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TOPICAL REVIEW

Acrylamide in biological materials and methods of the analytical determination

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Abstract: Acrylamide is a toxic water-soluble highly-reactive substance of anthropogenic origin. It is formed in foodstuffs containing asparagine and reducing carbohydrates during their thermal processing at high temperatures. The presence of acrylamide has been proven in numerous kinds of food; fried potatoes and cereal based products contained significant levels. Approximately one third of human daily energy intake is food with a higher content of acrylamide, which gives rise to high loading. It effects as neurotoxin in human organism and is suspected to be genotoxic and carcinogenic to humans.
In a relatively short time since the detection of acrylamide in human nutriment, many of scientific studies focused on contaminated food. Many indefinite findings resulted from all the data about its toxicity, negative effects and occurrence in biological materials.
Based on former monitoring intent on the quantitative determination of acrylamide in food chain, we would like to emphasize the importance of additional studies on its occurrence in human milk and other biological fluids. Upon the strength of results of acrylamide research up till now, the European Union has discussed setting of maximum levels for acrylamide in particular types of food *(Ref. 21)*. Full Text in free PDF *www.bmj.sk.*
Key words: acrylamide, biological material, chromatography, legislation.

People have used fire for meal preparation since time immemorial. In addition to an origin of compounds improving taste, aroma and coloring, there is a production of undesirable substances as well. One of them, recently aiming great sciencists´ attention, is acrylamide (5, 9). In april 2002, Swedish National Food Administration referred to the new toxin occurring in food.

Initially, acrylamide was used in industrial area for production of plastic, sizing materials and paper. Only by chance it was found out that the compound showed neurotoxic effects for workers operating with varnishes containing high quantity of acrylamide. Acrylamide is supposed to have genotoxic and carcinogenic effects on humans from animal tests. It forms haemoglobin adducts (3, 4, 8, 9) and the formed compounds are metabolised via a direct conjugation with glutathione or via oxidation into glycidamide, that is metabolised and eliminated in urine. Glycidamide is more effective carcinogen than acrylamide itself. In comparison with acrylamide it has a higher reactivity with nucleophilic compounds. It forms adducts with DNA that have been detected in biological fluids and placenta (21). For the analyses, gas and liquid chromatography and the mass spectrometry are employed.

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Acrylamide in biological materials and methods of detection and determination

Human body is exposed to vague amount of xenobiotics. Environmental components and food are the main sources of these substances. After entering organism, they can be transformed and a formation of more toxic substances in comparison to the primary toxicity cannot be excluded. One of such instance is acrylamide. It is dangerous for pregnant women and also for nursed children due to the possibility of breast milk contamination. Many scientific teams attend to study its malignance on human since the prenatal period.

One of relevant biochemical studies concerned mainly with the process of the transfer of acrylamide and glycidamide through placenta into a foetus was the study by Annol et al. The following findings are important. Placenta does not protect the foetus from the ability of toxic compounds such as acrylamide and glycidamide to cross the placental barrier. Their occurrence in mother´s body during pregnancy is due to ingestion of acrylamide-containing food such as French potatoes and potato chips but transplacental transfer of these substances does not depend on their concentrations in the mother´s blood. It is known that placenta contains active enzymes participating in the metabolism of xenobiotics.

Acrylamide and glycidamide can enter the fetus via the fetal circulation. Fetal exposure to these genotoxic and neurotoxic compounds through the placenta is possible in the exposed mother and these substances are dangerous for healthy development of

the fetus. However, in order to find out how these compounds effect on a fetus, there is a necessity for sequential studies (1, 12).

Sörgel et al analyzed the contents of acrylamide in biological fluids, placenta and human tissue, employing liquid chromatography coupled with mass spectrography (LC–MS/MS) (14). The limit of detection LOD for samples of human milk was 5 ng/ml, for urine 1 ng/ml. For the analyses of placenta, the method with LOD 2 ng/ml was used; the samples were prepared by means of liquid/liquid extractive procedure, evaporated in an inert nitrogen atmosphere at the temperature of 35 °C. Reversed-phase chromatography column was eluted with an isocratic solvent system consisting of water, acetic acid and an organic modifier.

Detection of acrylamide by utilizing the gas chromatography (GC) was performed for plasma and tissue homogenates after the ionic bromination into its 2,3-dibrompropionamide in water ambience.

GC-MS method was employed for detection and determination of acrylamide in blood and also for detection and determination of acrylamide adducts and their metabolites, from which glycidamide is more dangerous than other acrylamide adducts to haemoglobine (6).

Faca et al (8) published methods of determination of ^{13}C--labelled acrylamide in human serum utilizing LC-MS/MS and high-resolution FTICR mass spectrometry.

A toxicokinetic study with experimental animals using ^{14}C-labelled acrylamide was performed by Miller et al (1982). They proved that only 2 % of the applied acrylamide were eliminated without changes of the chemical structure. Sörgel et al (2002) described the elimination of acrylamide by human urine after consumption of 500 g potato chips. In that case, the amount of 5 μg of acrylamide was the maximum value obtained in urinary excrete isolated at the time of eight hours after the administration of acrylamide-containing food.

In 2002, a comprehensive review of the toxicity of acrylamide as well as methods of its detection and determination in blood but also in foodstuffs were published. Making a comparison between the analyses performed by GC-MS method after bromination and by a direct method LC-MS/MS with the electrone capture detector without bromination it was found that bromination brought some analytical problems due to creation of artifacts. GC and LC coupled with MS differed in their limits of quantification (LOQ) that was lower for GC-MS from 5 to 10 μg/kg in comparison with LOQ for LC-MS/MS, from 20 to 50 μg/kg (10).

Exposure of general population to acrylamide is via contaminated food and tobacco smoke. Both inhalation and peroral administration are important ways of penetration of the compound to the human body. Acrylamide contained in tobacco smoke was studied in saliva of smokers and the elimination was studied in urine (13, 21). Most of acrylamide was eliminated in urine mainly as conjugates with urinary mercapturic acids.

Among comparative studies, we extracted data on quantity of acrylamide excreted by smokers in comparison with non-smokers and data on its content in their blood as well. In the study, there were by means of LC-MS/MS and GC-MS determined three compounds established as biomarkers of acrylamide exposure. It resulted in the fact that smokers excreted 2.5-times higher amounts of mercapturic acid of acrylamide N-acetyl-S-(2-carbamoylethyl)-L-cysteine and 1.7-times higher amounts of its metabolite glycidamide in their urine and they had 3-times higher levels of N-terminal valine adduct of acrylamide in their blood (18).

Acrylamide in human milk

Acrylamide gets into human milk from various sources, first of all from the diet of nursing mothers containing food that either fulfill all the conditions for the toxin production due to their composition and either were treated at high temperature of 120 °C or higher (20). Besides, the smoking in nursing mothers can also contaminate human milk with acrylamide via inhalation of tobacco smoke (9).

Acrylamide is water-soluble what enables transmission of the compound into child organism due to the higher content of water in child bodies in proportion to their body mass, what is more dangerous for them than for the adults. Another reason is the fact that the blood system and internal organs particularly the brain of prenatal and new-born children are not developed sufficiently (15). Nutrition of children in the first year of life is essential for their healthy development (9, 14).

Authors Sörgel et al have worked out a chromatographic method of analyses of biological samples containing acrylamide - for breast milk, urine and placenta. Also the authors have confirmed a relation of acrylamide toxicity and the creation of compounds with haemoglobine and its ability to form compounds with other large molecules such as for instance DNA proteins. Similarly the acrylamide metabolit glycidamid can form haemoglobin adducts. It is not supposed that these adducts are the reason of human carcinogenity. Acrylamide is transformed into glycidamide by cytochrome P 450 activity (14).

Experiments of Fohgelberg et. al., who processed human breast milk of 15 mothers, proved the presence of acrylamide at concentration levels lower than the limit of detection of the used chromatographic method what was less than 0,5 μg/kg and 0,51 μg/kg in one sample (9).

German professor Soergel warns pregnant and nursing women against consumption of fried potatoes, potato chips and other acrylamide-containing foodstuffs. Each pregnant woman should minimize her daily consumption to the limit of 10 g of such foodstuffs which are supposed to contain maximum of 20 μg acrylamide (15). According to a reference in work of the author there was found out that breast milk of two mothers consuming chips four hours before analysis contained maximum acrylamide levels 19 μg/l a 5 μg/l, i. e. 25-multiple of the assumed medium daily intake of an adult individual.

Results of observation of foreign scientific teams have pointed out that surveillance of nutrition habits of people and recognition of dangerous components in the diet in order to minimize them is in place. It concerns particular groups such as population, pregnant women, nursing mothers, children, adolescents and the elders as well.

Occurrence of acrylamide in human diet

After previous studies of occupational exposure of human body to acrylamide it was found that the substance was present in numerous kinds of food. A great amount of acrylamide was proven in fried potatoes, potato chips, toasts and cereal based products heat-treated at high temperatures from 120 °C to 160 °C. In next steps, studies on acrylamide occurrence in biological materials increased (2, 5, 6, 7, 9, 11, 15, 16, 18, 19).

Besides human milk, also the products of infant nutrition can be the source of acrylamide. Small quantity was determined in porridge, five of six analysed samples contained acrylamide at the limit of quantification. The Norweigian Food Control Authority found a low but measurable amount in infant nutrition products based on cereals.

According to a study of the U.S. Food and Drug Administration, the acrylamide quantity of approximately 20 µg/kg was determined in some products for infant nutrition whereas the limit of detection of 10 µg/kg was reported (9).

Legal view of the *European Food Safety Authority* on acrylamide occurrence in food

Up till now, acrylamide as a contaminating substance has not have any maximum level limit neither in Slovak Republic nor at European Union level yet. The European Commission issued Recommendation No 2007/331/ES where encouraged the Member States to monitor the acrylamide quantity in foodstuffs.

On 19 April 2005 the Scientific Panel for Contaminants in Food Chain of the European Food Safety Authority (EFSA) took measure of acrylamide in food where gave its affirmative statement to judge the risk concerned acrylamide that was worked out by Joint WHO/FAO Expert Committee on Food Additives (JECFA) in February 2005.

JECFA concluded that the margins of exposure for average and deep consumers were low for a compound that is genotoxic and carcinogenic and that this might indicate a human health concern. Therefore, appropriate efforts to reduce acrylamide concentrations in food should continue.

It is necessary to collect reliable data on acrylamide levels in food over at least three-year time span across the Community in order to get a clear picture of the levels of acrylamide in those food types that are known to contain high acrylamide levels and/or contribute significantly to the dietary intake of the whole population and specific vulnerable groups, such as infants and young children.

It recommended that Member States should have performed annually in 2007, 2008 and 2009, in accordance with Annex 1 the monitoring of acrylamide levels in food referred to in that Annex (17).

Conclusion

Humans are able to participate in his healthcare purposely in a high degree by eating nutritious food with suitable energy and biological value. Another condition is its minimal contamination with xenobiotics.

In our study, we report of the occurrence of neurotoxic acrylamide in human body, biological fluids and materials and its penetration from sources containing the noxious agent. Among those, which are known for their high content of acrylamide, are French potatoes, products based on cereals and tobacco smoke. Besides, there are other tens of food types containing acrylamide that the adults consume daily. The substance is dangerous for each human organism, especially for the children.

In term of nutritional toxicology, children differ from adult individuals with less mature biotransformational processes. The blood system and internal organs, particularly the brain, are not developed sufficiently. Because of the fact that breast milk can be contaminated with acrylamide it is essential for each nursing mother to minimize consumption of food rich in the noxious substance.

The adolescents have special nutrition manners. More frequently they eat food richer in content of acrylamide, they prefer Fast Food nutrition. Their diet contains more salt or is too sweet and based on hydrogenated oils. Using of alcohol and smoked products is not unusual. They prefer beverage rich in additives such as caffeine, taurine, artificial sweeteners and energy drinks.

Current consumables often contain unknown xenobiotics. In the case of the occurrence of acrylamide not only the substance itself presents a risk for human but also the products of its metabolism, that are more toxic than the primary matter. The risk consists in formation of more toxic adducts of acrylamide or its metabolite glycidamide to deoxyribonucleic acid.

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