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REVIEW ARTICLE

ACRYLAMIDE IN HUMAN BREAST MILK – THE CURRENT STATE OF KNOWLEDGE

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ABSTRACT

Human milk is a first choice in infant nutrition. It not only provides all the nutrients necessary for the proper infant`s development but also contains bioactive factors that provide natural protection against infections. Unfortunately, chemical contaminants can pass to breast milk and pose a health risk for the breastfed infant's health. Acrylamide is a typical process contaminant and in food it is formed as a result of the Maillard reaction. Numerous studies have shown that acrylamide is a neurotoxic and carcinogenic compound. So far there have been published only three studies on the acrylamide content in human milk. In two of them, the acrylamide level in most of the tested samples did not exceed the value of 0.5 µg/L. In the third study, the authors assessed the circulation of acrylamide in the body of two breastfeeding women after consuming products with high acrylamide content. Depending on the time elapsed after the meal, the acrylamide content ranged from 3.17 µg/L to 18.8 µg /L. These studies show that the breastfeeding mothers' diet may have a significant influence on the level of acrylamide in their milk. However, it seems that the acrylamide content in breast milk is also influenced by the time of breast milk collection, including the time elapsed after the mother's meal. To assess the exposure of breastfed infants to acrylamide in human milk, more data is needed on the acrylamide content in human milk at different stages of lactation and using standard protocols for human milk sampling.

Key words: *acrylamide, human breast milk, breastfeeding mothers, diet, exposure*

INTRODUCTION

Breastfeeding is the best way to feed babies early in life. Human milk provides all the necessary nutrients in the amount that meets the needs at a given stage of baby's development. It is also a source of a wide range of bioactive factors that may prevent allergies and protect against infections. World Health Organization (WHO) recommends "exclusive breastfeeding for 6 months, followed by continued breastfeeding as complementary foods are introduced, with continuation of breastfeeding for 2 years or longer as mutually desired by mother and infant" [1]. The content of individual nutrients and bioactive compounds in breast milk is influenced by both maternal physiological factors and lifestyle, including eating habits during pregnancy and lactation [2]. Unfortunately, chemical contaminants derived from environmental pollution as well as generated during food processing may be transferred to breast milk and pose a risk for the breastfed infant's health.

Acrylamide (prop-2-enamide; CAS 79-06-1) is a vinyl monomer used on an industrial scale for the synthesis of polyacrylamide polymers which are widely used among others, as fillers for industrial and drinking water filters, in the petroleum, paper, textile and cosmetic industries [3]. In 2002, the Swedish National Food Agency in collaboration with scientists from the Stockholm University reported for the first time results with a high acrylamide content in heat-treated food [4]. Twenty years after the first reports, it is well known that acrylamide is a processing contaminant mainly formed in food as a product of the Maillard reaction between free asparagine and reducing sugars, especially glucose and fructose, under temperatures of more than 120°C [5, 6]. The main source of acrylamide in the human diet are thermally processed potato and cereal products as well as coffee and its substitutes. The content of acrylamide in food varies widely, from below 10 µg/kg in bread to even more than 7000 µg/kg in individual samples of coffee substitutes [7]. Recent studies by *Timmermann* et al. [8] show that the estimated median exposure to acrylamide from food worldwide varies from 0.02 µg/kg bw/day to 1.53 µg/kg bw/day. Another important source of human exposure to acrylamide

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is smoking [9, 10, 11]. In recent years, studies have been conducted on the fate of acrylamide during digestion in the human gastrointestinal tract [12] and the possibility of acrylamide formation from other intermediate products of the Maillard reaction, e.g. 5-hydroxymethylfurfural, released both during digestion in the gastrointestinal tract and present in thermally processed products [13, 14].

The International Agency for Research on Cancer classified acrylamide as „probable human carcinogen" (Group 2A) as early as 1994 [3], concluding that the carcinogenicity of acrylamide in animal studies is well documented, despite limited evidence for it in human studies. Acrylamide also demonstrates neurotoxic properties and can contribute to damaging the central and peripheral nervous system, both among experimental animals and people exposed to this compound at work [15, 16]. The margin of exposure (MOE) criterion is commonly used to assess the carcinogenic and neurotoxic risks of acrylamide [17]. The MOE is the ratio of the Benchmark Dose Lower Limit ($BMDL_{10}$) to the estimated human intake of the compound. Joint FAO/WHO Expert Committee on Food Additive (JECFA) [18] considered that 0.18 mg/kg bw /day (lowest value in the range of $BMDL_{10}$ values) for Harderian gland tumors in male mice and 0.31 mg/kg bw /day for breast tumors in female rats are appropriate for assessing the risk of carcinogenic effects of acrylamide and advises that a calculated MOE of less than 10 000, based on $BMDL_{10}$ from animal studies, may indicate that the compound poses a risk to human health.

In the human body, acrylamide is metabolized through two main metabolic pathways: epoxidation to glycidamide and glutathione conjugation to mercapturic acids. The conversion of acrylamide to glycidamide, its main metabolite, is catalyzed by an enzyme of cytochrome P450 (isoenzyme CYP2E1) [19] Both acrylamide and glycidamide form adducts with haemoglobin. Adducts of acrylamide and glycidamide with hemoglobin (AAVal and GAVal) are used to assess long-term exposure to acrylamide [20]. In turn, the assessment of recent exposure uses the metabolites of acrylamide and glycidamide in form of mercapturic acid derivatives, N-Acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and glycidamide to N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA), which are excreted in the urine [21, 21].

EFFECT OF DIETARY ACRYLAMIDE INTAKE DURING PREGNANCY ON OFFSPRING

Due to its very good solubility in water, acrylamide is quickly absorbed and transferred to various tissues. It is able to pass through the placenta barrier [23] posing a risk to the growing foetus. Several studies [24, 25, 26, 27] have confirmed the relationship between dietary intake of acrylamide by pregnant women and low birth weight, length and head circumference of newborns. They also demonstrated an increased risk of having a baby that is small for gestational age (SGA). On the other hand, it is worth noting, that *Nagata* et al. [28] did not confirm an inverse relationship between the consumption of acrylamide in the diet in a group of 204 Japanese pregnant women and the birth size of their newborns. At the same study, they found that higher acrylamide consumption was significantly positively associated with higher levels of umbilical cord estradiol during labor. The results of *Nagata* et al. [28] indicate other possible action of acrylamide.

TRANSFER OF ACRYLAMIDE FROM DIET INTO MILK AND EFFECTS ON OFFSPRING

Animal studies [29, 30] showed that oral administration of acrylamide to lactating female rats did not cause adverse effects in the offspring. The authors concluded that this is probably due to the limited transfer of acrylamide across the blood / milk barrier during lactation and the low level of acrylamide in the milk, which is insufficient to initiate toxic changes. In turn, *Pabst* et al. [31] determined the acrylamide content in cow feed and investigated the potential to carry-over of acrylamide from cattle feed to cow's milk. From the results obtained, they calculated that the mean carry-over of acrylamide was 0.24% of the amount taken from feed. The mean half-life of acrylamide was estimated to be 2.8 hours. The authors concluded that acrylamide was rapidly transformed in the cows. Considering the analytically determined levels of acrylamide in the commercial feed at 180, 145 and 140 µg/kg, they also estimated that the expected concentration of acrylamide in raw milk from cows fed with such feeds could be a maximum of approximately 0.2 µg/kg. It is worth noting that the metabolism of acrylamide in the human body is also described as fast, and the presence of AAMA and GAMA, the main metabolites of acrylamide, is found in the urine 2 hours after oral ingestion of acrylamide [32].

ACRYLAMIDE IN HUMAN BREAST MILK

So far there have been published three studies on the acrylamide content in human milk [33, 34, 35]. The first results was published by *Sörgel* et al. [33] already in 2002. They evaluated the transmission of acrylamide in case of two breastfeeding women who consumed potato chips (crisps) with the acrylamide

content at the level of 800 μg/kg and 1000 μg/kg. The determined acrylamide content in the breast milk samples of the subjects was high and ranged in the first subject from 4.86 μg/L after 4 h to 3.17 μg/L after 8 h from eating potato crisps and, respectively, 10.6 μg/L after 3 h to 18.8 μg/L after 4 h in the other subject. The authors found that the level of acrylamide in human milk correlated with the amount of this compound ingested by breastfeeding mothers and with the time elapsed after a meal. They concluded that acrylamide is transferred from mother's diet to her breast milk and could pose a risk to infant during breastfeeding.

On the other hand, *Fohgelberg* et al. [34], who determined acrylamide in breast milk samples taken from 15 women and in 4 pooled breast milk samples (10 mothers per pool) in Sweden found that in most of the samples acrylamide content was below the limit of quantification (LOQ = 0.5 μg/kg). Only in one sample, the acrylamide content was above LOQ and was equal to 0.51 μg/kg. According to the authors, this confirms the rapid digestion of acrylamide in the human body and the limitation of its penetration into breast milk. However, it is not described how the individual milk samples were taken and how much time elapsed between sample collection and the last meal of the breastfeeding mother.

In recently published study carried out in Poland [35] the results of determination of acrylamide content in human breast milk, in two lactation periods, were presented. As in the studies by *Sörgel* et al. [33] and *Fohgelberg* et al. [34] acrylamide content was determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). In Polish study, acrylamide content was determined in 47 samples of colostrum collected by healthy women in the 2nd – 3rd day after childbirth in the Obstetrics Ward and in 26 samples of mature breast milk collected by breastfeeding mothers at home. In the majority (77 %) of colostrum samples and in over 40% of the mature milk samples, the acrylamide level was below the limit of quantification (LOQ = 0.1 μg/L). Additionally, in the majority of the samples, both colostrum (93.6% of the samples) and mature milk (96.1% of the samples), the acrylamide level was not higher than 0.5 μg/L. The highest level of acrylamide was found in only one sample of human milk and it reached 1.0 μg/L. These results are similar to the data presented by *Fohgelberg* et al. [34] and are from several to several dozen times lower than found by *Sörgel* et al. [33]. Our study also showed for the first time a difference between the acrylamide content in different lactation periods. The median level of acrylamide in the mature milk was significantly ($p < 0.05$) almost three times higher than the median level of acrylamide in the colostrum (0.14 μg/L vs. 0.05 μg/L). Comparing acrylamide intake from hospital and home diets, we found that acrylamide intake from home diet was significantly ($p < 0.0001$) more than twice as high (16.9 μg/day vs. 7.3 μg/day). It seems to confirm the impact of the breastfeeding mother`s diet on the acrylamide level in their breast milk. A positive correlation, although modest and borderline significant, between the intake of acrylamide from the diet by breastfeeding women and the content in breast milk was also found but only in relation to colostrum. This is probably due to the small number of women (n = 26) who provided samples of mature milk. The results seem to confirm that acrylamide is transferred from the mother's diet into her milk. Additionally, clearly indicating that acrylamide levels in breast milk can be lowered by changing the breastfeeding mother`s diet. However, further studies are needed to corroborate this finding.

The results of the studies carried out so far indicate that the acrylamide content in breast milk is probably significantly influenced by the time elapsed after the mother's meal and the method of taking milk samples. This has been well demonstrated by *Sörgel* et al. [33], who took milk samples 3, 4 and 8 hours after eating foods high in acrylamide and found high levels of acrylamide in breast milk. In turn, in our research [35], milk samples were collected in the morning after the babies were first fed. Probably even before the first meal of the breastfeeding mothers. This means that the time that elapsed from the mother's last meal ranged from about 8 - 12 hours. As a result, in our research we found trace amounts of acrylamide in human milk samples. On the other hand, it should be remembered that the content of acrylamide in the diet of breastfeeding women participating in our study was several dozen times lower than in the studies by *Sörgel* et al. [33]. It also seems that the methodology of sampling has an influence on the analytically determined content of acrylamide in breast milk. Doing it after the baby is fed is often an ethical choice but the actual acrylamide content in breast milk appears to be higher. It should also be remembered that human milk is a water/fat emulsion. The initial phase is dominated by the water fraction and the final phase of feeding contains more fat. Acrylamide is a very good soluble in water and therefore there may be more of it in the initial phase of breast milk compared to the final phase. It is also worth remembering that babies consume different amounts of milk each time they are fed. It depends on their individual needs. All these factors could influence the variability of the acrylamide content in individual breast milk samples, which was clearly shown in the studies carried out in Poland [35]. It seems that more research is needed on the acrylamide content of human milk using standardized human milk sampling protocols.

EXPOSURE OF BREASTFED INFANTS TO ACRYLAMIDE FROM BREAST MILK

Infant exposure to acrylamide present in breast milk as assessed by *Sörgel* et al. [33], based on the determined acrylamide content in milk of two women after eating potato chips (crisps), was very high and ranged from 0.66 μg/kg bw/day to 3.3 μg/kg bw/day. A significantly lower exposure was estimated by *Fohgelberg* et al. [34]. Taking into account that in most of the tested milk samples the acrylamide content was below the LOQ (0.5 μg/kg), they used 0.25 μg/kg (half the LOQ value) to estimate the exposure of breastfed infants. They estimated that the mean acrylamide intake during the first six months for children who were exclusively breastfed was 0.04 μg/kg bw/day. Exposure to acrylamide in the group of Polish exclusively breastfed infants was estimated in two different lactation period based on a similar approach [35]. For the calculations, we used the actually determined values of acrylamide in breast milk for samples above the LOQ (0.1 μg/L) and half the LOQ value (0.05 μg/L) for samples below this value. In our study, estimated average (median) exposure to acrylamide ranged from 0.003 μg/kg bw/day to 0.018 μg/kg bw/day, depending on the lactation period. To assess the risk of carcinogenicity of acrylamide among breastfed infants, the above results were compared with Benchmark Dose Lower Limit ($BMDL_{10}$) derived for animals as a health reference value [17]. The calculated margins of exposure (MOEs) for neonates and infants at the 95th percentile were slightly below 10 000, indicating that in this study the acrylamide level in breast milk may be of concern for health [18] for 5 % of breastfed infants. It is worth noting that despite the presence of acrylamide in breast milk, the content of this compound found in studies in Sweden [34] and Poland [35] was considerably lower than in baby food [36, 37, 38]. The exposure arising from the presence of acrylamide in breast milk was also significantly lower than in case of infants fed with formulas [36, 37, 38].

CONCLUSIONS

In conclusion, it should be stated that the results of the studies on acrylamide content in human milk are limited by the different periods of sampling of milk for testing and the different sampling methodology. It seems that both the lactation period and the stage during a single feeding, as well as the time elapsed after the mother's meal, have a significant influence on the level of acrylamide in human milk. More research is needed on the acrylamide content in human milk using standardized milk sampling protocols. However, breastfeeding mothers should pay attention to the composition of their diet and avoid products that may contain acrylamide. It seems necessary to develop specific recommendations for breastfeeding women. The use of a metabolomic approach [39, 40] that takes into account the relationship between acrylamide intake by breastfeeding mothers and the content of acrylamide in breast milk and the level of its metabolites in other body fluids could also increase knowledge about the circulation of acrylamide in the human body.

Conflict of interest

Author declare no conflict of interest.

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