

# Assessing Vitamin D Levels in Fortified Foods and Supplements for Infants and Its Impact: A Review

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## Abstract

*In this study, the vitamin D levels in fortified foods (including follow-on formula, baby porridge, and curd cheese dessert) and dietary supplements designed for infants were examined. Analytical assessments uncovered variations in vitamin D concentrations compared to the labeled values, fortified foods contain anywhere from 50% to 153% for supplements 8% to 177%. Instant follow-on formula and oil-based supplements typically aligned with or surpassed the labeled values, whereas ready-to-eat baby porridge consistently registered below the declared values (74–81%). The study suggests that relying solely on label information may lead to inaccurate estimations of vitamin D intake in infants, with potential for both underestimation and overestimation. Furthermore, the discrepancies observed in nutrition D concentrations spotlight the importance of regular monitoring and quality management measures within the manufacturing and distribution of fortified meals and dietary dietary supplements for babies. Adequate nutrition D intake is critical for most effective bone health and immune function throughout infancy, making accurate estimation essential for preserving typical fitness and well-being. The findings underscore the need for greater transparency and accuracy in labeling practices in the infant nutrition industry. Additionally, healthcare experts must be vigilant in advising caregivers about capability variations in vitamin D content material and the significance of varied assets to ensure babies receive adequate vitamins for healthy increase and development. Further studies is warranted to discover the elements contributing to these discrepancies and to establish standardized tips for nutrition D fortification in little one foods and supplements.*

**Keywords:** Vitamin D, Fortified Foods, Infants, Dietary Supplements, HPLC

## INTRODUCTION

Vitamin D deficiency in infants may cause conditions like rickets. However, excessive intake poses risks such as hypercalcemia and kidney problems. Taking this in account, various institutes have established tolerable upper intake levels (UL) for all age groups, as well as infants. While vitamin D can be synthesized through skin exposure to UV light, young children, often shielded from direct sunlight, primarily rely on foods and dietary supplements [1]. Vitamin D supplementation for children up to 4 years has been recommended since 2012 in Netherlands. Previous studies estimated potential overages in vitamin D intake, with 4–11% of infants aged 7–11 months exceeding UL assumptions.

This research aims on examining the vitamin D levels in fortified foods and dietary supplements designed for infants. The objectives include evaluating discrepancies between declared and actual values, exploring variations between production batches, and discussing the potential impact on estimating the distribution of vitamin D intake [2]. The study acknowledges challenges in accurately determining micronutrient levels due to natural variations, processing changes, and storage effects, emphasizing the need for reliable data to prevent misinformation and guide nutritional recommendations [3].

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## MATERIALS AND METHODS

### Product Selection

In mid-2014, an assessment of vitamin D fortified foods and supplements for young children (6–12 months) in the Dutch market was conducted. The INNOVA database and manufacturers' websites were used, and stores were visited for insights [4]. Vitamin D fortified foods are comprised of infant formula, follow-on formula, baby porridge, infant milk, soy milk, yoghurt drink, instant chocolate milk, curd cheese dessert, and lemonades. Non-child-specific products and chewable supplements were excluded. On September 2015, 44 products of 18 follow-on formula, 10 porridges, 1 curd cheese dessert, and 15 supplements were Purchased from local stores, covering numerous brands and price classes [5, 28]. Curd cheese dessert, although designed for children over one year, was included due to frequent infant consumption. Products were within their best-before dates [6]. To assess batch variations, pairs of products with different best-before dates from the same brand were purchased (5 follow-on formulas and 5 dietary supplements), totaling 10 supplement brands and 13 follow-on formula brands. Table 1 depicts the Overview of fortified food & dietary supplement

Including 5 similar products (brand & type) with a different best before date (interval of 3–12 months)

### Labelled Vitamin D Concentration

The labeled vitamin D concentration (in  $\mu\text{g}$ ) was obtained from manufacturer labels, allowing a comparison with the analyzed vitamin D concentration [7]. Among the 29 fortified foods, 13 specified vitamin D3, while 16 lacked information on the vitamin D form. Labels provided a single value for vitamin D content without manufacturer-provided uncertainty ranges. Follow-on formulas and baby porridges declared vitamin D content per 100 g prepared product, recalculated to 100 g unprepared product based on preparation instructions. Dietary supplements displayed vitamin D concentration per number of drops per daily dose, not per mL or g [8–10].

### Chemical Analysis

Vitamin D3 chemical analysis followed European Standards (NEN-EN 12821) using high-performance liquid chromatography (HPLC) to measure cholecalciferol (D3). TNO Triskelion B.V. conducted the analysis, accredited for internal reference method TRIS/VIT/051. After purchase, samples were homogenized, aliquoted, and stored appropriately [11]. Analysis occurred within 3–5 weeks in duplicate for unprepared samples. Saturation during sample reprocessing was not observed. Saponification in alcoholic potassium hydroxide with antioxidants, followed by extraction and purification, was employed. Ultra performance liquid chromatography (UPLC) with diode-array detection confirmed vitamin D3 presence. The method's combined measurement uncertainty was 13% (1 SD) at 3900 IU/kg (9.8  $\mu\text{g}/100\text{ g}$ ), covering reproducibility, accuracy, and homogeneity [12–15].

**Table 1.** Overview of foortified food & dietyr supplimanet containg vitamin D concentration was chemically analyzed.

Food Group	Specification	No. of Products	No. of days until best-before date	Labelled age-category
Follow-on formula	Instant milk based (powdered)	16	68–688	6-10 months or 6-12 months
	Ready to eat, milk-based with cereals/yoghurt or fruit (liquid)	2	145–265	>6 months
Porridge	Instant with cereals (adding milk)	7	283–735	>6 months or >8 months
	Ready to eat, milk based with cereal	3	148–282	>6 months
Desert	Curd cheese with fruit	1	7	>1 year
Supplement	Water based, only containg vitamin D	12	85–955	0-4 years
	Oil-based, only containing vitamin D	3	355–895	0-4 years

The accuracy was high, ensuring complete vitamin D extraction. The valid range was 2–5000 IU/100 g or 100 mL, expressed in IU (1 µg = 40 IU) per kg unprepared food or supplement.

### Statistical Analysis

Vitamin D content in solid foods was recalculated to µg per 100 g, while for liquids, it was recalculated per 100 mL using density. For dietary supplements labeled with the number of drops, the weight of 30 drops was determined for recalculation from IU/kg to µg per advised number of drops. Re-analyses in January 2015 were conducted for products with significant deviations between measured and declared values [16]. The two-sided 95% confidence interval was calculated using the combined measurement uncertainty.

Measured vitamin D concentrations were expressed as a proportion of labeled values and compared with EU guidelines. Tolerance ranges were calculated based on potential rounding, with lower and higher bounds for fortified foods (65% and 150%, respectively) and dietary supplements (80% and 150%, respectively). To assess the impact of different production batches, measured vitamin D concentrations were plotted against the days until the best-before date for paired products with different best-before dates within the same brand. Table 2 depicts Overview of validation parameters [17–18, 29]

## RESULTS

### Measured Vitamin D Concentration and Comparison with Label Information

Table 3 summarizes vitamin D concentration statistics categorized by food or supplement type. Declared vitamin D content varied from 1.0 µg/100 mL for ready-to-eat follow-on formula to 16.5 µg/100 g for instant baby porridge. Supplements had a consistent advised daily dosage of 10 µg. Measured vitamin D content ranged from 4.5 to 21 µg/100 g for instant food and 0.9 µg/100 mL to 1.5 µg/100 mL for ready-to-eat foods. Follow-on formula exhibited less variation in labeled vitamin D content compared to instant baby porridge.

**Table 2.** Overview of validation parameters of method used to measure vitamin D concentration in infant formula.

Vitamin D level	Reproducibility (RSD (r))	Repeatability (RDS (r))	Accuracy
20 IU/L			100%
4000 IU/Kg	8%	12%	92%
49000 IU/Kg	4%	4%	98%

**Table 3.** Overview of labelled & measured vitamin D3 Contents (median; lowest; highest) in fortified foods supplements and proportion of labeled values (median: lowest: highest)

		Number of samples	Labelled vitamin D content µg/100g (number of drops)			Measured vitamin D3 Concentration µg/100g <sup>3</sup>			Proportion (%) of labelled value		
			Median	Range		Median	Range		Median	Range	
				Low	High		Low	High		Low	High
Follow on Formula	Instant	16	8.5	7.8	1.4	11	8.5	14	125	102	153
	Ready to eat	2	1.45	1	1.9	1.4	1.4	1.4	87	73	101
Baby porridge	Instant	7	7.5	5.6	16.5	5.8	4.5	21	96	50	127
	Ready to eat	3	1.7	1.2	1.8	1.3	0.9	1.4	78	74	81
Curd Desert		1	1.25	-	-	1.5	-	-	122	-	-
Supplement	Water based	12	10 (5)	10 (5)	10 (10)	10	0.89	11	101	8	111
	Oil-Based	3	10 (5)	10 (10)	10 (2)	11	9.9	18	113	99	177

Dietary supplements' measured vitamin D content ranged from 0.8 to 18 µg per daily advised dosage. For fortified foods, measured vitamin D content varied between 50% and 153% of the declared value, while for dietary supplements, the range was 8% to 177%. Instant follow-on formula and oil-based supplements generally matched or exceeded declared values, while all measured vitamin D concentrations for ready-to-eat baby porridge were below the declared value (74–81%).

### **Comparison with EU Tolerance Limits**

Among the foods with added vitamin D, the declared concentrations varied, leading to different tolerance values. Two out of 29 foods had measured vitamin D concentrations outside the tolerance range. For one follow-on formula (O\_03), the concentration was just above the upper bound tolerance, though not statistically significant.

A baby porridge (P\_05) had a measured vitamin D concentration below the lower bound tolerance, borderline statistically significant [19, 20].

All dietary supplements had identical declared vitamin D content and tolerance ranges. Four out of 15 supplements had measured concentrations outside the tolerance range. For three products (S\_01, S\_02, S\_03), the concentration was lower, and for one product (S\_13), it was higher. Statistically significant deviations were observed for two products, S\_01 and S\_02, which were the same products with a different best-before date. Re-analyses of these products confirmed statistically significant deviations from EU tolerance values [21–24].

### **Difference in Vitamin D Content of Same Products from a Different Production Batch**

For most follow-on formulas in pairs from the same brand but different batches (best-before dates 86–333 days apart), the vitamin D concentration was 15–18% higher in the product with more days until the best-before date. These deviations were within measurement uncertainty and not statistically significant. Dietary supplements showed minimal differences between pairs from the same brand but different batches (less than 4%; best-before date 88–365 days apart; data not shown). In one supplement pair (S\_01 and S\_02), the product with the highest best-before date had a vitamin D concentration over 250% higher than the product with the lower best-before date, but both significantly deviated from EU tolerance values [25–27].

## **DISCUSSION**

The study highlights potential variations between measured vitamin D concentrations and declared values in fortified foods and dietary supplements for infants. While most deviations fell within EU tolerance ranges, the findings underscore the importance of considering actual nutrient content rather than relying solely on label information. The study suggests that public health assessments, dietary intake estimations, and research outcomes may be impacted by such deviations.

The discussion emphasizes the need for improved accuracy in nutrient intake assessments, advocating for analytical measurements alongside label information, especially in intervention and epidemiological studies. It calls for awareness among researchers about potential biases introduced by relying solely on labeled values and their implications for dietary reference values and study interpretations.

## **CONCLUSIONS**

Measured vitamin D content in fortified foods and supplements may deviate from labels, often within tolerance limits. However, relying on label information for dietary intake estimations can lead to invalid results. Factors such as product variability, brand loyalty, and batch differences can result in under- or overestimations of vitamin D intake. Further research, employing advanced statistical methods like probabilistic modeling and prediction models, is needed to understand the impact on habitual vitamin D intake distribution.

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