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Molybdenum Content of Canadian and US Infant Formulas

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Abstract Molybdenum is an essential trace nutrient in the human diet. Our purpose was to provide a comprehensive analysis of Mo content of various types of powdered infant formulas across Canada and the USA. All infant formulas, available on the day of sampling, were purchased from random supermarkets in Grand Forks, ND, USA; San Diego, CA, USA; Washington, DC, USA; and Winnipeg, MB, Canada. Reference powdered milk, human milk (HM), and formula samples were weighed and acid-digested prior to analysis by graphite furnace atomic absorption spectroscopy. Mo content in all formulas ranged from 15.4 to 80.3 μg/L (mean±SE, 37.7±1.7 μg/L). HM Mo concentration ranged from 1.5 to 9.5 μg/L (5.09±0.81 μg/L). Formulas intended for full-term or for premature infants feeding contained, on average, more Mo than HM. Formulas intended for infants with special needs contained similar mean Mo levels to HM. No significant differences were detected between mean Mo values of formulas of a same type purchased from different brands and/or at different locations. High Mo intake may pose health risks, despite lower bioavailability of Mo from formula compared with HM.

Keywords Molybdenum · Infant feeding · Formula content

Introduction

Molybdenum is a trace element and an essential nutrient in the human diet. Mo is a cofactor for the enzymes xanthine oxidase, sulfite oxidase, and aldehyde oxidase involved in the

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metabolism of sulfur-containing amino acids, purines, and pyrimidines [1,2]. These three "molybdoenzymes" served as the foundation for the discovery of the essentiality of Mo [1,3].

Significant sources of Mo (as soluble molybdates) in the human diet are leafy vegetables, legumes, organ meats, grain products, cow's milk, and eggs [4]. Mo content provided by food sources varies depending on the amount in soil [5]. For infants before weaning, the primary sources of Mo are HM and infant formula, cow's milk- or soy-based.

The passive, non-mediated, system for Mo absorption is efficient at both very high and low intakes [1]. Absorption of Mo has been shown to be as high as 75% to 97% [4,6–8], even in premature infants [9]. Despite high levels of absorption, Mo does not bioaccumulate in tissues, and physiological levels decrease rapidly during low dietary intake [4]. Liver and kidney are the primary short-term storage sites of Mo, while lungs, brain, muscles, and blood store less significant amounts [5]. Mo tissue levels are not regulated by intestinal absorption [6]. The mineral is absorbed and rapidly cleared within hours or days through urinary excretion of molybdate [10], effectively maintaining Mo homeostasis [6]. Thus, dietary intake is correlated with urinary, but not fecal, excretion [11]. Mo is present at very low levels in fetal tissues at birth [6,12]; its content of the fetal liver is seven-fold lower than that of adult liver [12]. The lack of prenatal stores suggests that newborns have a low need for this mineral [6].

There is low risk of Mo toxicity in humans due to the high efficiency of urinary excretion, except possibly in premature infants [7]. The few known cases of Mo toxicity in adult humans were characterized by malaise, headache, irritability, reduced appetite, abdominal pain, joint and muscle pain, and weight loss [10]. No longitudinal human studies have evaluated the effects of prolonged exposure to high Mo concentrations. An overproduction of the enzyme xanthine oxidase, the cofactor of Mo, can react with oxygen to produce reactive oxygen species and subsequent tissue damage [4,5].

In the first year of life, infants receive the majority of nutrients from HM or manufactured formula. The European Commission [4] has set an upper intake level (UL) for Mo intake in infancy of 10 μg/kg/day. The Australia New Zealand Food Authority [13] specifies that a maximum of 69 μg Mo/100 kcal can be added to special formula (designed for full-term infants with specific issues, including colic, allergy, and lactose intolerance) but prohibits the addition of Mo to standard formula. In Canada [14] and the USA [15], Mo is not formally regulated in infant formula.

To date, only one study [16] has analyzed the concentrations of Mo in infant formulas obtained in multi-country studies. The purpose of the present study was to perform analysis of the Mo content of powdered infant formulas (cow's milk-based and soy-based) across both Canada and the USA. We collected a wide variety of common, commercially available formulas of seven different brands from four different geographical areas (central Canada; west, east, and central USA) to determine their Mo concentrations and assess the possible influence of different sources of raw materials that may be used in different manufacturing plants.

Materials and Methods

One can of each type and brand of powdered infant formulas that were available on the day of sampling in random supermarkets in Grand Forks, ND, USA; San Diego, CA, USA; Washington, DC, USA; and Winnipeg, MB, Canada was purchased. A total of 81 formulas from all locations (19, 13, 19, and 30, respectively) were analyzed for Mo concentration (Table 1). The four specific locations were selected to provide a representation of formulas from different manufacturing plants available to US and to Canadian families in different regions: in Eastern, Western, and the Southern United States and in Canada. Four US locations were chosen as they are served by multiple manufacturing plants, whereas Canadian infant formulas are manufactured in only one central location. Formulas were defined as either cow's milk-based (cow's milk protein source), soy-based (soy protein source), preterm (designed to meet the special needs of premature or low weight infants), or "special" (designed for full-term infants with specific issues, including lactose intolerance, allergy, and colic). In addition, ten samples of mature HM were collected from breastfeeding mothers at different stages of lactation, residing in Winnipeg, MB, Canada, and analyzed for Mo concentration for comparison with formulas' contents. The HM was collected using hand expression or mechanical pumps. These samples were obtained from an ongoing study in our laboratory for which ethical approval was granted by the University of Manitoba. For results validation, reference powdered milk with certified concentrations of Mo was purchased from the National Institute of Standards and Technology (SRM 8435, whole milk, Gaithersburg, MD, USA). A mean value of 0.28± 0.04 μg/g was obtained in our analyses for Mo content in the SRM 8435, in agreement with the certified Mo content, which was 0.29±0.13 μg/g.

HM samples were freeze-dried to powder form in acid washed Mo-free containers. Using established criteria [17], reference powdered milks and formula samples were weighed and acid-digested prior to analysis of Mo concentrations by graphite furnace atomic absorption spectroscopy (Varian Inc, Palo Alto, CA, USA). All samples were analyzed in triplicate. Sample blanks were used as a baseline. Mo concentrations were obtained per gram of dried sample. The concentrations of formula powder or powdered HM per liter of fluid were determined and converted to the concentration of Mo per liter of ready-to-feed product.

From an ongoing study of premature infants in hospital during early infancy, we recorded their daily intake of formula and breast milk. Based on the intake data and on the infants' average weight during mid-hospitalization, we calculated mean Mo intakes per day and per kilogram bodyweight.

A Kruskal–Wallis test with Dunn's post hoc test was performed to compare mean Mo concentrations by formula type using Graph Pad Prism version 5.00 for Windows, Graph Pad Software, San Diego, CA, USA. Significance was assigned to $P<0.05$.

Results

The results for Mo content of all formulas, analyzed in duplicate, can be found in Table 1. Mo content of all formulas from all locations ranged from 15.4 μg/L (Similac Sensitive, "special" type) to 80.3 μg/L (Earth's Best, soy type), with a mean±SE of 37.7±1.7 μg/L. Formula contained more Mo than HM (ranged from 1.5 to 9.5 μg/L (5.09±0.81 μg/L)). With respect to formula type, as can be seen from Fig. 1, "special" formulas' mean Mo content did not differ significantly from HM mean Mo content. Premature formulas' mean Mo content did not differ significantly from full-term and from soy-based formulas'. Full-term, premature, and soy-based formulas had significantly higher mean Mo content compared with HM and "special" formulas.

When the mean Mo content of all formulas from the same type (from all locations of purchase combined) was compared between different brands, no significant differences were detected (data not presented). For example, there were no significant differences between mean Mo content of all "special" type Enfamil, Similac, and Target formulas, purchased in all the locations combined.

Table 1 Molybdenum content of the analyzed formulas

Brand	Formula name	Purchase location				Type
		Winnipeg	Washington, DC	Grand Forks	San Diego	
		Molybdenum content (μg/L)[a]				
Nestle	Goodstart® (iron, omega 3 and 6, 0–12+months)	22.9				Full T
	Goodstart® 2 (iron, omega 3 and 6, 6–18+months)	20.7				Full T
	Goodstart® (iron, 0–12+months)	22.4	30.8			Full T
	Follow-up® Transition (iron, 6–18+months)	17.3				Full T
	AlSoy 2 (soy, calcium, omega 3 and 6, 6–24 months)	55.3				Soy
	AlSoy (iron, soy, omega 3 and 6, 0–12 months)	41.4				Soy
	Good Start Supreme (iron, easy to digest, 0–12 months)		22.5			Full T
	Good Start Supreme (iron, easy to digest, 0–12 months)		48.6			Full T
	Good start 2 Supreme (iron, calcium, DHA, ARA, 9–24 months)		51.2			Full T
	Good Start Supreme (iron, DHA, ARA, Bifidus, 0–12 months)			25.2		Full T
	Good Start Supreme (iron, comfort proteins, 0–12 months)			26.0		Full T
	Good Start Supreme (iron, soy, DHA, ARA, 0–12 months)				52.3	Soy
	Good Start Supreme (iron, natural cultures, 0–12 months)				40.3	Full T
	Good Start Supreme (iron, DHA, ARA, comfort proteins, 0–12 months)		43.4	35.1		Full T
Enfamil	Enfagrow (high iron and calcium, 12+months)	55.1				Full T
	Enfapro (iron, calcium, 6+months)	28.5				Full T
	A+ (iron, calcium, 6+months)	46.8				Full T
	A+ (iron, 0+month)	35.7				Full T
	(iron, 0+month)	34.0				Full T
	(Lower iron, 0+month)	48.1				Full T
	(Iron, lactose-free, 0–12 months)	16.7				Spec
	A+ (iron, DHA, ARA, 0–12 months)	19.7				Full T
	Lipil (iron, DHA, ARA, premature and LBW)		58.6			Prem
	LactoFree Lipil (iron, DHA, ARA, 0–12 months)		26.3	18.8	16.5	Spec
	Gentlease Lipil (iron, DHA, ARA, 0–12 months)		34.8	37.6		Spec

Table 1 (continued)

Brand	Formula name	Purchase location: Winnipeg	Washington, DC	Grand Forks	San Diego	Type
		Molybdenum content (μg/L)[a]				
	Nutramigen (hypoallergenic, 0–12 months)	26.9				Spec
	Nutamigen Lipil (iron, DHA, ARA, hypoallergenic, colic, 0–12 months)		19.8			Spec
	EnfaCare Lipil (iron, DHA, ARA, premature, and LBW, 0–12 months)				60.9	Prem
	(Iron, 0–12 months)		51.9			Full T
	Lipil (DHA, ARA, iron, 0–12 months)		54.9	47.7		Full T
	A.R. Lipil (iron, DHA, ARA, spitting up, 0–12 months)			27.1		Spec
	Nutramigen (iron, DHA, ARA, colic, 0–12 months)			20.0	20.7	Spec
	Next Step ProSobee Lipil (iron, soy, DHA, ARA, 9–24 months)		66.9			Soy
	(Soy, 0–12 months)	47.5				Soy
	ProSobee Lipil (iron, soy, 0–12 months)		63.4	58.7	31.6	Soy
	Next Step Lipil (iron, DHA, ARA, 9–24 months)		41.4			Full T
President's Choice	Organics® Biologique® Step 1 (iron, 0–12 months)	28.6				Full T
	(0–12 months)	24.7				Full T
	Organics® Biologique® Step 1 (soy, 0–12 months)	60.7				Soy
	(Iron, omega 3 and 6, 0–12 months)	35.7				Full T
Similac	Advance® (iron, 0–12 months)	31.0	41.1			Full T
	Advance® Step 1 (iron, omega 3 and 6, 0+month)	23.2				Full T
	Advance® Step 2 (iron, 6–18 months)	35.5				Full T
	Advance® Step 2 (iron, calcium, omega 3 and 6, 6–18 months)	31.3				Full T
	Advance® LF (lactose-free)	20.3				Spec
	Organic (iron, DHA, ARA, 0–12 months)		25.9			Full T
	NeoSure (iron, DHA, ARA, premature, and LBW)		43.9		31.6	Prem
	Sensitive (iron, DHA, ARA, lactose-free, 0–12 months)			15.9		Spec
	Advance (iron, DHA, ARA, 0–12 months)			35.7	31.1	Full T
	LactoFree Lipil (iron, DHA, ARA, lactose-free, 0–12 months)			22.4		Spec
	Alimentum (iron, DHA, ARA, hypoallergenic, 0–12 months)		23.1	22.3	24.4	Spec

Table 1 (continued)

Brand	Formula name	Purchase location				Type
		Winnipeg	Washington, DC	Grand Forks	San Diego	
		Molybdenum content (μg/L)[a]				
	Sensitive (iron, DHA, ARA, lactose-free, 0–12 months)				15.4	Spec
	Isomil Step 2 (soy, hypoallergenic, 6–24 months)	57.6				Soy
	Isomil (soy, 0–12 months)	63.9				Soy
	Isomil Advance (iron, soy, DHA, ARA, 0–12 months)	59.5	56.1	61.1	40.2	Soy
Parent's Choice	Infant Formula (iron, beta-carotene, nucleotides, 0–12 months)			37.3		Full T
	2 (iron, calcium, 6–18 months)	40.0				Full T
	(Iron, omega 3 and 6, 0–12 months)	38.3				Full T
	(Iron, soy, DHA, ARA, lipids, 0–12 months)			50.0		Soy
	(Iron, soy, DHA, ARA, 0–12 months)			43.0		Soy
Target	(Iron, DHA, ARA, fussiness/gas, 0–12 months)			24.9		Spec
	Next Stage Formula (iron, DHA, ARA, 9+months)			51.1		Full T
Earth's Best	(Iron, DHA, ARA, 0–12 months)				33.3	Full T
	(Soy, iron, 0–12 months)				80.3	Soy

[a] All samples were analyzed in triplicate

When the mean Mo content of all formulas of a same type (from all brands combined) was compared between different locations of purchase, no significant differences were detected between the locations (data not presented). For example, there were no significant differences between the mean Mo content of all full-term type formulas purchased in Winnipeg, Washington, Grand Forks, and San Diego.

Discussion

Mo Concentration in HM and Infant Formulas

Our results for Mo concentration in HM (5.1±0.81 μg/L) were in general accordance with other studies. Bougle et al. [18] reported Mo to be 1.4 to 10.2 μg/L in term and preterm HM of French mothers during the first month of lactation. Casey and Neville [19] reported Mo levels less than 2 μg/L in HM of US mothers more than 1 month after delivery. Biego et al. [20] reported Mo contents of 4±3 μg/L in mature HM from French mothers. As dietary intake influences Mo concentration in HM, in those populations with higher Mo intake, such as the Japanese, higher Mo contents will be found in the HM of local women [21].

Our results for the Mo concentration of infant formulas (15.4 to 80.3 μg/L) fell within previously reported ranges, which vary considerably. In Germany, Sievers et al. [7] reported Mo concentrations that ranged from 12 to 259 μg/L. In France, Bougle and coworkers [22]

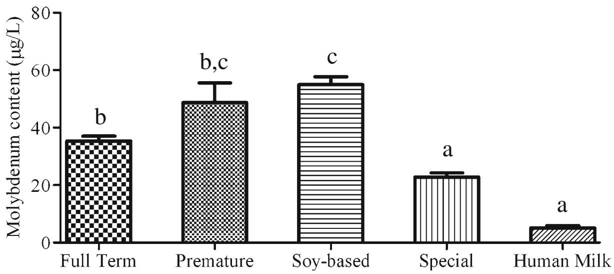

Fig. 1 Molybdenum content (mean±SE) of infant formulas and human milk. *Columns with different superscript letters* are significantly different ($p<0.05$)

detected Mo in the range of 18 to 204 μg/L. According to their results, some infant formulas may provide as much as 100 times more Mo than HM [22]. In contrast, six Japanese formulas were found to contain 2 to 3 μg/L of Mo [21].

Full-term, premature, and soy-based formulas in the present study contained significantly higher levels of Mo than HM. Soy-based formulas were found to contain significantly more Mo than full-term milk-based formulas. This is in agreement with Ikem et al. [16], who reported higher average levels in US soy-based formulas compared with the milk-based formulas from the USA, UK, and Nigeria. Similarly, in his study, Biego et al. [20] reported that the mean concentration of Mo in HM (4±3 μg/L) was approximately 40-fold less than in soy milk (160±80 μg/L) and nine times less than in cow's milk (30 to 35 μg/L). The Mo content of plant-based foods is determined by the element content of the soil; animal-sourced products generally contain low Mo content [2,5]. Therefore, the higher Mo content of soy-based formulas compared with cow milk-based formulas and with HM may be attributed to the substantial difference in the element content of the raw material. Additional Mo may be provided through water during preparation of the powdered formulas [9]. Mo levels in potable water usually do not exceed 10 μg/L [4].

"Special" formulas did not contain significantly different Mo concentration from HM. This is probably due to the usually practiced adulteration of the raw product of the protein base.

Mo Concentration by Brand

As no significant differences in Mo content were detected between same formula types of different brands, it may be concluded that the Mo content and the quality of the raw materials were consistent among the manufacturers of the studied products. It may also be implied that manufacturing practices and packaging containers do not influence final Mo content in the products.

Mo Concentration by Geographical Area

As no significant differences in Mo content were detected between same formula types purchased at different locations, we hypothesize that there is no influence of different sources of the raw materials that may be used in the manufacturing plants in different geographical areas across Canada and the USA. Infants fed by a same type of infant formula in this region receive same amount of Mo through feeding. Similarly, Ikem et al. [16] did not detect differences in Mo content of milk-based powdered formulas from three continents (USA, UK, and Nigeria). Therefore, we hypothesize that the type of the raw material on which the formula is based, rather than the source of it, influences the final Mo content.

Estimated and Recommended Mo Intakes of Full-Term Infants

In the present study, Mo concentrations of soy-based formulas were significantly greater than those of cow's milk-based formulas for full-term infants and for premature infants. These three formula types were significantly higher in Mo than HM. Full-term infants consume an average of 0.78 L of milk per day from birth to six months and an average of 0.6 L/day thereafter [1,2]. From data analyzed in the current study, exclusively breast- and formula-fed infants' Mo intakes are approximately 4 and 18–43 μg/day (depending on the type), respectively, from birth to 6 months of age, when diet is generally based on HM or formula alone. At seven to 12 months, the reduction in daily milk consumption with the introduction of other foods lowers Mo intake to 3 and 13–33 μg/day, for breast-fed and formula-fed infants, respectively.

Lopez-Garcia et al. [23] concluded that the bioavailability of Mo in HM is greater than that in milk-based formula. Bougle et al. [22] also showed that Mo is absorbed better from HM than from milk-based formula, even though the Mo concentration in the preterm formula was 60-fold greater than that in HM. According to the Institute of Medicine [1], soy products provide the most poorly absorbed Mo (~57%). Therefore, in order to achieve levels of absorbed Mo similar to that from HM, higher levels of Mo in both soy- and cow's milk-based formulas may be required. The data from our study suggest that the Mo intake of exclusively formula-fed Canadian and US full-term infants is approximately 4- to 11-fold greater than those who are exclusively breastfed from birth to 12 months. For full-term infants aged zero to 6 months and 7 to 12 months, the Dietary Reference Intakes are 2 and 3 μg/day, respectively [1]. Therefore, the actual intakes of Mo of formula-fed infants may be substantially higher than recommended and may pose health risks, even when taking into account lower bioavailability of Mo from formula. To our knowledge, the potential risks associated with these elevated intakes have not been studied. It may be that "special" formulas, containing low Mo, which is not well absorbed, could be considered for supplementation with Mo to a level yet to be established.

Estimated and Recommended Mo Intakes of Preterm Infants

In the current study, premature infant formulas were found to contain 48.7±6.8 μg Mo/L. From an ongoing study of premature infants in hospital, we calculated a mean daily intake of 115 mL HM and 75 mL formula from data obtained for 65 subjects. Average milk intake from both HM and formula provided approximately 4.25 μg Mo/day. Using mean weights from mid-hospitalization (1,799 g), we estimate mean Mo intakes to be 2.36 μg/kg/day. The Canadian Pediatric Society [24] has recommended an intake of 0.2–0.4 μg/kg/day for premature infants, while Friel et al. [17] suggested that an oral intake of 4 to 6 μg/kg/day would be adequate for low birth weight infants. These estimated and the recommended intakes fall well below the European Commission's UL of 10 μg/kg/day [4]. However, actual daily intakes of some infants may have been considerably higher than the estimated if they receive a greater proportion of feeds as formula.

Mo Toxicity and Upper Limits of Intake

The results of the current study suggest that the Mo intakes of Canadian and US formula-fed infants of 18–43 μg/day from birth to 6 months of age and of 13–33 μg/day at 7 to 12 months are within Mo toxicity guidelines established in other countries. Based on the 50th percentile of weight for age [25], the European Commission's no observed adverse effect level [4] for infants would be approximately 77 μg/day at 6 months and 93 μg/day at 1 year of age. The New Zealand Food Authority's estimated that safe and adequate value for infants is 30 μg Mo/day [4]. To our knowledge, no guidelines of Mo toxicity exist in Canada and in the USA.

An American study reported an estimated intake of 100 μg Mo/day from all food sources for infants aged 6 to 11 months [26]. The researchers estimated that complementary foods provide 33% of total dietary Mo. The significant additional contribution of these foods may increase the risk of toxicity for formula-fed infants, especially when formula Mo concentrations exceed those detected in the present study.

Conclusions

The mean Mo content of soy-based formulas and in formulas based on cow milk intended for full-term or for premature infants feeding is higher than in HM. Despite lower bioavailability of Mo from formula compared with HM, the high intake may pose health risks, especially for premature infants due to immaturity of their compensatory mechanisms. These health risks should be studied. Formulas intended for feeding of infants with special needs contain similar Mo levels as HM, but as it is less well absorbed, supplementation of this product should be considered.

No significant differences were detected in the mean Mo content between formulas of the same type purchased from different brands and/or at different geographical locations in Canada and in the USA. We conclude that infants fed by a same type of infant formula in all regions receive a consistent amount of Mo. We hypothesize that the source of the differences in the element content between formula types and HM originates in the type of the raw material and in the Mo added through water during preparation of the product for feeding rather than in the manufacturing practices or different source of the same raw material. The Mo intakes of Canadian and US formula-fed infants are within Mo toxicity guidelines, though contribution of complementary foods may increase the risk of toxicity.

Limitations of the Study

In the current study, only the formulas available for purchase were analyzed. No infant sample collections were made in order to estimate the Mo absorption.

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Conflict of Interest There is no conflict of interest for any author.

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