumers and total population respectively.



Nickel MOE graphs for consumers and total population at $BMDL_{10}$ = 1.1 µg/KgBw/day for acute exposures of developing eczematous flare up skin reactions for consumers and total population respectively.