

The Chair, Electronic Working Group on Infant Formula Composition
Codex Committee on Nutrition and Foods for Special Dietary Uses
Professor Dr. Hildegard Przyrembel
Federal Institute for Risk Assessment
P.O. Box 33 00 13

D-14191 Berlin, Germany

cc:

The Chair, Codex Committee on Nutrition and Foods for Special Dietary Uses
Dr. Rolf Grossklaus, Director and Professor
Federal Institute for Risk Assessment
P.O. Box 33 00 13

D-14191 Berlin, Germany

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Comments on the Circular Letter CL 2005/53-NFSDU and on the Synopsis of comments received until 30 April (prepared by Germany)

Dear Professor Przyrembel,

Thank you very much indeed for providing this very well structured material, and for the outstanding work that you have invested. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) wishes to respectfully submit the following comments:

- 1) **General Outline:** ESPGHAN supports the general outline and most of the details of the Draft Revised Standard for Infant Formula and greatly appreciates that many of the concepts and detailed suggestions made by the ESPGHAN coordinated International Expert Group report (1) have been adopted.
- 2) **3.1.3 - Maximum levels and “Guidance Upper Levels”:** ESPGHAN expresses its concern that the choice of the wording referring to “Guidance Upper Guidance Levels”, as proposed in the current draft, is rather vague and may lead to widespread use of formulae containing nutrient levels that exceed infant requirements by far and have not been subjected to a systematic evaluation of their suitability and safety in infants. Therefore, ESPGHAN suggests that the wording used should be reconsidered (see below).

- 3) ESPGHAN supports the conclusion of the International Expert Group report (1) that infant formulae should only contain components in such amounts that serve a nutritional purpose or provide another benefit. The inclusion of unnecessary components, or unnecessary amounts of components, may put a burden on metabolic and other physiologic functions of the infant. Those components taken in the diet, which are not utilised or stored by the body, have to be excreted, often as solutes in the urine. Since water available to form urine is limited and the infant's ability to concentrate urine is not fully developed during the first months of life, the need to excrete any additional solutes will reduce the margin of safety, especially under conditions of stress, such as fever, diarrhoea or during weight loss.
- 4) ESPGHAN generally supports the minimum and maximum values of nutrient contents in infant formulae suggested in the International Expert Group report (1) with the goal to provide safe and nutritionally adequate infant formula products that meet the nutritional requirements of healthy babies. These minimum and maximum values have been based, where available, on scientific data on infant requirements, the absence of adverse effects, and on scientific evaluations of upper safe levels of intake for infants or young children. In some cases where availability of scientific data was considered to be rather limited, maximum values for several nutrients have been suggested which equal 5 times the minimum adequate levels, taking into consideration that such a rather wide five-fold range should cover compositional variability of raw materials used in formula production and the possible decay of some nutrients during product shelf life. It was also taken into account that nutrient intakes exceeding 5 times the requirements have generally not been subjected to a systematic evaluation of safety in infants.
- 5) ESPGHAN is under the impression that differences exist in the **interpretation of minimum and maximum levels** for nutrients in infant formulae among the members and observers attending CCNFSDU. Some delegations apparently wish to use minimum and maximum levels for definition of a target range that infant formulae should aim for, while a certain level of tolerance is found acceptable for individual batches considering e.g. analytical imprecision. In contrast, other delegations appear to expect that analytical results should never be outside of the range from minimum to maximum levels. We suggest that this question, which may be important for deciding on specific values, be addressed by the Electronic Working Group.
- 6) ISDI has provided a report dated 31 March 2006 on "Industry Upper Levels of Nutrients for Infant Formulas: Data on Current Situation". ESPGHAN greatly appreciates the large amount of work that has been invested in assembling this report. The data presented on observed nutrient levels in a very large number of formula samples marketed worldwide in recent years is extremely valuable, and should help to make progress with respect to finding an agreement on the Infant Formula Standard. The data presented in the ISDI report indicate that the vast majority of infant formula currently produced provides nutrient intakes within the range recommended by the International Expert Group report (1). In those cases where values fall outside of the report, solutions often appear possible and will be addressed below when discussing the specific nutrient levels.
- 7) **Established history of apparently safe use.** The question arises whether the ranges of nutrient levels in infant formulae that are reported by ISDI, without documented occurrence of side effects, suffice to establish a "history of safe

use”, or even of adequacy of such nutrient levels for infant formulae. ISDI suggests that a history of apparently safe use of products might be based on the use of commercially produced infant formula and the monitoring of spontaneous consumer reports of observations that may indicate a problem with a specific batch of formula. In some areas, such as Europe, Israel and the USA, there are consumer phone line services have been established where parents may call in, usually free of charge, to place questions or complaints to the manufacturer or distributor of an infant formula. ISDI explains that such customer reports are monitored and should provide a tool for post-marketing surveillance of infant formula safety. Based on the evaluation of these consumer phone line services and the absence of detected serious side effects, ISDI implies that a history of safe use has been established for the nutrient levels reported in their compilation.

ESPGHAN wishes to emphasize that there is no evidence available to show that the evaluation of consumer phone line services is sensitive enough to detect adverse effects of infant formulae. On the contrary, for example the very severe adverse effects recently induced by an infant formula with inadequate contents of vitamin B1 (thiamine), which resulted in failure to thrive, severe neurological damage, severe lactic acidosis and even infant deaths (2-4), were not detected by the distributor’s consumer phone line services. The thiamine deficient formula was sold to caregivers of about 10,000 infants over a period of several months. The available 1-800 # got no calls that led to the detection of adverse effects. Only when 3 children were being treated at the same paediatric intensive care unit with severe and life-threatening symptoms, and it was detected that all three infants had been fed with the same formula, the inadequacy of the formula composition was detected and the connection to adverse side effects in these three and many other infants was made.

It is even less likely that an inadequate nutrient composition causing more subtle consequences, that still has significant adverse effects on health, would be detected by post marketing surveillance. For example, a randomized trial conducted in 1980 among 476 Dutch newborn infants examined the effects of a lower or higher content of salt/sodium in the infant diet on blood pressure during the first 6 months of life. Blood pressure was not only affected at the end of the 6 month of dietary intervention. The effects of early differences in nutrient intake persisted into adolescence, when individuals who had received a lower salt intake in the first months of life still had a systolic blood pressure that was lower by a mean 3.6 mm Hg, a difference that would clearly reduce the long-term risk to die from stroke (5). This long-term adverse effect of an excessive nutrient intake in early life, as well as other lasting effects of early feeding on long-term health (6), would certainly not be detected by post-marketing surveillance based on evaluation of consumer phone line services.

As another example, an evaluation of a commercial ready-to-feed formula that was heat processed in cans indicated that lack of bioavailability of copper from this formula, and induction of copper deficiency in infant primates (7), but this major inadequacy of a widely marketed product was not detected by post-marketing surveillance based on customer calls.

ESPGHAN concludes that the documentation of nutrient levels occurring in infant formulae sold on the market, as kindly provided by ISDI, does not demonstrate the adequacy or safety of these nutrient levels for infants.

- 8) ESPGHAN suggests that in cases where maximum values cannot be agreed upon, **Upper Guidance Levels** (not Guidance Upper Levels) should be set and defined in footnote 1 as:

“Upper Guidance Levels are set for some nutrients without sufficient information for a definite science-based risk assessment. These levels are derived on the basis of meeting nutritional requirements of infants and a history of apparently safe use. They may be adjusted based on relevant scientific or technological progress. Nutrient contents in infant formulae should usually not exceed the Upper Guidance Levels unless higher nutrient levels cannot be avoided due to high or variable contents in constituents of infant formulae or due to technological reasons.”

- 9) **3.1.3 a - Protein:** ESPGHAN supports the table and text, and suggests removing the square brackets around footnote 2).

In agreement with the conclusions of the expert reports of the Life Science Research Office of the American Societies for Nutritional Sciences (8), the EU Scientific Committee on Food (9), the International Expert Group (1) and the practice used by WHO/FAO, ESPGHAN supports the choice of one N-conversion factor of 6.25 for calculation of protein content in all types of infant formulae, and to account for the lower biological value of soy (and other plant) proteins by the use of a higher minimum requirement of protein content (2.25 rather than 1.8 g/100 kcal). The considerations put forward by the International Dairy Federation on the choice of nitrogen conversion factors for whole cows' milk are appreciated, but one needs to consider that unmodified milk cows' protein is hardly used any more for the production of modern infant formula, and hence a nitrogen conversion factor that may be appropriate for whole cows' milk would not be adequate for all types of infant formulae that contain a variety of different nitrogenous compounds derived from animal milks.

ESPGHAN also expresses its concern that the use of a nitrogen conversion factor that is higher than the factor 6.25 used by WHO/FAO for human protein and protein requirements, and for human milk protein, would suggest that the biological value of animal milk proteins is higher for infants than that of breast milk protein, which clearly is not the case.

The Codex General Principles clearly state that Codex Food Standards “... shall be based on the principle of sound scientific analysis and evidence, involving a thorough review of all relevant information, ..”. All recent reports of scientific experts in infant feeding (1,8,9) as well as the WHO's 2006 expert report on human protein requirements agree that a conversion factor of 6.25 should be used for calculating protein contents of human milk and infant formulae.

ESPGHAN agrees with some of the arguments submitted that the use of one and the same nitrogen conversion factor for all nitrogen sources used in infant formula is a compromise. A scientifically correct alternative would be to analyse

the contents of nitrogen and all amino acids in all different infant formula products, and to calculate a specific nitrogen conversion factor for each individual product, or even for each batch of a product. However, severe doubts exist whether this is a practically feasible approach on a global basis, hence the use of a nitrogen conversion factor of 6.25 is supported.

- 10) **3.1.3 a - Protein - Footnote 3):** ESPGHAN supports the wording proposed by New Zealand that would accommodate the potential use of milk proteins other than cows' milk protein, but suggest that this should not be extended to soy or other vegetable proteins. Therefore, we suggest the wording: "Infant formulae based on non-hydrolysed *milk* protein containing less than 2 g per 100 kcal and infant formulae based on hydrolysed protein containing less than 2.25 g protein per 100 kcal should be clinically evaluated".

ESPGHAN does not support the use of the term "partially hydrolysed", as suggested by the USA, because

a) there is no generally agreed definition of "partially hydrolysed", or agreed criteria that would allow a strict separation of "partially hydrolysed" and "extensively hydrolysed" products, and

b) there is no scientific reason to exclude the use of extensively hydrolysed proteins in infant formulae, for example with the aim of reducing allergy risks in otherwise healthy infants, if suitability and safety have been established, and indeed infant formulae with high degree hydrolysates are used in healthy children aiming at reduction of allergy risks.

- 11) **Total fat:** ESPGHAN supports the table and the text in footnote 5, including the 3 % maximum level for trans fatty acids, and suggests to remove the square brackets.

Linoleic acid, alpha-Linolenic acid, and Linoleic / alpha-Linolenic acid ratio: ESPGHAN supports the values in the table which provide for a balanced and adequate essential fatty acid intake.

The USA and ISDI have proposed a higher maximum level for linoleic acid of 1.4 g/100 kcal. The ISDI data evaluation reports that linoleic acid levels at or above 1.2 g/100 kcal, as proposed by the International Expert Group report (1) were found in 17,773 units of hypothetical infants fed for 3 months, i.e. only 0.05 % of the total amount of formula on which the ISDI report is based (35.306.321 units of hypothetical infants fed for 3 months).

The linoleic acid content in infant formula is a function of the choice of oil and fat blends used in formula production and thus can be adapted to meet the manufacturer's goals. The ISDI report mentions that soy oil, which is high in linoleic acid, is also a good source of alpha-linolenic acid. However, to reach the minimum or twice the minimum contents, respectively, of alpha-linolenic acid in infant formulae as suggested in the draft standard, only about 6.5 or 1.3 g/100 kcal, respectively, of soy oil (about 10-20 % of the fat blend) would suffice. Thus it is no apparent to ESPGHAN why there is a need to increase the linoleic acid maximum to a level higher than 1.2 g/100 kcal, which would provide for a less

balanced composition. If there is a true necessity to increase the maximum level for technological or cost reasons, or due to limited availability of suitable raw materials in some parts of the world, such arguments should be put forward. If such convincing reasons would be put forward, and the proposed ranges of Linoleic/alpha-Linolenic Acid Ratio and of Vitamin E are accepted, ESPGHAN would consider supporting a maximum level for linoleic acid of 1.4 g/100 kcal.

- 12) **c – Carbohydrates:** ESPGHAN supports the table and text, and suggests to removing the square brackets around footnote 6).
- 13) **Vitamin A:** ESPGHAN supports the values of 60-180 µg/100 kcal and the text of the footnote as proposed. Setting of a Maximum Level is supported because excessive intakes of vitamin A may induce severe adverse effects.

Both the USA and ISDI have proposed a higher maximum level for vitamin A of 225 µg/100 kcal. Vitamin A levels at or above 180 µg/100 kcal are reported in the ISDI data evaluation to occur only in a very small proportion of less than 0.05 % of the total amount of infant formula products reviewed. The ISDI report indicates that the vitamin A content in infant formula is derived principally from its specific addition at the time of manufacture, and loss at the end of shelf life is reported not to exceed 30 % (without presenting the mean and range of losses, or the data on which this estimate is based). Thus, one would conclude that levels above 180 µg/100 kcal can be avoided with good manufacturing practice.

Maximum levels for preformed vitamin A in infant formulae of 150 µg/100 kcal (8) and of 180 µg/100 kcal (1,9) have been set based on the observations of adverse effects of chronic high vitamin A intakes and science based evaluations of tolerable upper intake levels for children 1 to 3 year old (800 µg RE/day), calculating that consumption of formulae with vitamin A contents greater than 180 µg/100 kcal may result in intakes higher than the tolerable upper intake level. As mentioned before, the Codex General Principles clearly state that Codex Food Standards “... shall be based on the principle of sound scientific analysis and evidence, involving a thorough review of all relevant information, ..”, an aim the ESPGHAN coordinated International Expert Group report strived to achieve (1). Scientific arguments to justify higher maximum levels, and their safety, have not become known to ESPGHAN.

- 14) **Vitamin D:** ESPGHAN supports the suggested values of 1-25 µg/100 kcal.

ESPGHAN also supports the proposal of Norway to indicate in the footnote that vitamin D₃ should be used. No conclusive evidence is available to allow a comparative assessment of the biological activity of dietary vitamin D₃ and vitamin D₂ in infants, therefore it is recommended to continue to use vitamin D₃ in infant formulae, rather than vitamin D₂, until such comparative data might become available (1).

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN as-

sumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 15) **Vitamin E:** ESPGHAN supports the suggested values of 0.5-5 mg/100 kcal and the respective footnotes.

A Vitamin E level in infant formula up to 10 mg/100 kcal has been considered to induce no adverse effects (10). Very high intakes of vitamin E may induce adverse effects, and levels markedly higher than 5 mg have not been subjected to a systematic analysis of its effects in infants. Therefore, ESPGHAN favours setting a Maximum Level rather than an Upper Guidance Level.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN assumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 16) **Vitamin K:** ESPGHAN supports the suggested values of 4-25 µg/100 kcal and would accept if 25 µg/100 kcal is regarded as an Upper Guidance Level rather than a Upper Maximum Level, considering that oral supplementation of infants with several mg vitamin K is given during the first weeks of life in different countries without any indication of untoward effects in large surveillance studies.

- 17) **Thiamine:** ESPGHAN supports the suggested values of 60-300 µg/100 kcal and proposes to set a maximum value, but could also accept setting an Upper Guidance Level if convincing arguments were put forward that this is really necessary.

The ISDI report indicates that thiamine levels at or above 300 µg/100 kcal occur only in a very small proportion of 0.024 % of the total amount of formula reviewed, with a reported range of maximum values (means + 2 SD) of 121-315 µg/100 kcal. The report states that more than 90 % of thiamine content in formula originate from the vitamin mix added during formula production. Loss at the end of shelf life is reported not to exceed 40 % (without presenting the mean and range of losses, or the data on which this estimate is based). Thus, one would conclude that levels above 300 µg/100 kcal can be avoided with good manufacturing practice.

If there is a true necessity to increase the maximum level to either 315 or 330 µg/100 kcal for technological or cost reasons, or due to limited availability of suitable raw materials in some parts of the world, such arguments should be put forward. If there are technological or cost reasons to increase the maximum levels only for soy protein based formulae one might wish to discuss the option of a separate maximum level for these products, but in this case again such arguments should be put forward. In this ESPGHAN would review these arguments and consider acceptance of a maximum level of 330 µg/100 kcal.

- 18) **Riboflavin:** ESPGHAN supports the suggested values of 80-400 µg/100 kcal and proposes to set a Maximum Value, but would not oppose setting an Upper Guidance Level.

The ISDI report indicates that levels at or above 400 µg/100 kcal occur in 0.52 % of the total amount of formula reviewed, with a reported range of maximum values (means + 2 SD) of 138-519 µg/100 kcal. The report states that riboflavin is one of the more variable nutrients in infant formulae because of different levels in various ingredients such as milk protein and lactose, and the effects of form and package on stability. Loss at the end of shelf life is reported not to exceed 60 % (without presenting the mean and range of losses, or the data on which this estimate is based). In view of these challenges for formula production and given that riboflavin appears virtually non-toxic when given orally, with excess excreted in urine, ESPGHAN does not oppose a maximum level of 600 µg/100 kcal.

- 19) **Niacin:** ESPGHAN supports the suggested values of 300-1500 µg/100 kcal which refer to preformed niacin (1), and proposes to set a Maximum Value but would not oppose setting an Upper Guidance Level.

The ISDI report indicates that levels at or above 1500 µg/100 kcal occur in 0.095 % of the total amount of formula reviewed, with a reported range of maximum values (means + 2 SD) of 898-2285 µg/100 kcal. The report indicates that variability was not related to product type or form, therefore, there appears to be no reason to set different levels for formulae based on milk or soy proteins, respectively. Loss at the end of shelf life is reported not to exceed 27 % (without presenting the mean and range of losses, or the data on which this estimate is based). The report states that nearly all niacin in formula originates from the vitamin mix added during formula production. Thus, one would conclude that levels above 1500 µg/100 kcal can easily be avoided with good manufacturing practice.

- 20) **Vitamin B6:** ESPGHAN supports the suggested values of 35-175 µg/100 kcal, and proposes to set a Maximum Value but would not oppose setting an Upper Guidance Level.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN assumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 21) **Vitamin B12:** ESPGHAN supports the suggested values of 0.1-0.5 µg/100 kcal, and proposes to set a Maximum Value but would not oppose setting an Upper Guidance Level.

The ISDI submission reports that levels at or above 0.5 µg/100 kcal occur in 2.4 % of the total amount of formula reviewed, with a reported range of maximum values (means + 2 SD) of 0.5-1.5 µg/100 kcal. Losses at the end of shelf life are

reported not to exceed 55 % (without presenting the mean and range of losses, or the data on which this estimate is based). The report states that milk protein may contribute up to 0.14 µg/100 kcal of finished product and indicates considerable imprecision of analytical determination of vitamin B12. Considering these reported challenges for formula production and monitoring of its composition, and lack of known untoward effects with high oral doses of cobalamin given to infants for therapeutic purposes, and the urinary excretion of excessive amounts, ESPGHAN does not oppose a higher Maximum Level but considers that a Maximum Level of 1.0 µg/100 kcal could accommodate for the content arising from intrinsic contents in raw materials used of up to 0.14 µg/100 kcal and a loss during shelf life of up to 55 %.

- 22) **Vitamin C:** ESPGHAN supports the suggested values of 10-30 mg/100 kcal, and proposes to set a Maximum Value.

The ISDI submission indicates levels at or above the suggested Maximum Value occur only in a very small fraction of 0.067 % of the total formula production reviewed, with a reported range of maximum values (means + 2 SD) of 18-72 mg/100 kcal. Loss at the end of shelf life is reported to range up to 65 % (without presenting the mean and range of losses, or the data on which this estimate is based). The report further states that virtually all of the vitamin contents in formula originate from the vitamin mix added during formula production. The wording chosen in the ISDI suggests that vitamin C levels in the suggested range of 10-30 mg/100 kcal are feasible for powdered products while this is more of a challenge in liquid products due to a much greater degradation over the course of shelf life.

The ISDI submission suggests that there would be “a history of safe use well above 30 mg/100 kcal” but no data are presented to establish safety, such as lack of interference with infant copper absorption and copper balance at such levels. High intakes of vitamin C have been shown to reduce copper absorption and also to affect intracellular copper metabolism and transport of copper to the liver (11). Limited data from a small balance study in 6 formula fed preterm infants with low-birth-weight supplemented with ascorbic acid (2 daily doses of 31 mg/kg&d) showed no significant effects on iron, copper, zinc and nitrogen balance under these conditions (12), but no data were obtained on copper metabolism and transport in these preterm infants, and also conditions may differ in larger infants where both iron and copper absorption may be affected by vitamin C.

While there are some indications of possible adverse effects of high ascorbic acid intakes, the existing technological challenges are acknowledged. Considering that liquid formulae contribute a rather small proportion to total formula production (12.8 % of total formula production reviewed in the ISDI report), that liquid formulae may be of benefit to the recipient infants primarily under poor hygienic conditions, and expecting that manufacturers will strive for improved production methods to reduce high vitamin C levels that could interfere with the absorption of other essential nutrients, ESPGHAN could accept setting separate Maximum Values of 45 mg/100 kcal for powdered formulae and of 90 mg/100 kcal for liquid formulae, respectively.

- 23) **Iron:** ESPGHAN supports the suggested values of 0.3-1.3 mg/100 kcal for infant formulae based on milk protein and its hydrolysates, and of 0.45-1.9 mg/100 kcal for infant formulae based on soy protein.

The ISDI submission reports levels at or above the suggested Maximum Value in 2.38 % of the total formula production reviewed, with a reported range of maximum values (means + 2 SD) of 1.9-2.4 mg/100 kcal. Iron levels are reported to be determined primarily by the amount of iron specifically added by the manufacturer, and losses at the end of shelf life are reported to be 0 %. Thus, here the choice of minimum and maximum levels does not need to take further technological limitations into account but should be led by physiological and nutritional considerations.

Current scientific evidence clearly indicates that iron levels of 0.3 mg/100 kcal in infant formulae suffice to meet infant iron requirements (1). During the period when infant formula may be fed exclusively, i.e. before the introduction of complementary foods, infant formulae based on cows' milk protein supplying 0.25 mg, 0.6 mg and 1.0 mg per 100 kcal resulted in similar iron status and haematology results, and there were no infants with inadequate iron status in either group (1).

After the age of 6 months, infant formula is unlikely to be fed exclusively, and the introduction of complementary feeding/Beikost and the stepwise introduction of foods from adult diets are recommended. The ESPGHAN coordinated International Expert Group addressed the question whether formula feeding together with diets having very low iron contents might induce a risk of developing iron deficiency anaemia during this time period. In a study from Chile, infants were fed formulae with about 0.34 mg and 1.9 mg/100 kcal, respectively, from 6 to 12 months of age. As these Chilean infants received little additional iron from complementary feeding, this study evaluates whether the lower level of iron fortification would be inadequate in a poor setting. There was no significant difference in prevalence of iron deficiency anaemia between groups, thus formulae with about 0.34 mg/100 kcal can prevent iron deficiency anaemia even in a poor setting (1).

In the past it was often considered that a lower iron bioavailability from formula than from breast milk might justify that the minimum level should be kept higher. It has commonly been assumed that iron absorption from breast milk is much higher (about 5-fold) than from infant formula. However, such data were generated more than two decades ago, and there were several methodological problems with these studies. For example, a commonly cited study by Saarinen et al (cited in 1) used an extrinsic labelling technique that is not valid, and what they called "formula" was a home-made product made from cows' milk. Infant formulae have developed during the last two decades and recent studies show that iron absorption from both breast milk and modern infant formulae is about 15-20 %; i.e. there is no major difference in iron absorption between modern infant formulae and human milk (1). Therefore, a breast-fed infant consuming 750 ml of milk will absorb 20 % of 0.2-0.3 mg/L = 0.03 – 0.05 mg of iron per day. A formula-fed infant consuming 500 kcal/day would absorb 15-20 % of 0.3 mg/100 kcal (proposed minimum for cows' milk based formulae) equal to 0.22 – 0.3 mg

of iron per day. Thus, infants fed the proposed minimum level of 0.3 mg/100 kcal would absorb 4-10 (four to ten!) times more iron than breast-fed infants.

The ESPGHAN coordinated International Expert Group considered potential risks associated with providing too much iron in early life (1). Infants fed formulae with higher iron levels (1.5 mg/100 kcal and 1 mg/100 kcal, respectively) showed reduced copper absorption and status with functional outcome effects (Haschke et al and Lönnerdal and Hernell, cited in 1). In a more recent study on Swedish and Honduran infants aged 4-9 months, iron supplementation (1 mg/kg & d) to Swedish breast-fed infants with adequate iron status significantly reduced their length growth (13). This marked adverse side effect of a high iron intake in infants was not observed for the Honduran cohort as such, but when dividing these infants according to iron status which varied much more in Honduras, Honduran infants with adequate iron status given iron supplements also had significantly lower length gain. Infants with adequate iron status who were given iron had a significantly higher prevalence of diarrhoea and a marginally higher prevalence of upper respiratory infections. Infants receiving iron supplements from 4-9 mo also had significantly lower enzyme activity of erythrocyte copper-zinc superoxide dismutase at 9 months of age (0.95 ± 0.27 vs 1.08 ± 0.24 U/mg Hb, $p=0.023$) than those receiving placebo (14). These data demonstrate that in both settings, one affluent and one poor population, providing excess iron to infants caused relevant adverse effects and should be avoided.

While it may be argued that the supplemental iron was given in free form and not in formula, basic studies on iron homeostasis in infants suggest that there may be reasons for concern regardless of the form of iron provided. In the Swedish cohort described earlier, iron absorption studies with stable isotopes have been performed (15). Iron absorption at 6 months was identical in infants who had received iron supplements for 2 months and those who had not been supplemented. Thus, at this age there is no homeostatic down-regulation of iron absorption as would occur in adults. By 9 months of age, iron absorption was significantly lower in Fe-supplemented infants than in non-supplemented infants. This shows that regulation of iron absorption is immature at a young age and does not start reaching adult levels until about 9 months of age. This was further supported by the fact that haemoglobin and serum ferritin of infants with adequate iron status increased to the same extent as they did in non-supplemented infants (16), i.e. whatever amounts of iron given will be absorbed and accumulated in the body raising the possibility of iron excess. Whether the adverse effects of excess iron are due to pro-oxidative events caused by Fe, interactions with zinc which may affect insulin like growth factor 1 and thereby growth, or the immune system and be related to infection risks, or other factors cannot be determined with certainty at this time. However, the observed effects warrant caution with respect to supplying iron exceeding requirements. Iron contents higher than 1.3 mg/100 kcal provide no additional benefit, but adverse effects on copper status have been observed.

A further question addressed was whether a minimum iron content of 0.3 mg/100 kcal would be appropriate for all populations. Various bodies, including the World Health Organisation, have made efforts to improve the micronutrient supply of infants with complementary foods globally. In many parts of the world, weaning foods containing meat and iron fortified baby foods with a good

bioavailability of iron are commonly used between 6-12 months. Thus, many infants receive quite substantial quantities of iron in their diet, and there may be good reasons to allow formula manufacturers to use a level close to 0.3 mg/100 kcal. Setting minimum values of 0.45 mg/kcal, as proposed by Australia, or 0.6 mg/100 kcal as proposed by the USA, would eliminate the choice of using infant formulae with an iron supply that is fully adequate to meet nutrient needs, based on controlled trials with adequate methodology, and is more similar to the physiological model of breast feeding. These choices of higher minimum levels would also implicitly suggest that full breastfeeding, providing about 6-15 or 8-20 times less absorbed iron than formulae with these suggested minimum iron contents, respectively, would supply an inappropriately low amount of absorbed iron for healthy infants in the first weeks and months of life and thus would need to be supplemented with iron, a notion that ESPGHAN cannot support. Therefore, ESPGHAN supports the suggested values of 0.3-1.3 mg/100 kcal for infant formulae based on milk protein and its hydrolysates.

- 24) **Calcium:** ESPGHAN supports the suggested values of 50-140 mg/100 kcal and setting a Maximum Value, considering that higher calcium intakes may induce adverse effects such as gastrointestinal and renal side effects.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN assumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 25) **Phosphorus:** ESPGHAN supports the suggested values of 25-90 mg/100 kcal for infant formulae based on milk protein and its hydrolysates, and of 30-100 mg/100 kcal for infant formulae based on soy protein, regardless of the presentation of the latter value in the table or in a footnote.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN assumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 26) **Calcium/Phosphorus-Ratio:** ESPGHAN supports the suggested values of 1-2:1 and setting both a Minimum and a Maximum Value, considering that extreme ratios may induce adverse effects.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN assumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 27) **Magnesium:** ESPGHAN supports the suggested values of 5-15 mg/100 kcal and setting both a Minimum and a Maximum Value.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN assumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 28) **Sodium:** ESPGHAN supports the suggested values of 20-60 mg/100 kcal and setting both a Minimum and a Maximum Value. High sodium intakes in infants were shown to increase blood pressure not only during the period of feeding, but persisting well into adolescence, suggesting that excessive sodium intake in early life may affect the long-term risk to die from stroke (5). In view of these possibly severe adverse effects of excessive intakes, a maximum level appears needed.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN assumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 29) **Chloride:** ESPGHAN supports the suggested values of 50-160 mg/100 kcal and setting both a Minimum and a Maximum Value.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN assumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 30) **Potassium:** ESPGHAN supports the suggested values of 50-160 mg/100 kcal and setting both a Minimum and a Maximum Value.

The ISDI submission indicates levels at or above the suggested Maximum Value occur only in a very small fraction of 0.019 % of the total formula production reviewed, with a reported range of Maximum Values (means + 2 SD) of 114-164 mg/100 kcal, i.e. hardly exceeding the suggested Maximum Value. Loss at the end of shelf life is reported as 0 %, and analytical precision is high. Considering the variability of potassium contents in cows' milk and the use of various potassium containing compounds for formula production, ESPGHAN does not oppose a maximum level of 180 mg /100 kcal.

- 31) **Manganese:** ESPGHAN supports the suggested Minimum Level of 1 µg/100 kcal and does not oppose setting a Maximum Level of 50 µg/100 kcal for infant formulae based on milk protein and its hydrolysates, and of 100 µg/100 kcal for infant formulae based on soy protein.

The ISDI submission indicates levels at or above 50 µg/100 kcal occur only in a very small fraction of 0.28 % of the total cows milk protein based formula production reviewed with a reported range of Maximum Values (means + 2 SD) of 14-61 µg/100 kcal, and in 0.33 % of the total soy protein based formula production reviewed with a reported range of Maximum Values (means + 2 SD) of 39-59 µg/100 kcal. The report indicates that the higher levels soy protein based formulae originate from manganese contents of the soy protein isolates used and may be difficult to avoid with current manufacturing technology.

The recommended Minimum Level of 1 µg/100 kcal is in the order of human milk concentrations, taking into account that there is no major difference in manganese bioavailability between breast milk and formulae. Very high manganese intakes should be avoided, because manganese excretion is immature in infants and hence very high intakes may cause accumulation in tissues including brain with potential adverse consequences, such as neurodevelopmental abnormalities observed in newborn animals (17). Feeding of a soy protein based infant formula with 300 µg manganese/L to infant rhesus monkeys tended to alter neurotransmitter levels in cerebrospinal fluid and led to significantly less play behaviour, more affiliative clinging in social dyadic interactions, shorter wake cycles, and shorter periods of daytime inactivity, than observed in controls fed a soy protein based infant formula with 50 µg manganese/L or a cows milk protein based infant formula. Given these indications of marked effects of very high manganese intakes on early neurodevelopment, a Maximum Value should be set that should not be higher as can be achieved with reasonable efforts and standard manufacturing technology.

- 32) **Iodine:** ESPGHAN supports the suggested Minimum Level of 10 µg/100 kcal and does not oppose setting a Maximum Level of 75 µg/100 kcal.

The ISDI submission indicates levels at or above 50 µg/100 kcal occur in 0.13 % of the total formula production reviewed. The report indicates that iodine content in milk and milk proteins differs considerably, which may make it difficult to always achieve levels not exceeding 50 µg/100 kcal.

Iodine levels up to about 75 µg/100 kcal occur in human milk, depending on the iodine intake of lactating women (8,9). The FAO/WHO Joint Expert Consultation set probable safe upper limits of 150 and 140 µg/kg/day in infants 0-6 months and 7-12 months, respectively, and suggested that the upper limit probably should be one that provides a daily iodine intake of no more than 100 µg/kg (18). Considering these data and the current technological constraints, ESPGHAN does not oppose setting a Maximum Level of 75 µg/100 kcal.

- 33) **Selenium:** ESPGHAN supports the suggested Minimum and Maximum Levels of 1-9 µg/100 kcal.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN as-

sumes that observed nutrient levels are within the range proposed by the International Expert Group report.

Upper Safe Levels of selenium intake have been set as 45 µg/day in 0-6 month infants (equivalent to 9 µg/100 kcal at an energy intake of 500 kcal/day) and as 60 µg/day in 7-12 month infants by the US Food and Nutrition Board, and as 60 µg/day by the EU Scientific Committee on Food (cited in 9). A Maximum Level of 9 µg/100 kcal is suggested to avoid exceeding the Upper Safe Levels of intake.

- 34) **Copper:** ESPGHAN supports the suggested Minimum Level of 35 µg/100 kcal and setting a Maximum Level of 80 µg/100 kcal.

The ISDI submission indicates that levels at or above 80 µg/100 kcal occur in 0.18 % of the total formula production reviewed, with a reported range of Maximum Values (means + 2 SD) of 93-189 mg/100 kcal. It is reported that the majority of copper is added during manufacture, and there is no loss during storage, hence manufacturers can largely modify copper levels. The report indicates that copper contents are higher in U.S. and non-European global products, than in European products, and also tend to be higher in soy formulae than in cows' milk protein based formulae.

Diets which contain excess copper have been reported to lead to toxicity and liver damage during childhood, which have occurred particularly in infants fed formulae prepared with tap water with a high copper content (9,11). For children 1-3 years, a tolerable upper intake level for copper has been set of 1 mg/day (equivalent to about 200 µg/100 kcal). To meet this tolerable upper intake level, one needs to take into account both the intake from formula products and from tap water which may contain up to 2 mg/L following WHO standards. Thus, a Maximum Level of 160 µg/100 kcal, as suggested by the USA, appears acceptable for liquid infant formulae, considering that infants fed with such formulae will only consume limited amounts of additional tap water. Infants fed with powdered formulae may receive some 750 ml or more of tap water per day which may contain 2 mg/L copper and sometimes even higher levels. Therefore, a Maximum Level of 80 µg/100 kcal should be set for powdered infant formulae to avoid excessive copper intakes.

- 35) **Zinc:** ESPGHAN supports the suggested Minimum Level of 0.5 mg/100 kcal and the suggested Maximum Level of 1.5 mg/100 kcal.

It is noted that the ISDI report does not provide data to show whether the proportion of formulae exceeding the proposed maximum level would be in the same order of magnitude as, or different from, the reported proportions of other nutrients exceeding the recommended maximum levels. Thus, ESPGHAN assumes that observed nutrient levels are within the range proposed by the International Expert Group report.

- 36) **Chromium and Molybdenum:** ESPGHAN concludes that at this time there are no sufficient biological or nutritional data to define minimum and maximum levels for infant formulae.

- 37) **Choline, Myo-Inositol, L-Carnitine:** ESPGHAN supports the suggested values.
- 38) **Optional ingredients:** ESPGHAN supports the levels as proposed and supports deletion of the square brackets.
- 39) **Carrageenan:** ESPGHAN notes that carrageenan is included in the provisional list of accepted food additives for infant formulae of the current draft of the Infant Formula Standard. Carrageenan is used as a thickener, stabilizer, and texturer in a variety of processed foods. In animals, particularly degraded carrageenan with low molecular weight (polygeenan) induces strong inflammatory reactions in the intestine and is used to induce experimental colitis. As a component of a barium enema solution, carrageenan produced allergic reactions (cited in 1). Given the lack of adequate information on possible absorption of carrageenan by the immature gut in young infants and its biologic effects in infancy, it appears inadvisable to use carrageenan in infant formulae intended for feeding young infants, including those in the category of foods for special medical purposes.

Thank you very much indeed for considering our comments. Yours sincerely



Berthold Koletzko, M.D., Professor of Paediatrics
Chair, ESPGHAN Committee on Nutrition

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