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► To cite this version:

Erika J. Mitchell, Seth H. Frisbie, Stéphane Roudeau, Asuncion Carmona, Richard Ortega. How much manganese is safe for infants? A review of the scientific basis of intake guidelines and regulations relevant to the manganese content of infant formulas. *J.Trace Elem.Med.Biol.*, 2021, 65, pp.126710. 10.1016/j.jtemb.2020.126710 . hal-03115779

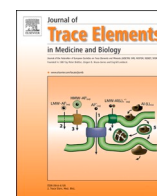
HAL Id: hal-03115779

<https://hal.science/hal-03115779>

Submitted on 25 Oct 2021

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Review

How much manganese is safe for infants? A review of the scientific basis of intake guidelines and regulations relevant to the manganese content of infant formulas

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ARTICLE INFO

Keywords:

Toddler formula
Neurotoxicity
Intellectual development
Tolerable daily intakes
Drinking water guidelines
Codex Alimentarius

ABSTRACT

Background: Recent research has uncovered the potential for excess manganese (Mn) intakes causing significant neurotoxic effects for early brain development.

Methods: We identified the Mn tolerable intakes (TI) published by the U.S. Institute of Medicine (IOM), World Health Organization (WHO), *Agence nationale de sécurité sanitaire* (ANSES), and U.S. Environmental Protection Agency (US EPA) and examined the primary studies on which regulatory TIs are based. We converted the TIs to μg of Mn/kg/day using standard assumptions specific to each agency. We estimated μg of Mn/kg/day intakes due to formulas. Using our estimates for formula intakes, weights, and kcal content, we converted regulatory maxima and minima from μg of Mn/100 kcal to estimates of μg of Mn/kg/day.

Results: Except for the proposed ANSES TI for drinking water, none of the primary studies on which Mn intake guidelines and regulations are based measured health outcomes. Some infant formulas may exceed the regulatory TIs, especially if prepared with water containing considerable concentrations of Mn (e.g. 250 $\mu\text{g}/\text{L}$), even while meeting national and international regulatory standards or guidelines.

Conclusions: Infant formula regulations must be revised to reduce the potential for excess manganese intakes and the practice of manganese supplementation of infant formulas should be ceased.

1. Background

Comparisons between breast-fed infants and infants fed by alternative means have always shown and continue to show superior survival rates and health outcomes for breast-fed infants, which is why the World Health Organization (WHO) stresses that “infants should be exclusively breastfed for the first six months of life to achieve optimal growth, development and health” [1]. In the early 1800s, before the discovery of

pathogenic microorganisms, the mortality rate for infants who were not breast-fed was nearly 88 % [2].

It is possible that the higher Manganese (Mn) content of present-day infant formulas compared to breast milk could be associated with adverse neurological effects for some formula-fed infants. Infant formulas currently have Mn content up to 1,000 times that of breast milk [3], and formula-feeding results in higher Mn intake than breast-feeding [4]. Exposures to high concentrations of Mn in drinking water have been

Abbreviations: AAP-CON, Committee on Nutrition of the American Academy of Pediatrics; AI, Adequate Intake; ANSES, *Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail*; EC, European Commission; ESGHAN, European Society for Paediatric Gastroenterology Hepatology and Nutrition; EU, European Union; FAO, Food and Agriculture Organization; FDA, Food and Drug Administration; GUL, Guidance Upper Level; HA, Health Advisory; IOM, Institute of Medicine; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LOAEL, Lowest Observed Adverse Effect Level; LS, *limite de sécurité*; LSRO, Life Sciences Research Organization, Inc; Mn, Manganese; NAM, National Academy of Medicine; NFA, National Food Agency; NOAEL, No Observed Adverse Effect Level; RfD, Reference Dose; SCF, Scientific Committee on Food; TDI, Tolerable Daily Intake; U.S. EPA, United States Environmental Protection Agency; UF, uncertainty factor; UL, Tolerable Upper Intake Level; US, United States; WHO, World Health Organization.

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<https://doi.org/10.1016/j.jtemb.2020.126710>

Received 1 September 2020; Received in revised form 25 November 2020; Accepted 20 December 2020

Available online 25 December 2020

0946-672X/© 2020 The Author(s).

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associated with adverse neurological outcomes in school-aged children [5–8].

In this paper, we review the tolerable intake levels for Mn used by international, US, and French regulatory and advisory agencies, identifying and examining the original studies used to set these tolerable intake levels. We then compare these regulatory tolerable intake levels to the regulations and guidelines that apply to infant formulas and young child nutritional beverages (follow-on/-up and toddler formulas) in the US and France. We note that there is very little evidence that the tolerable intake levels used by regulatory bodies are protective of the neurological health of young infants; nevertheless, current infant formula regulations allow for exceedances of these questionable tolerable intake levels in some cases. Consequently, products on the market in France and the US can satisfy local regulations yet lead to excess Mn intakes that might harm the neurological development of infants and young children.

2. Material and methods

2.1. Sufficiency and tolerable intakes

We searched for tolerable intakes established by national and international governmental or advisory agencies pertinent to residents of the United States and France using governmental databases, Google Scholar, and Google. For each of the published tolerable intakes that we found, we examined the supporting documentation for the tolerable intake and identified the key study or studies that formed the basis or bases cited for the tolerable intake. We reviewed each of these key studies to determine the type of study, the type and duration of exposure, the number and age of subjects, and whether health effects were measured.

2.2. Infant formula and young child nutritional beverage regulations and guidelines

We identified the national and international regulations and guidelines that apply to infant formulas and young child nutritional beverage products sold in the United States and France. We searched the revision history and background documentation for each regulation or guideline to identify when the current minima or maxima were first established, whether and when they were revised, and the bases for each specific regulation or guideline.

2.3. Estimating weights and intakes

Tolerable intake levels for Mn used by regulatory and advisory agencies are expressed in terms of μg of Mn/day for adults [9–11]. We converted these figures to $\mu\text{g}/\text{kg}/\text{day}$ to extrapolate these tolerable intake levels to infants and young children. Some authors have argued that infants and young children may be more susceptible to effects of excess Mn than adults, so extrapolating adult tolerable intakes to apply to infants and young children may yield estimates that are too high for this vulnerable population [8,12,13]. We calculated the extrapolated equivalences of adult tolerable intakes solely to enable comparison of the agency adult tolerable intakes levels with Mn intake estimates due to formula. When calculating Mn intakes due to formula products in units of $\mu\text{g}/\text{kg}/\text{day}$, we used weight and intake estimates for infants and young children at ages 3 weeks, 4.25 months, 7 months, and 18 months from breast milk, infant formulas, and young child nutritional beverage products from Mitchell et al. [4], summarized in Table 1. Data on the Mn content of infant formula and young child nutritional beverages were drawn from our market basket study of infant formula and young child nutritional beverage products sold in the US and France [3].

3. Results and discussion

3.1. Manganese sufficiency level estimates

The United States National Academy of Medicine (NAM) is a non-governmental organization that provides advice on health and medicine to the US and the international community; it was formerly called the Institute of Medicine (IOM) [20]. Under the name IOM, it published well-known guidelines on nutrient sufficiency and tolerability levels [9]. The IOM notes that Mn deficiency has never been reported in exclusively breast-fed infants, so it assumes that breast milk provides sufficient Mn for development in young infants, 0–6 months [9]. The IOM Adequate Intake (AI) for 0–6 month-old-infants is $3\mu\text{g}$ of Mn/day based on an estimated Mn concentration in breast milk of $3.5\mu\text{g}/\text{L}$ and a reference weight of 7 kg for infants 2–6 months old [9]. The European Food Safety Authority (EFSA) has not set a specific AI for Mn intake for 0–6 month-olds [21].

The standard regulatory units for infant formulas are μg of Mn/100 kcal of prepared product. These units allow for automatic scaling by type of product and the nutritional needs of the child. For comparison to the Mn content in infant formulas expressed in regulatory units, $3.5\mu\text{g}$ of Mn/L in breast milk would be equivalent to $0.50\mu\text{g}$ of Mn/100 kcal in breast milk, assuming an average gross energy of $697\text{ kcal}/\text{L}$ of breast milk at 3 months of lactation; see Eq. (1) [17].

$$\begin{aligned} \frac{\text{IOM Concentration of Mn in Breast Milk Expressed in Regulatory Units}}{100 \text{ kcal}} &= \frac{1 \text{ L of Breast Milk}}{697 \text{ kcal}} \times \frac{3.5 \mu\text{g of Mn}}{1 \text{ L of Breast Milk}} \\ &= \frac{0.50 \mu\text{g of Mn}}{100 \text{ kcal}} \end{aligned} \quad (1)$$

The estimated Mn concentration in breast milk of $3.5\mu\text{g}/\text{L}$ used by the IOM in 2001 was based on 5 studies with a total of 81 mothers conducted from 1984 to 1986 [9]. In our more extensive review of 21 studies published between 1984–2017 consisting of 5,423 samples, the mean Mn concentration of breast milk was $7.7\mu\text{g}$ of Mn/L, equivalent to $1.1\mu\text{g}$ of Mn/100 kcal [4].

3.2. Tolerable intakes for manganese used by international, United States, and French regulatory and advisory agencies

3.2.1. The United States Institute of Medicine tolerable Upper intake Level for manganese ingestion

In 2001, the IOM set a tolerable Upper intake Level (UL) of 11 mg of Mn per day “for adults based on a no-observed-adverse-effect level for Western diets” [9]. The IOM used their 11 mg of Mn per day UL for adults to develop ULs for the ingestion of Mn in food by children at different ages through extrapolation by body weight [9].

The IOM 11 mg of Mn per day UL for adults [9] was set at the maximum Mn intake of data presented in a 1999 review article, rounded up to 11 mg/day from the $10.9\text{ mg}/\text{day}$ stated in the article [22]. The study in the review article that mentioned $10.9\text{ mg}/\text{day}$ as a maximum intake was a 3-day dietary survey by Gibson and Scythes of 100 pre-menopausal Canadian women from a university community that did not measure any health outcomes and did not measure the weight of its subjects [22,23]. In the Gibson and Scythes study, only 10 % of subjects had an intake over 5 mg of Mn per day for these 3 days; that is, no more than 10 subjects exceeded 5 mg of Mn per day for the 3 days of the survey, and the published histogram of subject Mn intakes indicates that most likely only 2 out of 100 subjects had an intake of approximately 10 mg of Mn/day for these 3 days [23]. Nevertheless, the intake of these 2 subjects over the 3 days of the survey has become the basis for several international dietary and drinking water regulations and guidelines.

No health outcomes were measured in this 1982 study of adult women [23], so the study does not provide information about possible health effects at the reported dietary extremes. Since no attempt was

Table 1

Estimated daily manganese (Mn) intakes for infants and young children at 3 weeks, 4.25 months, 7 months, and 18 months from breast milk, and minimum and maximum intakes from infant formulas and young child nutritional beverage products sampled in a market basket survey of products available on the US and French markets [3,4]. These estimates assume that the products are reconstituted with water containing 0 µg of Mn/L.

Feeding Method	Market	Minimum, Mean, and Maximum	Mean Body Weight (kg)	Mean g of Breast Milk or Product/kg of Body Weight/Day	Mean g Breast Milk or Product/Day	Mean Total kcal/kg/Day	Mean Total kcal/Day	Mean % Energy/Day from Milks	Mean kcal/Day from Breast Milk or Product	Mean kcal/L	Density (g/L)	Mean L/Day	µg of Mn/L	µg of Mn/day	µg of Mn/kg of Body Weight/Day	µg of Mn/kg of Body Weight/Day Relative to Mean Breast Milk
3 Weeks																
Breast milk			3.91 ^a	159 ^a	622		420			697 ^d	1,031 ^f	0.603				
		Min											0.17 ^g	0.10	0.026	0.022
		Mean											7.7 ^g	4.6	1.2	1.0
		Max											30.27 ^g	18.3	4.67	3.9
Formula			3.84 ^a	163 ^a	626		414			662	1,010	0.620				
	US	Min											210	130	34	28
		Max											830	520	130	110
	France	Min											240	150	38	32
		Max											560	340	90	76
4.25 Months																
Breast milk			6.5 ^a	123 ^a	800		540			697 ^d	1,031 ^f	0.78				
		Min											0.17 ^g	0.13	0.020	0.022
		Mean											7.7 ^g	6.0	0.92	1.0
		Max											30.27 ^g	23	3.6	3.9
Formula			6.89 ^a	146 ^a	1,010		668			662	1,010	1.00				
	US	Min											210	210	30	33
		Max											830	830	120	130
	France	Min											240	230	34	37
		Max											560	550	80	88
7 Months																
Breast milk			8.066 ^b			91	730	58.6	430	741.8 ^e		0.58				
		Min											0.17 ^g	0.10	0.012	0.022
		Mean											7.7 ^g	4.5	0.55	1.0
		Max											30.27 ^g	18	2.2	3.9
Formula			8.421 ^b			91 ^c	770	58.6 ^c	450	669		0.67				
	US	Min											210	140	17	30
		Max											830	560	66	120
	France	Min											200	140	16	29
		Max											560	370	44	80
18 Months																
Breast milk			10.889 ^b			87.4	952	21.3	203	880.3 ^e		0.230				
		Min											0.17 ^g	0.039	0.0036	0.022
		Mean											7.7 ^g	1.8	0.16	1.0
		Max											30.27 ^g	6.97	0.640	3.9
Formula			11.189 ^b			87.4 ^c	978	21.3 ^c	208	622		0.335				
	US	Min											160	54	4.8	30
		Max											2,800	950	85	520
	France	Min											200	68	6.1	38
		Max											560	190	17	100
Medical beverage	US		11.189 ^b			87.4 ^c	978	100.	978	1,000						

(continued on next page)

Table 1 (continued)

Feeding Method	Market Minimum, Mean, and Maximum	Mean Body Weight (kg)	Mean g of Breast Milk or Product/ kg of Body Weight/Day	Mean g Breast Milk or Product/ Day	Mean Total kcal/ kg/Day	Mean Total kcal/ Day	Mean % Energy/ Day from Milks	Mean kcal/Day from Breast Milk or Product	Mean kcal/L	Density (g/L)	Mean L/ Day	µg of Mn/L	µg of Mn/day	µg of Mn/kg of Body Weight/ Day	µg of Mn/kg of Body Weight/ Day	Relative to Mean Breast Milk
	Min															880
	Max															1,100

(a) Sievers et al. [14] (German breast-fed and formula-fed infants).
(b) Dewey et al. [15] (US breast-fed and formula-fed infants; means calculated from results originally reported separately for boys and girls).
(c) Fantino and Gourmet [16] (French formula-fed infants; energy originally reported in kilojoules, kJ).
(d) Nommensen et al. [17] (US mothers; energy measured at 3 months lactation).
(e) Mandel et al. [18] (Location of study not specified; energy originally reported in kJ/L; 741.8 kcal/L is calculated for 2–6 months; 880.3 = kcal/L is calculated for 12–36 months).
(f) Macey [19].
(g) Mitchell et al. [4].

made to measure health effects, it is entirely unknown whether the women at the dietary intake extremes experienced health effects at similar or different rates than those with mean dietary intakes. It is possible that women at the intake extremes could have experienced subtle health effects. In a typical epidemiological study designed to determine whether exposure to a substance affects risk, health effects are measured and incidence of health effects is compared across exposure levels [e.g. 24,25]. Since the Gibson and Scythes [23] study did not attempt to measure any health effects or compare outcomes at different intake levels, the study cannot be legitimately said to support the assumption that an adult intake of 11 mg of Mn day is tolerated without adverse effects.

In addition, since the Gibson and Scythes study [23] was based on young to middle-aged adults, it does not provide information about possible health effects of Mn intakes for older adults or infants and young children, who may be more sensitive to high Mn intakes than middle-aged adults [8]. The IOM recognized this increased sensitivity for infants and stated, “For infants, the UL was judged not determinable because of lack of data on adverse effects in this age group and concern about the infant’s ability to handle excess amounts. To prevent high levels of manganese intake, the only source of intake for infants should be from food or formula” [9].

For children 1–3 years old, the IOM assumed a 13 kg body weight and extrapolated a 2 mg Mn per day (2,000 µg Mn/day) UL; see Eq. (2) [9].

$$\begin{aligned} \text{Upper Level} \\ (1 - 3 \text{ years old}) &= \left(13 \text{ kg of body weight} \times \frac{11 \text{ mg of Mn}}{70 \text{ kg of body weight} \times \text{day}} \right) \\ &= \frac{2 \text{ mg of Mn}}{\text{day}} = \frac{2,000 \text{ } \mu\text{g of Mn}}{\text{day}} \end{aligned} \tag{2}$$

More generally, using the IOM assumption of an adult body weight of 70 kg [9], the IOM UL would be equivalent to 160 µg Mn/kg/day; see Eq. (3) [9].

$$\begin{aligned} \text{Upper Level} \\ \left(\frac{\mu\text{g of Mn}}{\text{kg of body weight} \times \text{day}} \right) &= \frac{11 \text{ mg of Mn}}{70 \text{ kg of body weight} \times \text{day}} \\ &\times \frac{1,000 \text{ } \mu\text{g of Mn}}{1 \text{ mg of Mn}} \\ &= \frac{160 \text{ } \mu\text{g of Mn}}{\text{kg of body weight} \times \text{day}} \end{aligned} \tag{3}$$

However, as discussed above, the 2 mg of Mn per day UL for 1-3-year-old children is based on the untested assumptions that people consuming high intakes of Mn have no different health outcomes than those consuming mean intakes and that children can tolerate Mn intakes equivalent by weight as adults.

Despite its potential inadequacy as a measure of tolerable intake, several of the products that we analyzed in our recent market basket survey of French and US infant formulas and young child nutritional beverages had Mn concentrations that would likely lead to exceedances of this IOM UL when used as directed [3,4].

3.2.2. The World Health Organization No Observed Adverse Effect Level for manganese ingestion

The United Nations Food and Agriculture Organization (FAO) and the Joint Expert Committee for Food Additives (JECFA) have not established a tolerable intake level for Mn in food. However, the WHO has published a Health Based Value (HBV) for Mn in drinking water [26]. The derivation of this HBV references a No Observed Adverse Effect Level (NOAEL) of 11 mg Mn/day for adults based on dietary exposures [11]. This 11 mg Mn/day adult NOAEL ultimately comes from the same study [23] that the IOM UL of 11 mg Mn/day is based on [9,22,26].

Using the WHO’s standard assumption of adult weight as 60 kg [26], the NOAEL of 11 mg of Mn/day would be equivalent to 180 µg/kg/day;

see Eq. (4).

$$\begin{aligned} \left(\frac{\text{NOAEL}}{\frac{\mu\text{g of Mn}}{\text{kg of body weight} \times \text{day}}} \right) &= \frac{11 \text{ mg of Mn}}{60 \text{ kg of body weight} \times \text{day}} \\ &\times \frac{1,000 \mu\text{g of Mn}}{1 \text{ mg of Mn}} \\ &= \frac{180 \mu\text{g of Mn}}{\text{kg of body weight} \times \text{day}} \end{aligned} \quad (4)$$

The WHO used this 11 mg of Mn/day adult dietary NOAEL to calculate a tolerable daily intake (TDI) for drinking water of 0.06 mg of drinking water Mn/kg of body weight/day; see Eq. (5) [11,26].

$$\text{TDI} = \frac{\left(\frac{11 \text{ mg of Mn}}{60 \text{ kg of body weight} \times \text{day}} \right)}{3 \text{ uncertainty factor}} = \frac{0.06 \text{ mg of Mn}}{\text{kg of body weight} \times \text{day}} \quad (5)$$

This derivation uses an uncertainty factor that applies specifically to water, “to allow for the possible increased bioavailability of manganese from water” [11] since the NOAEL was originally drawn from dietary exposures [23]. The National Food Agency (NFA) of Sweden stated that, “this TDI for manganese was based on weak scientific evidence and should not be considered as a definite level under which there is no reason for concern” [27].

Since the study from which the 11 mg/day NOAEL was drawn included only young to middle-aged adults, it does not provide information about adverse possible health effects of Mn intakes for older adults or infants and young children, who may be more sensitive than middle-aged adults to high Mn intakes [8]. Therefore, the assumed NOAEL from this survey and the resulting TDI for drinking water might not protect sensitive populations, such as infants, children, and the elderly [8]. Nevertheless, as noted above, despite its potential inadequacy as a measure of tolerable intake, this NOAEL, extrapolated by weight to apply to 1–3 year olds, would be exceeded by some young child nutritional beverage products sold on the US market [4].

3.2.3. The United States Environmental Protection Agency Reference Dose for manganese ingestion

The United States Environmental Protection Agency (U.S. EPA) has established a Lifetime Health Advisory (HA) for Mn [28] of 0.3 mg of Mn/L of drinking water, based on a Reference Dose (RfD) of 0.14 mg Mn/kg body weight/day [10,28]. This calculation assumes a 70 kg adult, a 2 L drinking water/day intake, a 20 % relative source contribution factor, and a “3 fold modifying factor to account for increased bioavailability from drinking water”; see Eq. (6) [10,28].

$$\begin{aligned} \text{HA} &= \frac{\left(\frac{70 \text{ kg of body weight}}{\text{body weight}} \times \frac{\text{day}}{2 \text{ L of drinking water}} \times \frac{0.14 \text{ mg of Mn}}{\text{kg of body weight} \times \text{day}} \right) \times 20\%}{3 \text{ modifying factor}} \\ &= \frac{0.3 \text{ mg of Mn}}{\text{L of drinking water}} = \frac{300 \mu\text{g of Mn}}{\text{L of drinking water}} \end{aligned} \quad (6)$$

The 0.14 mg Mn/kg/day RfD was derived from a 10 mg Mn/day NOAEL based on dietary information [10].

In deriving this RfD, the U.S. EPA assumed an adult body weight of 70 kg and an Uncertainty Factor (UF) of 1; see Eq. (7) [10].

$$\text{RfD} = \frac{\left(\frac{10 \text{ mg of Mn}}{70 \text{ kg of body weight} \times \text{day}} \right)}{1 \text{ uncertainty factor}} = \frac{0.14 \text{ mg of Mn}}{\text{kg of body weight} \times \text{day}} \quad (7)$$

In justifying the UF of 1, the U.S. EPA wrote, “The use of 1 as the UF is based on the following considerations. Manganese is an essential trace element for human health. The information used to derive the RfD was collected from many large human populations consuming normal diets

over an extended period of time. The available data suggest that as long as physiological systems are not overwhelmed, humans exert effective homeostatic control over manganese so that body burden is kept relatively constant even when the concentration of manganese in the diet varies” [10].

The U.S. EPA notes that the RfD for ingestion of Mn is based on “dietary information described by WHO (1973), Schroeder et al. (1966), and NRC (1989)” [10]. The National Research Council (NRC) 1989 reference [29] used by the U.S. EPA [10] to support its NOAEL of 10 mg of Mn/day was a review of Mn as an essential element and an analysis of recommended daily intakes of Mn. In this analysis, the NRC noted, “In humans, toxicity has been observed only in workers exposed to high concentrations of manganese dust or fumes in air, but not as a consequence of dietary intake by people consuming 8–9 mg of manganese per day in their food (WHO, 1973) In view of the remarkably steady tissue concentrations of manganese in the U.S. population (Schroeder et al., 1966) and the low toxicity of dietary manganese, an *occasional* intake of 10 mg/day by adults can be considered safe” [29 (emphasis added)]. That is, this NRC review [29] referred to the other 2 references cited above, WHO (1973) [30] and Schroeder et al. (1966) [31], but did not cite any additional research to support its statement that an occasional intake of 10 mg of Mn/day by adults could be considered safe.

The Schroeder et al. (1966) reference [31] used by the U.S. EPA [10] to support its NOAEL of 10 mg of Mn/day was a review of Mn as a trace element in nutrition. This review included a summary table of Mn in sample diets showing diets that ranged from 2.2 mg of Mn/day to 8.8 mg of Mn/day [31]. Both dietary extremes came from the same 1941 Mn balance study by Kent and McCance in which 2 people in England were fed experimental diets for an unspecified amount of time [31,32]. The reported minimum intake of 2.2 mg of Mn/day came from 1 of the 2 subjects whose diet was manipulated to include large amounts of “white” flour, and the 8.8 mg maximum intake of Mn/day came from the remaining subject whose diet was manipulated to include large amounts of “brown” flour. That is, the 8.8 mg of dietary Mn/day derived from this study [32], cited in Schroeder et al., (1966) [31] and assumed by the U. S. EPA [10] as a maximum intake of dietary manganese came from a single subject fed an experimental diet for an unspecified amount of time in 1941; the original research by Kent and McCance [32] did not attempt to measure and did not report any potential health effects of this dietary Mn intake.

The WHO (1973) reference [30] used by the U.S. EPA [10] to support its NOAEL of 10 mg/day was a review of trace elements in nutrition, in which it was stated, “Investigations of adult diets in Europe, India, and the USA have revealed manganese intakes ranging from 2.0 to 8.8 mg/day.^{1,2}...It ... seems logical to assume that manganese intakes of 8–9 mg/day are perfectly safe, since there was no evidence of manganese toxicity in the individuals consuming these high-manganese diets” [30]. Reference [1] was to a chapter in a textbook of human and animal nutrition [30,33]; the maximum daily Mn intake noted in reference [1] was the 8.8 mg of Mn/day from the 1941 2-person experimental balance study in England [32,33]. Reference [2] was to a 1969 water and dietary survey in India by Soman et al. in which total intakes of Mn were estimated based on analyses of drinking water and 31 “representative samples of the total daily intake of food”, which were 24-h duplicate dietary samples from volunteers residing in Bombay chosen to represent “different groups of society...coming from different parts of the sub-continent” [30,34]; a mean dietary intake of 8.1 mg of Mn/day was reported for all subjects [34]. No health data were reported for the subjects in the Soman et al. study [34].

Thus, it is not clear from the 3 references listed by the U.S. EPA where their 10 mg of Mn/day NOAEL came from [10]. The highest daily intake of Mn mentioned in the cited references is 8.8 mg of Mn/day [32]. While the NRC suggests, based on the maximum intake of 8.8 mg/day reported in its review, “an *occasional* intake of 10 mg/day by adults can be considered safe,” [29 (emphasis added)], 10 mg of Mn/day was adopted by the U.S. EPA as its NOAEL for *chronic* ingestion of Mn by humans

[10]. In any case, it can be concluded from these 3 references and the references that they cite that the most recent original research that could have been included in selecting the 10 mg of Mn/day NOAEL is the 1969 Soman et al. study with the mean 8.1 mg of dietary Mn/day intake [34].

Since this NOAEL was selected before more recent research that examined the link between the ingestion of Mn in drinking water and neurological harm to children [5–8,35–37], this important body of research on childhood manganese exposures through drinking water was not considered by the U.S. EPA when they established their NOAEL [10]. Therefore, this 10 mg of Mn/day U.S. EPA NOAEL is outdated.

Thus, the U.S. EPA 0.14 mg of Mn/kg of body weight/day was derived from a 10 mg of Mn/day NOAEL that was estimated to cover known dietary extremes [10]. The studies used to document the dietary extremes contained a total of 31 Indian subjects following ordinary diets in Bombay for 24 h in 1969 [34] and 2 subjects in England fed experimental diets for an unspecified amount of time in 1941 [32]. This is not consistent with the U.S. EPA description of “The information used to derive the RfD was collected from many large human populations consuming normal diets over an extended period of time” [10]. It is also not consistent with the following U.S. EPA definition of an RfD, “The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious health effects during a lifetime, and is derived from the NOAEL (or LOAEL [Lowest Observed Adverse Effect Level]), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s)” [10].

In addition, as with the WHO and IOM NOAELs discussed above [9, 11], the studies on which the U.S. EPA 10 mg of Mn/day NOAEL is based appear to have only included adults and did not report any investigation of health effects at the dietary extremes (the Soman et al. study describes subjects as “volunteers” but does not provide ages of subjects) [34]. Thus, the U.S. EPA 10 mg of Mn/day NOAEL may not be sufficiently protective for infants or young children.

3.2.4. The European Union declined to set tolerable intake levels for manganese ingestion

EFSA is an independent risk assessment agency funded by the European Union (EU) that has provided scientific advice on risks associated with the food chain to the EU since 2003 [38,39]. Until May 2003, the Scientific Committee on Food (SCF) of the European Commission (EC) provided advice on food safety to the EC [38]. EFSA recommends Tolerable Upper Intake Levels for vitamins and minerals in foods for the European Parliament [40]. By definition, an Upper intake Level (UL) is “an estimate of the highest level of intake which carries no appreciable risk of adverse health effects” [40]. Repeating the conclusions of the SCF [41], EFSA states, “Oral intake of manganese despite its poor absorption in the gastrointestinal tract has also been shown to cause neurotoxic effects. The limitations of the human data and the non-availability of NOAELs for critical endpoints from animal studies produce a considerable degree of uncertainty. Therefore, an upper level cannot be set” [40, 41]. EFSA reiterates the SCF [41] conclusion, “oral exposure to manganese beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit” [40].

The Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail proposed guideline for Mn in drinking water

The Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES; French Agency for Food, Environmental and Occupational Health & Safety) “is a public organisation reporting to the Ministries of Health, Agriculture, the Environment, Labour and Consumer Affairs” [42]. In 2016, ANSES followed EFSA regarding setting an upper limit for Mn intake, stating “L'Efsa confirme que les études menées chez l'animal n'ont pas permis d'identifier une NOAEL, et compte tenu des données limitées chez l'homme, il n'est pas possible de proposer une limite de sécurité pour le manganèse (EFSA confirms that animal studies have not been able to identify a NOAEL, and due to the

Table 2

Comparison of daily Mn intakes according to regulations and research in standard units of µg of Mn/kg/day.

Tolerable Levels for Dietary Exposure				
Organization	Publication	UL / NOAEL	µg of Mn/kg/day	Basis Study/Studies
EFSA	EFSA, 2006 [40]	Cannot be set		
ANSES	ANSES, 2018 [13]	25 mg/kg/day ^b	55	Kern et al., 2010 [46]
U.S. EPA	U.S. EPA, 2004 [10]; U.S. EPA, 2012 [28]	10 mg/day	140 ^c	Kent and McCance, 1941 [32]; Soman et al., 1969 [34]
IOM	IOM, 2001 [9]	11 mg/day	160 ^c	Gibson and Scythes, 1982 [23]
WHO	WHO, 2004 [11]; WHO 2017 [26]	11 mg/day	180 ^d	Gibson and Scythes, 1982 [23]
Regulations for Infant/Follow-on Formulas				
Organization	Publication	Minimum Mn content	µg of Mn/kg/day	Applies to
Codex Alimentarius	Codex Alimentarius, 2016 [50]	1 µg/100 kcal	1.07 ^e	infant formula
EU	EC, 2006 [51]	1 µg/100 kcal	1.07 ^e	infant and follow-on formula
France	ECEC0771649A, 2018 [52]	1 µg/100 kcal	1.07 ^e	infant and follow-on formula
US	21 CFR 107, 2017 [53]	5 µg/100 kcal	5.35 ^e	infant formula
EU	EC, 2006 [51]	Maximum Mn content 100 µg/100 kcal	107 ^e	infant and follow-on formula
France	ECEC0771649A, 2018 [52]	100 µg/100 kcal	107 ^e	infant and follow-on formula
Codex Alimentarius	CAC, 2016 [50]	100 µg/100 kcal	107 ^{e,f}	infant formula
US	21 CFR 107, 2017 [53]	None	Not set	
Intake Estimates for Formula Samples [10, 11]				
Type of product	Water Mn content	Maximum Mn content	µg of Mn/kg/day	
France infant formula	0 µg/L	93 µg/100 kcal	90 ^e	
France infant formula	250 µg/L	93 µg/100 kcal	120 ^e	
US infant formula	0 µg/L	120 µg/100 kcal	130 ^e	
US infant formula	250 µg/L	120 µg/100 kcal	170 ^e	
France follow-on formula	0 µg/L	55 µg/100 kcal	17 ^g	
France follow-on formula	250 µg/L	55 µg/100 kcal	23 ^g	

(continued on next page)

Table 2 (continued)

US toddler formula	0 µg/L	860 µg/ 100 kcal	85 ^g
US toddler formula	250 µg/L	860 µg/ 100 kcal	91 ^g
US pediatric medical formula	0 µg/L	240 µg/ 100 kcal	180 ^g
US pediatric medical formula	250 µg/L	240 µg/ 100 kcal	200 ^g

a) median of highest quintile.

b) LOAEL in rats.

c) assumes standard adult weight of 70 kg [9].

d) assumes standard adult weight of 60 kg [11].

e) 3-week old infant, 3.84 kg, 0.620 l/day (Table 1).

f) Guidance Upper Limit, not maximum level.

g) 18-month-old child, 11.189 kg, 0.978 L/day [4].

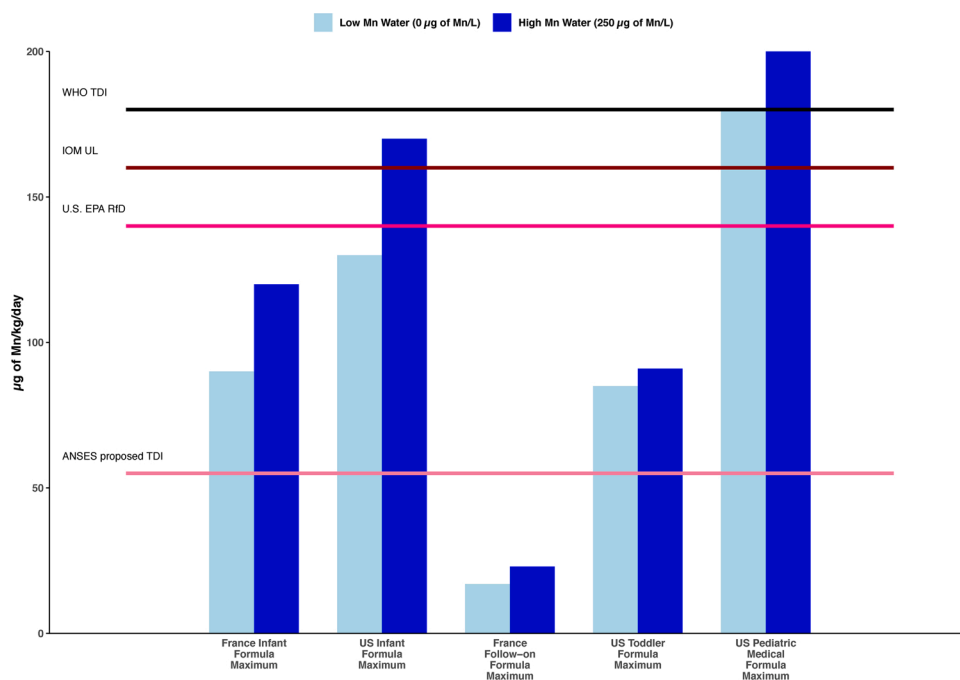


Fig. 1. Intakes of Mn per kg/day from infant formula and young child nutritional beverages compared to regulatory tolerable intake values. Ideally, powdered infant formula should be prepared with low Mn water (0 µg Mn/L). However, some consumers prepare it with tap water, which may contain considerably more Mn, such as 250 µg Mn/L, which is relatively high, but still less than the WHO 400 µg Mn/L Health-Based Value (HBV) for Mn [26] and the U.S. EPA 300 µg Mn/L Health Advisory (HA) for Mn [4,28]. Intake estimates drawn from Mitchell et al. [4] and Frisbie et al. [3]. Tolerable intake values are from ANSES proposed Tolerable Daily Intake (TDI) for drinking water [13], IOM Upper Limit (UL) for dietary intake [9], U.S. EPA Reference Dose (RfD) for drinking water [28], and WHO TDI for drinking water [11]; all except ANSES [13] are extrapolated from adult intake data and may not be sufficiently protective for the special vulnerabilities of developing infants.

Table 3

Summary of studies used as the basis for dietary and drinking water regulations and guidelines for manganese.

Study	Type of exposure	Exposure level	Duration of study	Number of subjects at exposure level or above	Mean age of subjects	Observed Health Effects	Basis for which regulations/guidelines
Kent and McCance, 1941 [32]	Dietary, humans	8.8 mg Mn/day = maximum ingested	unspecified	1	adults	not studied	U.S. EPA HA for Mn in drinking water [28]
Soman, et al., 1969 [34]	Dietary, humans	8.1 mg Mn/day = mean ingested	1 day	~16	"volunteers"	not studied	U.S. EPA HA for Mn in drinking water [28]
Gibson and Scythes, 1982 [23]	Dietary, humans	10.8 mg Mn/day = maximum ingested	3 days	~2	30 years	not studied	IOM UL for dietary Mn [9]; WHO HBV for Mn in drinking water [26]
Kern et al., 2010 [46]	Drinking water, rats	25 mg/kg/day = LOAEL	21 days postnatal	~40	0–21 days	Learning and behavioral deficits	ANSES proposed maximum concentration in drinking water [13]; Health Canada proposed MAC for drinking water [45]; Quebec proposed DWG for drinking water [54]; Minnesota short term HBV for drinking water [55]

cover uncertainty due to intra- and inter-species variation, use of a LOAEL instead of a NOAEL, and database deficiencies, yielding a TDI for humans of 55 µg of Mn/kg of body weight/day; see Eq. (8) [12].

$$\text{Tolerable Daily Intake (TDI)} = \frac{\left(\frac{25 \text{ mg of Mn}}{\text{kg of body weight} \times \text{day}} \times \frac{1,000 \text{ µg of Mn}}{1 \text{ mg of Mn}} \right)}{450 \text{ uncertainty factor}} = \frac{55 \text{ µg of Mn}}{\text{kg of body weight} \times \text{day}} \quad (8)$$

This amount of Mn would be provided by an infant formula with 340 µg of Mn/L when consumed by a 3-week-old infant weighing 3.84 kg and fed 0.620 L formula per day; see Eq. (9) [4].

$$\begin{aligned} \text{Tolerable Daily Intake (TDI)} &= \frac{3.84 \text{ kg of body weight}}{\text{body weight}} \times \frac{55 \text{ µg of Mn}}{\text{kg of body weight} \times \text{day}} \times \frac{1 \text{ day}}{0.620 \text{ L of prepared product}} \\ &= \frac{340 \text{ µg of Mn}}{\text{L of prepared product}} \end{aligned} \quad (9)$$

In standard regulatory units, the TDI of 340 µg of Mn/L would be equivalent to an infant formula with approximately 51 µg of Mn/100 kcal; see Eq. (10) [4].

$$\begin{aligned} \text{TDI Expressed in Regulatory Units} &= 100 \text{ kcal} \times \frac{3.84 \text{ kg of body weight}}{\text{body weight}} \times \frac{55 \text{ µg of Mn}}{\text{kg of body weight} \times \text{day}} \\ &\quad \times \frac{1 \text{ day}}{0.620 \text{ L of prepared product}} \times \frac{1 \text{ L of prepared product}}{662 \text{ kcal}} \\ &= \frac{51 \text{ µg of Mn}}{100 \text{ kcal}} \end{aligned} \quad (10)$$

3.2.5. Summary of tolerable intakes established by national and international agencies

The tolerable daily intakes for Mn set by national and international agencies are summarized in Table 2 and Fig. 1. These tolerable daily intakes are converted to standard units of µg of Mn/kg/day to facilitate comparison to infant formula regulations and infant intakes due to infant formula. The basis studies for the tolerable daily intakes are summarized in Table 3, together with the number of subjects and the durations of the studies.

3.3. International, French and United States regulations for manganese in infant formulas and young child nutritional beverages

3.3.1. The joint World Health Organization/Food and Agriculture Organization Codex Alimentarius Commission standards for manganese in infant formula and follow-up formula

The joint WHO/FAO Codex Alimentarius Commission develops “international food standards, guidelines and codes of practice [that] contribute to the safety, quality and fairness of this international food trade” [56]. The Codex Alimentarius publishes standards for infant formulas, follow-up formulas, and formulas for special medical purposes intended for infants [50,57,58].

For infant formula nutrients such as Mn, standards are stated in mass of nutrient/100 kcal of prepared formula to automatically scale to infant energy requirements according to infant body weight, to varying types of formula (powder or liquid), and to varying masses of powder used to prepare a volume of formula. The Codex Alimentarius states that infant formula prepared for consumption shall contain between 60 kcal (250 kJ) and 70 kcal (295 kJ) of energy per 100 mL, a minimum of 1 µg of Mn/100 kcal, and not exceed the Guidance Upper Level (GUL) of

100 µg of Mn/100 kcal [50,57]. The Codex Alimentarius does not provide guidance for the Mn content of formula products labelled for young children ages 1-year and older, which it terms “follow-up formulas” [58].

In our recent study of infant formulas and young child nutritional beverages available on the US and French markets [3], the mean energy content of the 21 powdered products labeled for use by 0–6 month-olds was 662.4... kcal/L of prepared formula. (Nonsignificant digits, such as 7..., are shown as a subscript followed by an ellipsis. These nonsignificant digits are included in all steps of a calculation to prevent rounding error.) Therefore, the 1 µg of Mn/100 kcal minimum would equal approximately 6.62 µg of Mn/L of prepared product; see Eq. (11).

$$\begin{aligned} \text{Estimated Minimum Volume Basis} &= \frac{1 \text{ µg of Mn}}{100 \text{ kcal}} \times \frac{662 \text{ kcal}}{\text{L of prepared product}} \\ &= \frac{6.62 \text{ µg of Mn}}{\text{L of prepared product}} \end{aligned} \quad (11)$$

When used as the sole source of nutrition for a 3-week old infant, a formula containing 6.62 µg of Mn/L would provide an intake of 1.07 µg of Mn/kg/day; see Table 1 and Eq. (12) [4].

$$\begin{aligned} \text{Daily Mn Intake at } 1 \text{ µg of Mn / 100 kcal} &= \frac{\frac{1 \text{ µg of Mn}}{100 \text{ kcal}} \times \frac{662 \text{ kcal}}{\text{L of prepared product}} \times \frac{0.620 \text{ L of prepared product}}{1 \text{ day}}}{\frac{3.84 \text{ kg of body weight}}{\text{body weight}}} \\ &= \frac{1.1 \text{ µg of Mn}}{\text{kg of body weight} \times \text{day}} \end{aligned} \quad (12)$$

Similarly, the 100 µg of Mn/100 kcal GUL would equal approximately 662 µg of Mn/L of prepared product in this study; Eq. (13).

$$\text{Estimated GUL Volume Basis} = \frac{100 \text{ µg of Mn}}{100 \text{ kcal}} \times \frac{662 \text{ kcal}}{\text{L of prepared product}} = \frac{662 \text{ µg of Mn}}{\text{L of prepared product}} \quad (13)$$

When used as the sole source of nutrition for a 3-week old infant, a formula containing 662 µg of Mn/L would provide an intake of 107 µg of Mn/kg/day; see Table 1 and Eq. (14) [4].

$$\begin{aligned} \text{Daily infant Mn Intake at } 100 \text{ µg of Mn/100 kcal} &= \frac{\frac{100 \text{ µg of Mn}}{100 \text{ kcal}} \times \frac{662 \text{ kcal}}{\text{L of prepared product}} \times \frac{0.620 \text{ L of prepared product}}{1 \text{ day}}}{\frac{3.84 \text{ kg of body weight}}{\text{body weight}}} \\ &= \frac{107 \text{ µg of Mn}}{\text{kg body weight} \times \text{day}} \end{aligned} \quad (14)$$

Prior to 2007, the Codex Alimentarius Standard for Infant Formula (Codex Standard 72-1981) [59] had specified a minimum of 5 µg of Mn/100 kcal and no maximum Mn content, which was identical to the requirements established for the US by the United States Congress in 1980 [59,60]. In 2004, the Codex Alimentarius requested recommendations from the Committee on Nutrition of the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) to update Codex Standard 72-1981 on Infant Formula [61]. ESPGHAN responded with a recommendation to lower the minimum concentration to 1 µg of Mn/100 kcal to be “in the order of human milk concentrations”, and to establish a maximum level of 50 µg of Mn/100 kcal. The specified maximum level was set not according to health data, but rather to be “equivalent to that of unsupplemented soy formula” [61], possibly to allow soy formulas to remain on the market without reformulation. At the time, it was recognized that the new maximum level of 50 µg of Mn/100 kcal would be “about 60 times higher than breast milk levels” [61]. In 2007, this recommendation by ESPGHAN to lower the minimum

to 1 µg of Mn/100 kcal was adopted by the Codex Alimentarius in its 2007 revision of its Standard for Infant Formula [57,59]. However, instead of establishing a maximum level of 50 µg of Mn/100 kcal according to the recommendations of ESGHAN [61], the Codex Alimentarius established the new criterion of Guidance Upper Limit (GUL), and created a GUL of 100 µg of Mn/100 kcal [57].

By definition, GULs “are values derived on the basis of meeting nutritional requirements of infants and an established history of apparent safe use. ... The purpose of the GULs is to provide guidance to manufacturers and they should not be interpreted as goal values. Nutrient contents in infant formulas should usually not exceed the GULs unless higher nutrient levels cannot be avoided due to high or variable contents in constituents of infant formulas or due to technological reasons” [50]. That is, GULs are stated to provide guidance to manufacturers, but are distinguished from maximum values, which presumably cannot be exceeded for a product to be in compliance with the Codex Alimentarius Standard for Infant Formula.

GULs were first distinguished from maximum values in 2005 at a meeting of the Codex Committee on Nutrition and Foods for Special Dietary Uses according to a “proposal of the Delegation of the United States to introduce wording “Guidance Upper Levels” for Vitamins and Minerals where values were derived on the basis of established history of apparently safe use” [62]; the Delegation of the United States to that meeting included representatives from the International Formula Council and Nestlé USA, as well as the U.S. Food and Drug Administration and other governmental and non-governmental agencies [62]. Following this meeting, other meeting participants observed that “the US delegation requested that both maximum values and guidance values should not be lower than values used for formulas already on the market, even if such levels have not been subjected to systematic evaluation of their biological effects and safety. The underlying concept that levels of exposure determine safety is unknown in science, be it in toxicology, pharmacology, or nutrition” [63]. The 100 µg of Mn/100 kcal GUL is about 120 times higher than the Mn concentration of human breast milk and about 2 times higher than the Mn concentration of unsupplemented soy formula in our recent study of infant formulas and young child nutritional beverages [3,57,61].

The Codex Alimentarius standards are not used to regulate infant formulas or young child nutritional beverages in the United States [53]. In our recent market basket survey of infant formula and young child nutritional beverage products available in the US and France, 2 out of 17 infant formula (12 %) products purchased in the US exceeded the 100 µg of Mn/100 kcal GUL [3].

3.3.2. The European Union regulations for manganese in infant formula and follow-on formula

In the European Union (EU), formula products labeled for infants ages 0–1 years are termed infant formulas and products labeled for young children older than 1 year are termed follow-on formulas. The European Parliament regulates infant formulas and follow-on formulas within the European Union [51,64]. The current EU regulation for infant and follow-on formulas stipulates a minimum content of 1 µg of Mn/100 kcal and a maximum content of 100 µg of Mn/100 kcal [64]. These minimum and maximum specifications were adopted in 2006, following the 2003 recommendations of the Scientific Committee on Food (SCF) [51,65]. The 2003 SCF recommendation for a minimum of 1 µg of Mn/100 kcal was based on “the mean content of manganese in human milk of 3–4 µg/l (~0.5 µg/100 kcal) and on the lower absorption of manganese in cows’ milk and in soy-formula” [65]. In justifying the maximum of 100 µg of Mn/kcal, the SCF noted “there is increasing evidence of the neurotoxicity of high exposure to manganese. Therefore, a maximum manganese content of 100 µg/100 kcal is proposed, which is below the estimated Lowest Observed Adverse Effect Level (LOAEL) in

adults for manganese contents in water (4.2 mg/L)” [65]. The choice of the specific number, 100 µg of Mn/100 kcal, was not justified specifically in this document, but it is identical to the recommendation for maximum Mn content in infant formula made by the Life Sciences Research Organization, Inc. (LSRO), which was mentioned earlier on the same page of the 2003 SCF recommendations [65,66]. The LSRO supported their recommendation for a maximum of 100 µg of Mn/100 kcal by stating, “The proposed maximum of 100 µg/100 kcal is significantly below the estimated LOAEL in adults for manganese in water and is far beyond the range likely to encountered in milk-based formulas. The Expert Panel [of the LSRO] concluded that this level is above the content likely to be associated with isolated soy protein formulas” [66].

The validity of the LSRO’s claim that the 100 µg of Mn/100 kcal maximum is significantly below the estimated LOAEL in adults for Mn in water value and source cannot be evaluated since the value and reference for this LOAEL are not specified [66]. In contrast to the LSRO, in supporting their recommendation for a 100 µg of Mn/100 kcal maximum, the SCF did mention a specific LOAEL value, stating “100 µg/100 kcal is proposed, which is below the estimated LOAEL in adults for manganese contents in water (4.2 mg/L)”, but they did not state the source for this 4.2 mg/L LOAEL [65]. Ljung and Vahter [67] note that the “4.2 mg” recalls a proposed U.S. EPA adult LOAEL for Mn in drinking water, but the correct units for the proposed U.S. EPA LOAEL refer to mg/day, not mg/L [67,68]. That is, the “U.S. EPA refers to milligrams per day and the SCF refers to milligrams per liter” [67]. Using the standard U.S. EPA assumption of a 2 L/day intake for drinking water, the correct conversion for this proposed U.S. EPA 4.2 mg/day LOAEL is 2.1 mg of Mn/L of drinking water; see Eq. (15) [67,68].

$$\begin{aligned} \text{Correctly Converted} \\ \text{U.S. EPA LOAEL} &= \frac{4.2 \text{ mg of Mn}}{\text{day}} \times \frac{\text{day}}{2 \text{ L of drinking water}} \\ \text{Volume Basis} &= \frac{2.1 \text{ mg of Mn}}{\text{L of drinking water}} = \frac{2,100 \text{ µg of Mn}}{\text{L of drinking water}} \end{aligned} \quad (15)$$

The proposed U.S. EPA 4.2 mg/day LOAEL for adults was correctly converted by the U.S. EPA to 0.060 mg of Mn/kg of body weight/day (60 µg of Mn/kg of body weight/day); see Eq. (16) [67,68].

$$\begin{aligned} \text{Correctly Converted} \\ \text{U.S. EPA LOAEL} &= \frac{4.2 \text{ mg of Mn}}{70 \text{ kg of body weight} \times \text{day}} \\ \text{Weight Basis} &= \frac{0.060 \text{ mg of Mn}}{\text{kg of body weight} \times \text{day}} = \frac{60 \text{ µg of Mn}}{\text{kg of body weight} \times \text{day}} \end{aligned} \quad (16)$$

For a 3-week-old formula-fed infant weighing 3.84 kg (Table 1) and consuming 0.6197... L of prepared product per day [4], the estimated concentration of prepared product that gives this U.S. EPA LOAEL is 3.71...x10² µg of Mn/L of prepared product; see Eqs. (15) and (17).

$$\begin{aligned} \text{Estimated Concentration of} \\ \text{Prepared Product that gives} \\ \text{this U.S. EPA LOAEL} &= \frac{3.84 \text{ kg of}}{\text{body weight}} \times \frac{\text{day}}{0.6197... \text{ L of}} \\ \text{(3-week-old)} &\quad \text{prepared product} \\ \text{formula-fed infant)} &\quad \times \frac{60 \text{ µg of Mn}}{\text{kg of}} \\ &\quad \text{body weight} \times \text{day} \\ &= \frac{3.71... \times 10^2 \text{ µg of Mn}}{\text{L of prepared}} \\ &\quad \text{product} \end{aligned} \quad (17)$$

In our recent study of infant formulas and young child nutritional beverages available on the US and French markets, the mean energy content of the 21 powdered products labeled for use by 0–6 month-olds was 662.4... kcal/L of prepared formula [3,4]; therefore, a prepared

product with $3.7_{1...} \times 10^2 \mu\text{g}$ of Mn/L would provide approximately $56 \mu\text{g}$ of Mn/100 kcal; see Eq. (18).

$$\begin{aligned} \text{Estimated Concentration of} \\ \text{Prepared Product that gives} \\ \text{this U.S. EPA LOAEL} \\ \text{(3-week-old} \\ \text{formula-fed infant)} \\ = 100 \text{ kcal} \times \frac{3.7_{1...} \times 10^2 \mu\text{g of Mn}}{\text{L of prepared product}} \\ \times \frac{\text{L of prepared product}}{662 \text{ kcal}} \\ = \frac{56 \mu\text{g of Mn}}{100 \text{ kcal}} \end{aligned} \quad (18)$$

Thus, this proposed U.S. EPA $60 \mu\text{g}$ of Mn/kg of body weight/day LOAEL, which was presumably the same LOAEL referred to by the SCF for comparison of its proposed maximum $100 \mu\text{g}$ of Mn/100 kcal for infant formula and follow-on formula, would likely be exceeded by formulas containing more than $56 \mu\text{g}$ of Mn/100 kcal and would certainly be exceeded by formulas with $100 \mu\text{g}$ of Mn/100 kcal, contrary to the claims of the SCF [65,68]. The magnitude of this discrepancy, $100 \mu\text{g}$ of Mn/100 kcal versus $56 \mu\text{g}$ of Mn/100 kcal, is approximately a factor of 2, which is consistent with the reported error made by the SCF of confusing “milligrams per day” with “milligrams per liter” and not dividing the stated LOAEL by 2; see Eq. (15) [67,68].

It must be kept in mind that this U.S. EPA proposed LOAEL represents a level at which adverse health effects were noted; see Eq. (15) [68,69]. When a guideline or standard is based on a LOAEL rather than a NOAEL, an additional uncertainty factor is usually included in the derivation of a tolerable intake threshold [26,70,71]. If the U.S. EPA proposed LOAEL for drinking water of $60 \mu\text{g}$ of Mn/kg of body weight/day were used for a comparison of infant formula (Eq. (16)), then $56 \mu\text{g}$ of Mn/100 kcal (Eq. (18)) would not represent a tolerable intake level, but rather, a level at which adverse health effects would be expected. However, this proposed U.S. EPA LOAEL was specifically derived for Mn intake from water, and it was posited that “manganese in drinking water may be absorbed more completely than that in food” [68].

The derivation for this proposed U.S. EPA LOAEL and the RfD that was derived from this proposed LOAEL for Mn in drinking water was published in a 1994 monograph containing risk assessments for essential elements [68]; it was never formally enacted by the U.S. EPA for regulating drinking water. The official U.S. EPA RfD for drinking water is set at 0.14 mg of Mn/kg/day based on dietary studies [28,72]. This official U.S. EPA RfD was apparently not referred to by the SCF for comparison of its proposed maximum level for Mn [65]. Ljung and Vahter [67] noted that the 1994 proposed U.S. EPA LOAEL of $60 \mu\text{g}$ of Mn/kg/day for drinking water was based on a study in which all subjects were 50 years of age and older [68,69], so it is unknown whether basing a tolerable intake level on this adult LOAEL would provide sufficient protection for infants and young children [67].

3.3.3. Republic of France standards for manganese in infant formula and follow-on formula

The *République Française* (Republic of France) publishes regulations covering *préparations pour nourrissons et aux préparations de suite* (infant formulas and follow-on formulas) in France. The Republic of France has set a $1 \mu\text{g}$ of Mn/100 kcal minimum and a $100 \mu\text{g}$ of Mn/100 kcal maximum concentration for Mn in infant and follow-on formulas [52]. This $100 \mu\text{g}$ of Mn/100 kcal maximum is in accordance with the regulations set by the European Parliament for infant and follow-on formulas within the European Union (EU) [51,64].

However, it must be noted that consumption of a product containing the maximum $100 \mu\text{g}$ of Mn/100 kcal by an 3-week-old would lead to a daily intake of $107 \mu\text{g}$ of Mn/kg/day (Eq. (14)), twice the maximum daily tolerable intake of $55 \mu\text{g}$ of Mn/kg/day established by ANSES in their proposed maximum health-based value for Mn in drinking water [13]. In contrast, the maximum value for Mn of $50 \mu\text{g}$ of Mn/100 kcal

originally proposed by ESPGHAN would not lead to exceedances of the new ANSES maximum daily tolerable intake of $55 \mu\text{g}$ of Mn/kg/day if the product is reconstituted with water with a low native Mn concentration [4,13,61].

In our recent survey of infant formula products on the French and US markets, none of the 17 products that we tested from the French market exceeded the French regulatory maximum of $100 \mu\text{g}$ of Mn/100 kcal in infant and follow-on formulas [3]. However, 5 of 17 products exceeded the proposed ESPGHAN maximum of $50 \mu\text{g}$ of Mn/100 kcal, of which 2 products were labelled for use by infants and would lead to exceedances of the new ANSES maximum daily tolerable intake of $55 \mu\text{g}$ of Mn/kg/day [3,13,61]. It should also be noted that if the formula powders are reconstituted with water containing substantial quantities of Mn, additional exceedances of the ANSES proposed maximum daily tolerable intake of $55 \mu\text{g}$ of Mn/kg/day would be expected [4,13].

3.3.4. The United States nutritional standards for manganese in infant formula

The Federal Food, Drug, and Cosmetic Act passed by the United States Congress (U.S.C.) regulates infant formulas in the US [53]. Formula products labeled for use by infants ages 0–1 year are termed “infant formulas”. Products labeled for use by young children older than 1 year do not have a legally defined term and are not regulated as formulas; they are often labeled as “toddler formulas” [73]. According to the Federal Food, Drug, and Cosmetic Act, the Minimum Level for Mn in prepared infant formula is $5 \mu\text{g}$ /100 kcal, and no Maximum Level is specified [53]. The $5 \mu\text{g}$ of Mn/100 kcal Minimum Level is about 6 times higher than the Mn concentration of human breast milk [61]. This Minimum Level of $5 \mu\text{g}$ of Mn/100 kcal prepared formula has not been updated since it was originally established in the Infant Formula Act of 1980 [60].

The initial list of nutrients in the 1980 statute was based on recommendations from the Committee on Nutrition of the American Academy of Pediatrics (AAP-CON) [74,75]. More specifically, in 1976 the AAP-CON recommended a $5 \mu\text{g}$ of Mn/100 kcal Minimum Level in infant formula because it is “approximately the level in commonly used milk-based infant formulas” [75]. No data were provided to support the claim that milk-based infant formulas had a level of approximately $5 \mu\text{g}$ of Mn/100 kcal at that time [75]. A 1971 survey of milk products reported that 2 out of the 2 milk-based infant formulas tested had a Mn concentration of 0.18 mg of Mn/kg of prepared product ($180 \mu\text{g}$ of Mn/kg of prepared product), which would be equivalent to $28 \mu\text{g}$ of Mn/100 kcal, assuming an energy content of $662.4_{...}$ kcal/L of prepared product and a density of $1.017_{...}$ kg of prepared product/L; see Eq. (19) [3,76].

$$\begin{aligned} \text{Mn Concentration} \\ \text{of Milk – Based} \\ \text{Infant Formulas} \\ \text{in 1971} \\ = 100 \text{ kcal} \times \frac{180 \mu\text{g of Mn}}{\text{kg of prepared product}} \times \frac{1.017_{...} \text{ kg of prepared product}}{1 \text{ L of prepared product}} \\ \times \frac{\text{L of prepared product}}{662 \text{ kcal}} \\ = \frac{27 \mu\text{g of Mn}}{100 \text{ kcal}} \end{aligned} \quad (19)$$

At the time the AAP-CON recommendation was made [75], it was not noted that adopting for a minimum a level “commonly used” in products could serve to increase the mean level used in all of the products covered by this recommendation.

In our recent study of infant formulas and young child nutritional beverages available on the US and French markets, the 21 products labeled for use by 0–6 month-olds had a mean energy content of 662 kcal/L of prepared product, so $5 \mu\text{g}$ of Mn/100 kcal would correspond to approximately $33 \mu\text{g}$ of Mn/L when prepared according to

labeled instructions; see Eq. (20), while the mean Mn content of the 25 cow-milk-based products with and without supplemental Mn that we tested was 54 µg of Mn/100 kcal, or 334 µg of Mn/L [3].

$$\begin{aligned} \text{Estimated Minimum Level} &= \frac{5 \text{ } \mu\text{g of Mn}}{100 \text{ kcal}} \times \frac{662 \text{ kcal}}{\text{L of prepared product}} \\ &= \frac{33 \text{ } \mu\text{g of Mn}}{\text{L of prepared product}} \end{aligned} \quad (20)$$

4. Conclusions

4.1. Supplementation is not required for manganese sufficiency

Since Mn deficiency has never been reported in exclusively breast-fed infants, it is assumed that Mn intakes of exclusively breast-fed infants are sufficient [9]. The IOM AI for 0–6 month-old-infants is 3 µg of Mn/day based on an estimated Mn concentration in breast milk of 3.5 µg/l and a reference weight of 7 kg for infants 2–6 months old [9]. Expressed in the infant formula regulatory units of µg of Mn/100 kcal, this AI would be equivalent to 0.50 µg of Mn/100 kcal (Eq. (1)). All infant formula products that we tested in our market basket survey of products on the French and US markets far exceeded this amount; the product with the minimum Mn content that did not contain supplemental Mn supplied 36 µg of Mn/100 kcal [3]. Thus, Mn supplementation of infant formula is not necessary to insure adequate intake.

4.2. Regulatory tolerable intakes of manganese by ingestion

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has not established a tolerable intake for Mn in food. The WHO has an HBV of 400 µg of Mn/L in drinking water assuming a NOAEL of 11 mg of Mn/day for adults [26]. This NOAEL was derived from the maximum Mn intake in a 1982 3-day dietary study that did not measure any health outcomes [11,22,23,26,77], but was simply a dietary survey of 100 Canadian women [23]. The United States Institute of Medicine (IOM) set a Tolerable Upper Intake Level (UL) of 11 mg of Mn/day for adults based on the same dietary survey [9,23].

Similarly, the U.S. EPA derived a tolerable intake level from 2 studies that did not measure any health outcomes. The U.S. EPA Reference Dose (RfD) for dietary Mn is 0.14 mg of Mn/kg of body weight/day [10,28] and is derived using an assumed NOAEL of 10 mg of Mn/day for adults. This assumed NOAEL is ultimately based on a 1969 24-h dietary study of 31 people in India [34] and a 1941 2-person experimental balance study in England in which 1 person was fed a diet with relatively high Mn content [29–32]; no health outcomes were reported in these studies. At least 2 of the 42 products in our recent market basket survey of infant formulas and young child nutritional beverages could exceed these regulatory NOAELs when used as directed [3].

The European Food Safety Authority (EFSA) does not have a tolerable intake level for Mn [40]. In contrast, ANSES has proposed a daily tolerable intake level of 55 µg of Mn/kg/day in drinking water in France based on neurodevelopmental effects observed in rats [43]. Two of 4 French products in our market basket survey labelled for use by 0–6 month-olds would exceed the proposed ANSES proposed tolerable daily intake of 55 µg of Mn/kg/day [3].

4.3. Infant formula and follow-on/follow-up formula standards and regulations

The Codex Alimentarius Commission (Codex Alimentarius) has a 1 µg of Mn/100 kcal Minimum and a 100 µg of Mn/100 kcal Guidance Upper Level (GUL) for formulas fed to infants not more than 12 months

of age [50]. The Codex Alimentarius does not provide guidance for Mn content in follow-on formulas [58]. The Codex Alimentarius GUL for infant formulas was not established on the basis of toxicity research, but rather “on the basis of established history of apparently safe use” [62]. In our recent market basket survey of infant formulas and young child nutritional beverages, 28 % (7 out of 25) of the US products exceeded the 100 µg of Mn/100 kcal GUL [3].

The current European Union (EU) regulation for infant and follow-on formulas stipulates a minimum content of 1 µg of Mn/100 kcal and a maximum content of 100 µg of Mn/100 kcal [64]. The Republic of France uses these same minimum and maximum values [52]. The minimum of 1 µg of Mn/100 kcal was based on “the mean content of manganese in human milk of 3–4 µg/l (~0.5 µg/100 kcal) and on the lower absorption of manganese in cows’ milk and in soy-formula” [65]. The maximum of 100 µg of Mn/100 kcal is likely derived from a proposed, but not enacted U.S. EPA LOAEL for Mn in water [67,68]; however, this derivation incorporated a mathematical error due to incorrect interpretation of units [67]. A corrected derivation for a maximum Mn content in infant formula based on this LOAEL would yield 56 µg of Mn/100 kcal (Eq. (18)) [67].

None of the 17 infant and follow-on formula products that we purchased in France and evaluated in our recent market basket survey of infant formulas and young child nutritional beverages exceeded the EU maximum content of 100 µg of Mn/100 kcal [3,64]. However, 1 of the 4 newborn infant formula products purchased in France for our market basket study exceeded 56 µg of Mn/100 kcal, the maximum when calculated with the SCF mathematical errors corrected (Eqs. (15)–(18)) [3].

To stay within the ANSES proposed tolerable intake for Mn in water, the regulatory maximum for infant formula would need to be set at 51 µg Mn/100 kcal (Eq. (10)), similar to the regulatory maximum of 50 µg Mn/100 kcal originally proposed by ESPGHAN [61]. Two of the 4 newborn infant formula products purchased in France for our market basket study contain less than 51 µg Mn/100 kcal, demonstrating that it is possible to produce infant formula with less than 51 µg Mn/100 kcal using current production methods [3].

The US government requires a Minimum Level of 5 µg of Mn/100 kcal in infant formula and has no Maximum Level [53]. It does not have special regulations beyond the ordinary food regulations for formulas labelled for young children 1 year and older [53]. Manufacturers often use the word “toddler” on labels of these products to clarify to the Food and Drug Administration (U.S. FDA) that they are not to be regulated as infant formulas, although parents are often unaware of this labelling distinction [73]. The minimum 5 µg of Mn/100 kcal in infant formula corresponds to approximately 34 µg of Mn/L (Eq. (20)), far higher than the estimated 3.5–7.7 µg Mn/L in breast milk [4,9]. This Minimum Level has not been updated since it was originally set in the Infant Formula Act of 1980 [60,74] and is based not on health, but rather, on “the level in commonly-used milk-based infant formulas [in 1976]” [75].

4.4. Comparison of manganese tolerable intakes, formula regulations, and intake estimates due to formulas

Daily Mn intakes according to regulations and guidelines discussed above are summarized in standard units of µg of Mn/kg/day in Table 2 and Fig. 1. These estimated intakes include tolerable intakes assumed by regulatory and advisory agencies, minimum and maximum levels allowed in infant and follow-on formulas, and maximum levels found in our recent market basket survey of US and French infant formulas and young child nutritional beverages [3,4]. When examining these intake levels, it must be kept in mind that intakes apparently tolerated by adults

may be less tolerable to infants due to maturational factors [8,78,79], and that the studies on which the adult “tolerable” intakes are based made no attempt to measure tolerability and were published more than 35 years ago (Table 3) [23,32,34].

4.5. Recommendations

No instances of Mn deficiency have ever been reported with exclusively breast-fed infants, and all recent studies of Mn content in infant formulas and young child nutritional beverages have found them to contain substantially more Mn than human breast milk [3,80–86]. Thus, it is highly unlikely that exclusive feeding with infant formulas or complementary feeding with young child nutritional beverages would lead to Mn deficiency. In contrast, no benefits have ever been reported for consuming Mn in excess of basic requirements, but current research on environmental, occupational, and total parenteral feeding (TPN) exposures to Mn strongly suggest the possibility of adverse health effects, especially neurological effects, with excess Mn exposures [5–8, 35–37,86–89]. Toxicity thresholds for Mn exposures in neonates and young children have not yet been identified; however, limited capacity for Mn excretion in neonates suggests that Mn toxicity thresholds could be lower for this age stage than for older children or adults [8]. The concentration of Mn is higher in infant brains than adult brains [90,91]; the exact mechanism for this difference is under debate. Some researchers have suggested this difference may be due to immaturity of the blood-brain barrier in early infancy [91,92], but others have noted increased expression of metal transporter proteins in the days after birth [93,94]. Regardless of the exact mechanism, the enhanced accumulation of Mn in infant brains during early post-natal development underscores that this is a sensitive population that must be protected from excess Mn exposure.

Given the lack of evidence for either Mn deficiency or the potential for benefit of additional Mn consumption in neonates, infants or young children, and the strong likelihood that excess Mn may be toxic, stricter upper limits for Mn content in infant formulas should be immediately considered by regulators. In France, the maximum content for Mn in infant formula should be updated to be consistent with the recently proposed tolerable daily intake for Mn used to derive a health-based maximum for Mn in drinking water [13]. In the US, the 39-year-old requirement for minimum Mn content in infant formulas [53,60] is in urgent need of updating, and a maximum Mn content for infant formulas and young child nutritional beverage products should be established for the US market as soon as possible. In addition, the practice of adding supplemental Mn to infant formula to ensure regulatory compliance should be discouraged since it is unnecessary and may lead to excess Mn intakes and consequent adverse neurodevelopmental effects.

Author statement

This study did not involve any human or animal subjects.

Declaration of Competing Interest

The authors have no competing interests to report. EJM's affiliation is with Better Life Laboratories, a nonprofit organization that conducts scientific research and provides technical expertise, equipment, and training to help needy people around the world. Better Life Laboratories received no specific funding for this project from any donors. Donors to Better Life Laboratories provided no input in choosing the subject matter of this project, the method of analysis, the research findings, the decision to publish, or the manner of disseminating the results. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. This study received institutional support from Better Life Laboratories, Inc., Norwich University, the Centre National de la Recherche Scientifique (CNRS), the Institut National de Physique Nucléaire et de Physique des Particules (IN2P3), and the Centre d'Etudes Nucléaires de Bordeaux Gradignan (CENBG). We thank Deborah Ahlers and Tammy Hunt from Norwich University for their valuable assistance with this project.

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