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INVESTIGATION OF IODINE IN STARTING, FOLLOW-ON, SPECIAL FORMULAE AND CEREAL PRODUCTS FOR INFANTS[1]

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Newborn babies are entirely dependent on mother's milk and in special cases on infant formulae, follow-on formulae and special formulae as the only iodine source. In the present work we investigated the iodine content in various starting infant formulae, follow-on formulae, special formulae and various other samples intended for preparation of baby food. We compared the values obtained with the declared values where possible, and with data found in the literature with the purpose of examining improvements made during last decade. Radiochemical neutron activation analysis was employed to determine the content of iodine in the selected samples. This method is based on ignition of the irradiated sample in an oxygen atmosphere, followed by absorption of iodine in a reducing acid solution

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containing 1 M H_2SO_4 and 10 % Na_2SO_3. Extraction of iodine was achieved with chloroform via the classical redox reaction with 10 % $NaNO_3$ and 2.5 M H_2SO_4. Stripping of the organic phase via 10 % Na_2SO_3 to purify iodine followed, which was then re-extracted with chloroform and transferred to a 10 ml vial for gamma-ray measurement of the induced radionuclide ($^{127}I(n, \gamma)^{128}I$, $t_{1/2}$ = 25 min, E_γ = 422.9 keV). The chemical yield of the procedure for each sample aliquot was determined spectrophotometrically. The values of iodine obtained in eighteen different infant formulae, with the exception of two, showed insufficient agreement with the declared values and there is still a great need for stricter quality control of infant formulae. The median iodine content of eighteen infant formulae was 136 $\mu g\ l^{-1}$, implying that newborns would reach the newest recommended daily requirements for iodine (50 $\mu g\ day^{-1}$) when they have consumed approximately 0.5 l of prepared infant formula.

Introduction

The important role of iodine as an essential element for man, especially in the early stages of life, has been well recognized and studied. The only known physiological role for iodine is the synthesis of thyroid hormones by the thyroid gland. Thyroid hormones are involved in normal growth and development of the brain and central nervous system from the 15th week of gestation to the age of three years, and they also control some metabolic processes such as carbohydrate, fat, protein, vitamin and mineral metabolism. Iodine deficiency affects all stages of human life, but pregnant women, lactating women, women of reproductive age and children younger than three years are considered to be at high risk [3].

The role of iodine becomes important already in pregnancy when the need for iodine is increasing. The most important consequences of iodine deficiency during pregnancy (foetus development) and in the early stages of infant and child development are neuromotor and cognitive impairments. Newborn infants identified with hypothyroidism usually display normal neurodevelopment if they are treated promptly with iodine supplementation [4].

The foetal thyroid starts the synthesis of thyroid hormones from about the 12th week of gestation but until then the human foetus depends entirely on the maternal thyroid hormone status, which plays a crucial role in foetal growth and differentiation [5,6]. After birth human milk (breastfeeding) is the best nutrition and primary source of iodine needed for normal growth and development of babies and infants. The iodine content in breast-milk depends on the iodine intake in the diet of breast-feeding mothers, which shows an interindividual variability [7]. In many countries adequate dietary iodine intake is achieved through the iodisation of salt, but a healthy, well balanced diet can avoid the need for dietary supplementation of minerals. The quantities of minerals necessary to maintain health are addressed as Recommended Dietary Allowances (RDAs) [8,9]. Insufficient concentrations of bioavailable iodine can lead to iodine deficiency disorders (IDD), a term that refers to all of the ill effects of iodine deficiency in a population, and that can be prevented by ensuring an adequate intake of iodine [10].

Dairy infant formulae, from 0 – 6 months, and later follow-on formulae, 6 – 12 months, are an alternative and adequate substitute for human milk in cases when breastfeeding is not possible. In some cases when babies are born prematurely or they are allergic to the normal constituents of milk, special formulae have to be used. Later on dairy products are the main source of iodine for children [11]. In the case of feeding of infants with human milk substitutes, appropriate concentrations of iodine should be provided.

The recommendations for minimum daily iodine intake ($\mu g\ day^{-1}$) during the last ten years are listed in Tables I and II. Regarding the Codex Alimentarius, infant formula should contain, per 100 available calories, 5 μg of iodine, which is also stated by the European Union guidelines [12,13].

Commonly used techniques for measuring iodine are colorimetry based on catalytic reactions, gas chromatography, ion selective electrodes, X-ray fluorescence spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), isotope exchange and neutron activation analysis (NAA) [14]. But to determine iodine at low concentrations the two methods sensitive enough are radiochemical neutron activation analysis and accelerator mass spectrometry [15].

Table I Changes in recommendations for daily iodine intake (in $\mu g\ day^{-1}$) during past the 10 years

Group	Age	before 1992[1]	from 1992[2]	Age	2001[3]
Preterm infants		> 30 $\mu g\ kg^{-1}\ day^{-1}$			
Infants	0 – 5 months	35 – 40	90	0 – 6 months	110
	5 – 12 months	50	90	7 – 12 months	130
	1 – 3 years	70	90		90
	4 – 6 years	90	90	4 – 8 years	90
	7 – 10 years	120	120	9 – 13 years	120
Adults		150	150		150
Pregnant women		175	200		220
Lactating women		200	200		290

[1]RDA from Food and Nutrition Board of the National Academy of Sciences [1]
[2]Recommendations on iodine nutrition made by ICCIDD [1]
[3]RDA from Food and Nutrition Board [8]

Table II Newest recommendations for daily iodine intake in μg day^{-1} [16]

Age	Iodine Germany Austria	Iodine WHO Switzerland
Infants		
0 to under 4 months	40	50
4 to under 12 months	80	50
Children		
1 to under 4 years	100	90
4 to under 7 years	120	90
7 to under 10 years	140	120
10 to under 13 years	180	120
13 to under 15 years	200	150
Adults	200	150
Pregnant women	230	200
Lactating women	260	200

The aim of our work was to investigate the iodine content of different starting infant formulae (seven samples), follow-on formulae (seven samples), special formulae (four samples) and various other samples intended for young children's food preparation (six samples). We compared the values obtained with the declared values, where possible, and with data from the literature to see the improvements made during the last decade.

Experimental

Reagents

For preparation of solutions and sample treatment ultra-pure water (Milli Q, Millipore, USA) and the following chemicals were used: 96 % H_2SO_4 (Carlo Erba Reagenti, ISO), $CHCl_3$ (Carlo Erba Reagenti, ISO), 25 % NH_3 (p.a.), KIO_3 (Merck, s.p.), KI (Scharlau, ISO, Ph Eur), 10 % Na_2SO_3 (Kemika-Zagreb, p.a.), 10 % $NaNO_2$ (Merck, p.a.), cellulose powder (Whatman), NH_4NO_3 (Alkaloid-Skopje, p.a.).

Stock standard solution of KIO_3 in NH_3 (1 mg I g^{-1} in 5 % solution of NH_3): KIO_3 was dried for 2 hours at 105 °C, then 0.4216 g was transferred to a 250 ml flask, dissolved in 50 ml of a 25 % solution of NH_3 and diluted with Milli Q water to 250 ml. The stock solution was diluted (dilution factor was 100) to prepare the working solution (10 μg I g^{-1} in 5 % solution of NH_3). The working standard solution was not subjected to chemical treatment.

Carrier solution of KI in NH_3 (50 mg I g^{-1} in 5 % solution of NH_3): 3.270 g of KI was transferred to a 50 ml flask, dissolved in 10 ml of a 25 % solution of NH_3 and diluted with Milli Q water to 50 ml. The carrier was used to prevent the loss of iodine, to ensure identical separation conditions and to allow the chemical yield of iodine separations to be determined.

Working standard solution of I_2 in $CHCl_3$ (0.150 mg I g^{-1} in $CHCl_3$): the appropriate amount of iodine was transferred to a 250 ml flask and dissolved in $CHCl_3$. 15 ml of stock solution was transferred to a 100 ml flask and again diluted with $CHCl_3$ to the mark.

Samples

From each product 2 – 4 aliquots were taken to determine the content of iodine. They were all analysed within the shelf life of the products, which are available on the Slovene and Polish market and can be bought at pharmacies. The samples investigated were as follows:

- Starting formulae (intended for babies from 0 to 6 months of age): Pikomil 1 (Krka, Slovenia), Comformil 1 (Nutricia, Holland), HIPP 1 (Hipp, Germany), Bebiko 1 (Nutricia, Poland), Bebilon 1 (Nutricia, Holland), NAN 1 (Nestle, Poland), Beba (Nestle, Germany)
- Follow on formulae (intended for babies from 6 months on): Milumil 2 (Milupa, Germany), NAN H.A. (Nestle, Germany), HIPP 2 (Hipp, Germany), Bebiko 2 (Nutricia, Poland), Bebilon 2 (Nutricia, Holland), NAN 2 (Nestle, Poland), Beba 3 (Nestle, Germany)
- Special formulae (intended for premature babies and babies with special needs (hypoallergenic, diarrhea) from 0 to 6 months of age): Neocate (SHS International, UK), Progamin AS (Milupa, Germany), HN25 (Milupa, Germany), Aptamil prematil (Milupa, Germany)
- Others (products intended for the preparation of meals for young children): Powdered milk, Granulated milk, Wheat bran, Oats flakes, Wheat flour, Barley porridge

Irradiation of Samples and Standards

The method for iodine determination by RNAA is described in more detail elsewhere [2].

Approximately 200 – 300 mg of sample was heat sealed in an acid-cleaned plastic tube and irradiated simultaneously with an appropriate aliquot of working standard solution (0.100 mg of 10 $\mu g\, g^{-1}$ I^- in 5 % solution NH_3) in a polyethylene container (rabbit) for approximately 2 – 3 minutes in the pneumatic system of our TRIGA Mark II Reactor at a neutron fluence of 4×10^{12} n cm^{-2} s^{-1}.

The irradiated sample and 90 – 100 mg of iodine carrier (solution of KI in NH_3) were then combusted in an oxygen atmosphere (4 l Schöniger flask) containing a reducing acid solution which consisted of 0.05 M H_2SO_4 and 10 % Na_2SO_3.

The solution with absorbed iodine in form of iodide (I^-) was transferred to an extraction funnel to separate the iodide from the matrix with chloroform *via* the redox reaction with $NaNO_2$ in H_2SO_4 medium. Stripping of iodine from the organic phase with Na_2SO_3 followed to separate iodine from any traces of bromine and chlorine. The purified iodine was then re-extracted into chloroform and filtered into a vial through a Whatman phase separating paper for gamma-ray measurement in a well-type HPGe detector. The whole procedure of isolation after irradiation took about 18 minutes; the half-life ($t_{1/2}$) of the isolated ^{128}I nuclide ($^{127}I(n, \gamma)^{128}I$; E_γ = 0.443 MeV) is 25 minutes.

After the gamma-ray measurement the organic phase was quantitatively transferred to 25 ml flask and diluted with chloroform to the mark for spectrophotometrical chemical yield determination at 517 nm with an MA 9525 – Spekol 210 spectrophotometer.

Results and Discussion

During foetal and neonatal growth and development the developing foetus and newborn is totally dependent on iodine coming from its pregnant or lactating mother. In some cases lactation ceases and the only alternatives to human milk are starting infant and follow-on formulae, and for babies with special nutritional requirements, special formulae. Infant milk formulae are continuously being improved to achieve all the functional effects in formula-fed infants that are observed in infants fed with breast milk [13].

The values of iodine obtained in the present study for selected starting, follow-on, special formulae and other samples are given in Table III. The iodine values of cereal products intended for meal preparation for young children, are too low and these cereal products cannot be the only iodine source for infants.

From the results obtained for infant formulae it is clear that only two samples (Beba (Nestle, Germany) and Bebiko 2 (Nutricia, Poland)) were in good agreement with the declared values. For the most critical group of milk products intended for babies with special needs, namely special formulae, there was no case of agreement with the declared value. These results are of concern and there is a necessity to ensure strict quality control of milk formulae to improve this area, a statement that had already been made by Dermelj *et al.* in 1999 [17]. They investigated declared values for iodine in various infant formulae samples and came to a similar conclusion that only two samples of infant formulae were in

Table III Iodine content and declared values in investigated products

	Product	Iodine content $\mu g\ g^{-1}$	Declared value $\mu g\ g^{-1}$
Starting formulae	Pikomil 1	0.35 ± 0.02	0.51
	Comformil 1	0.97 ± 0.05	0.79
from 0 – 6 months	HIPP 1	0.81 ± 0.04	0.7
	Bebiko 1	0.31 ± 0.01	0.91
	Bebilon 1	0.60 ± 0.02	0.77
	NAN 1	1.18 ± 0.05	0.78
	Beba	0.74 ± 0.02	0.77
Special formulae	Neocate	0.65 ± 0.04	0.47
	Progamin AS	0.18 ± 0.01	0.57
from 0 – 6 months	HN25	0.89 ± 0.05	-
	Aptamil prematil	1.82 ± 0.04	0.75
Follow on formulae	Milumil 2	0.52 ± 0.03	0.75
	Beba 3	0.97 ± 0.04	0.79
from 6 months further	HIPP 2	1.25 ± 0.07	0.81
	Bebiko 2	0.89 ± 0.04	0.88
	Bebilon 2	0.95 ± 0.02	0.82
	NAN 2	0.85 ± 0.03	1
	NAN H.A.	1.17 ± 0.02	1.0
Other	Powdered milk	0.25 ± 0.02	
	Granulated milk	1.22 ± 0.03	-
	Wheat bran	0.014 ± 0.002	-
	Oats flakes	0.009 ± 0.003	-
	Wheat flour	0.003 ± 0.001	-
	Barley porridge	0.006 ± 0.001	-

The results are given with measurement uncertainty

good agreement with the declared values, others showing considerably higher values than those labelled and declared by the producers. The difference between the values obtained by these authors and the declared values was in some cases a factor of one hundred.

Infant formulae analysed in this study did not show such large differences and they were of the same order of magnitude. Similar conclusions were drawn by Kunachowicz *et al.* [11]. They investigated the iodine content in five different infant formulae (starting, follow-on and special formulae). Analysis of the results obtained by them also demonstrated differences between the determined iodine content and the labelled one, but they did not vary more than a factor of two. So we can say that there is a slight improvement during the last decade.

There is an obvious lack of data in the literature regarding the iodine content of infant, follow-on and special formulae. Ares *et al.* [1] investigated the iodine content of infant formulae and the iodine intake of premature babies. They determined the iodine content of mother's breast milk, 32 formulae of different brands used in Spain and 117 formulae used in other countries. The results obtained for formulae showed a very high variability around the mean value for each brand. Another factor increasing variability was the country where the formula was sold (Table IV). They came to the conclusion that many preparations present then on the market do not even contain the minimum amount of iodine recommended before 1992, making the iodine content of many infant formulae inadequate [1].

Table IV Mean iodine content of infant formulae used in different European countries [1]

Year of study	Country	No of products	Iodine content μg l⁻¹	Range μg l⁻¹
1994	Austria	16	66	33 – 170
	Czech Republic	3	87	60 – 140
	France	17	105	40 – 150
	Germany	5	79	59 – 104
	Italy	16	75	20 – 120
	Poland	2	45	-
2000	Poland [11]	5	116	58 – 129
2004	This study	18	136	25 – 255

Conclusion

The iodine intake of newborn babies is entirely dependent on the iodine content of breast milk or of infant formula preparations, when breast feeding is not possible for various reasons. The producers of infant formulae should follow the newest recommendations to further improve infant formulae, making them a good replacement for mother's milk, but there is still a great need for stricter quality control of infant formulae. The values of iodine obtained in this study for eighteen different infant formulae, with the exception of two, showed insufficient agreement with the declared values, although the discrepancy between the analysed and declared values is becoming smaller than it was a decade ago.

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