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REVIEW

Acrylamide: a Common Food Toxin Related to Physiological Functions and Health

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Summary

Acrylamide (AA) is a highly reactive organic compound capable of polymerization to form polyacrylamide, which is commonly used throughout a variety of industries. Given its toxic effect on humans and animals, the last 20 years have seen an increased interest in research devoted to the AA. One of the main sources of AA is food. AA appears in heated food following the reaction between amino acids and reduced sugars. Large concentrations of AA can be found in popular staples such as coffee, bread or potato products. An average daily consumption of AA is between 0.3-2.0 μg/kg b.w. Inhalation of acrylamide is related with occupational exposure. AA delivered with food is metabolized in the liver by cytochrome P450. AA biotransformation and elimination result in formation of toxic glycidamide (GA). Both, AA and GA can be involved in the coupling reaction with the reduced glutathione (GSH) forming glutathione conjugates which are excreted with urine. Biotransformation of AA leads to the disturbance in the redox balance. Numerous research proved that AA and GA have significant influence on physiological functions including signal propagation in peripheral nerves, enzymatic and hormonal regulation, functions of muscles, reproduction etc. In addition AA and GA show neurotoxic, genotoxic and cancerogenic properties. In 1994, International Agency for Research on Cancer (IARC) classified acrylamide as a potentially carcinogenic substance to human.

Key words

Acrylamide • Neurotoxicity • Genotoxicity • Reproductive toxicity • Oxidative stress

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Introduction

Food should deliver all the ingredients necessary for the organism to function properly. Organic and inorganic compounds present in food are used by the organism as energetic, regulatory and/or building substances. Unfortunately, food consumed by people is often a source of harmful substances. Acrylamide (AA) is one of the most common toxins in food. It occurs in food containing high concentrations of hydrocarbons subjected to high temperature (Mottram *et al.* 2002). High concentration of acrylamide may be found in food products such as potato chips, fried potatoes, cornflakes or bread. Thus acrylamide is present in every day diet of most people. To make matters worse, some of the products containing acrylamide are attractive to children and young people.

In 2001, the Scientific Committee on Toxicity, Ecotoxicity and the Environment demonstrated its neurotoxicity, genotoxicity, carcinogenicity and reproductive toxicity (Keramat *et al.* 2011, Carere 2006). Toxic effects of acrylamide are mediated by the formation of genotoxic metabolites, oxidative stress, affected propagation of neural signals, ultrastructural and histological defects in central neural system (LoPachin 2004, El-Sayyad *et al.* 2011, Pingot *et al.* 2013). The

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International Agency for Research on Cancer (IARC 1994) classified acrylamide as potentially carcinogenic substance to human.

Most of the mechanisms of AA toxicity are well recognized. Nonetheless some aspects of acrylamide toxicity remain still unclear. First of all, the doses used in animal experiments are much higher than mean acrylamide intake in food. Thus it is not known whether acrylamide ingested with everyday diet pose real risk to consumers' health. On the other hand, the known incidences of occupational exposure were probably related with the intoxication with very high acrylamide doses (Pennisi *et al.* 2013). The studies conducted in chemical plants revealed that personal breathing zone air samples contained up to 984 µg of AA per m^3 of air. Acrylamide and polyacrylamide production operators had hemoglobin AA adducts in the concentration up to 1884 pmol/g, whereas hemoglobin AA adducts level in administrative workers was 97.9 pmol/g (Moorman *et al.* 2012). It is still unclear whether toxic effects of chronic exposure to acrylamide may accumulate in the organism in the long term. Very little is known about the risk of fetal exposure to AA and their potential effects to the prenatal and postnatal development. Thus the aim of this article is to bring together data from different studies in order to analyze the risk of exposure to AA and related health risks.

Acrylamide in food

Acrylamide (CH_2=CH–CO–NH_2, according to IUPAC: 2-propenamid) is a highly reactive, organic, white and crystal substance, with molecular weight of 71.08 g (Żyżelewicz *et al.* 2010). AA is a polar substance which easily dissolves in water or other polar solvents, e.g. in methanol or ethanol (Jankowska *et al.* 2009). High reactivity of AA is connected with the double bond and amide group. The compound may create hydrogen bonds and can react both with amide and vinyl groups (Girma *et al.* 2005, Żyżelewicz *et al.* 2010). Acrylamide is polymerized under the influence of temperature and UV radiation. These reactions result in creation of new chemical compounds called polyacrylamides.

Recent years revealed a considerable increase in investigation of acrylamide as a potentially dangerous substance to people. In early 2000s, Swedish researchers proved that certain foods might contain large concentrations of acrylamide (Lofstedt 2003). The research by Tareke *et al.* (2002) indicated that food processing has influence on acrylamide formation. The factors influencing the occurrence of AA in the food are: temperature, exposure time to high temperature, the amino acids content and their types and the content of carbohydrates in the food (Becalski *et al.* 2003, Konings *et al.* 2003). AA is formed during frying, deep frying and baking foods rich in carbohydrates and especially in amino acid – asparagine. High concentrations of AA are found in processed foods like: chips (50-3500 µg/kg), frites (170-2287 µg/kg), coffee (170-350 µg/kg), bread (70-430 µg/kg) or corn flakes (30-1400 µg/kg) (Friedman 2003). Acrylamide concentrations in selected foodstuffs together with methods of measurements of acrylamide concentration in food are presented in Table 1.

The mechanism of AA formation in food has not been clearly described yet. Numerous research has shown only hypothetical ways in which AA is being formed in comestible products (Edegaard *et al.* 2008, Mestdagh *et al.* 2008). Most of the research point to asparagine presence as a significant factor contributing to AA formation (Zhang *et al.* 2009, Taeymans *et al.* 2004). The reaction between glucose (reducing sugar) and asparagine gives a product responsible for the food's flavor and color. This reaction is known as a Millard's reaction and it has a higher rate at the temperature exceeding 120 °C (Friedman 2003, Tareke *et al.* 2002). The content of AA increases considerably during frying, grilling and roasting. Popular foodstuffs such as coffee, high-in-starch potato products and cereal products contain large amounts of AA (Claus *et al.* 2008, Tajner-Czopek *et al.* 2012). People are also exposed to harmful effects of AA by consuming natural unprocessed products rich in asparagines, including asparagus, cocoa beans or cereals (Rachwał and Nebesny 2012). According to the European Food Safety Authority (EFSA) report, the level of AA in food ranges from under 30 µg/kg to 4700 µg/kg, depending on the product (EFSA 2009, Mojska and Gielecińska 2012). Research also shows that exposure to AA varies and depends mainly on the population, age of consumers and their eating preferences. In European populations, mean daily intake of acrylamide goes from 0.14 to 1.31 µg/kg body weight. Similar mean intake (0.43-1.1 µg/kg body weight per day) was indicated in the United States (Dybing and Sanner 2003). The research conducted in Kraków, Poland by Jankowska *et al.* (2009) indicated that AA was excessively consumed by children and teenagers. Among children and adults, bread – a product eaten on a daily basis, is the main source of AA. Other Polish research indicated that

Table 1. Acrylamide content in analyzed products.

Author	Number of food samples	Analysis of acrylamide in food	Acrylamide content	Products with the highest acrylamide content	Highest mean acrylamide content (µg/kg)
Claeys *et al.* 2010	1725	LC-MS	34-2814 µg/kg (mean)	Coffee substitute	2814±1045
				Instant coffee	694±81
				Potato crisps	525±477
				Gingerbread	431±455
Sirot *et al.* 2012	192	LC-MS	2-954 µg/kg (range)	Potato chips	954±240
				French fries	724±358
				Cocktail biscuits (salted)	697±430
				Chocolate biscuits	139±100
Konings *et al.* 2003	341	LC-MS-MS	<30-3100 µg/kg (range)	Potato crisps	1249±656
				Cocktail snacks	1060±950
				Gingerbread	890±393
				Chips (deep-fried)	351±297
Mojska and Gielecińska 2012	111	GCQ-MS/MS LC-MS/MS	2-516 µg/kg (range)	Follow-on formula	73±78
				Infant biscuits	219 ±139

LC-MS – liquid chromatography-mass spectrometry, LC-MS-MS – liquid chromatography tandem mass spectrometry, GCQ-MS/MS – gas chromatography with tandem mass spectrometry, LC-MS/MS – liquid chromatography with tandem mass spectrometry.

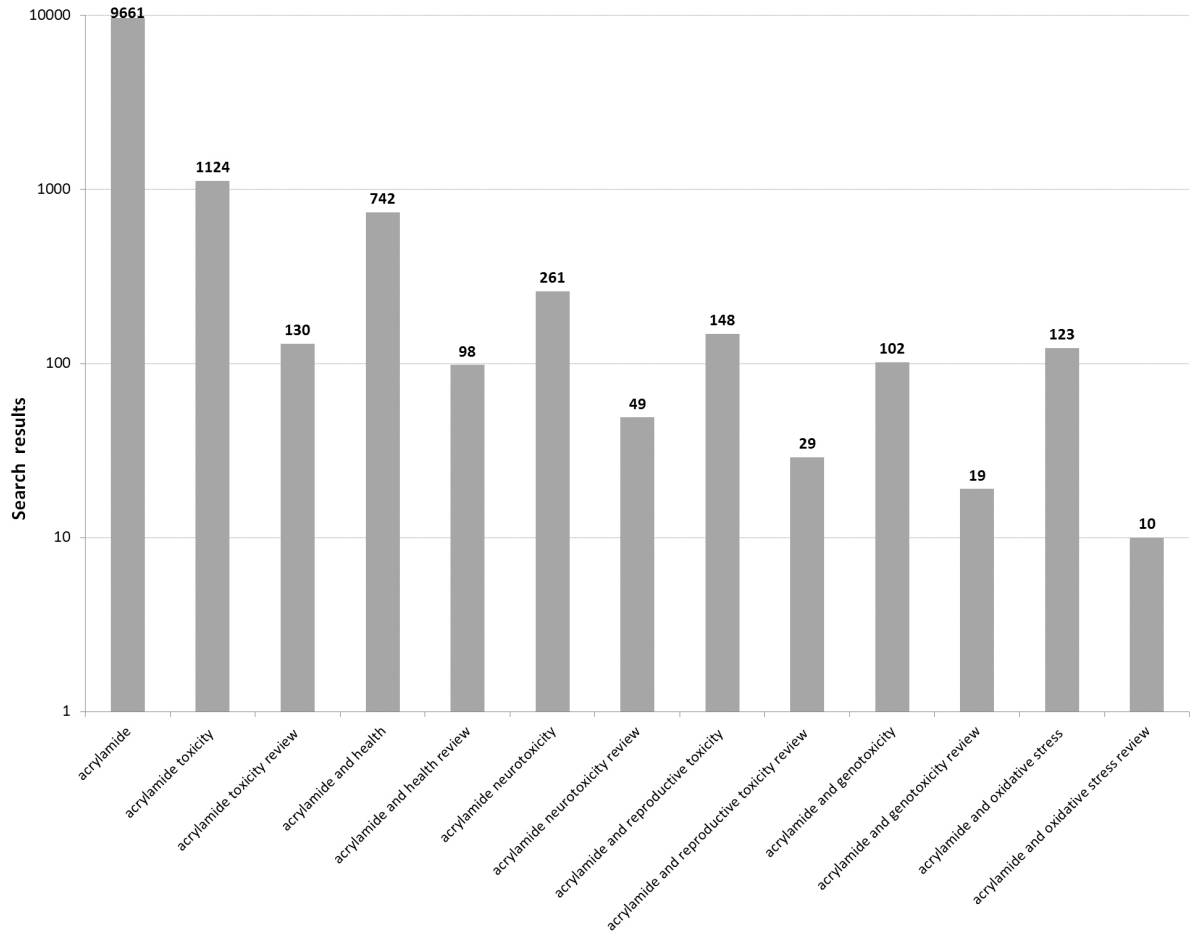

Fig. 1. Acrylamide studies according to PubMed (data from 1958 till 2016-08-23).

Table 2. Exposure of human population to acrylamide.

Authors	Method used	Population (n, total population)	Age years/months (n, group size)	Daily doses ingested	Contribution of food in daily acrylamide exposure
Zając *et al.* 2013	Semi-quantitative food frequency questionnaire	Polish (n=1470)	6-12 years (n=300)	mean: 1.51 µg/kg b.w.	Baked goods 42 % Crisps 25 % Cookies 14 %
			13-19 years (n=296)	mean: 0.89 µg/kg b.w.	Baked goods 46 % Cookies 25 % French fries 12 %
			20-30 years (n=296)	mean: 0.61 µg/kg b.w.	Baked goods 55 % Cookies 15 % Coffee 9 %
			31-41 years (n=278)	mean: 0.56 µg/kg b.w.	Baked goods 55 % Coffee 16 % Cookies 15 %
			42-60 years (n=300)	mean: 0.67 µg/kg b.w.	Baked goods 38 % Crisps 22 % Coffee 16 %
Claeys *et al.* 2010	Probabilistic approach 'Monte Carlo Risk Analysis Programme'	Belgium (n=662)	2.5-6.5 years (n=662)	mean: 0.72 µg/kg b.w.	Biscuits 26 % French fries 25 % Bread & rolls 20.2 %
BfR* 2003	Questionnaire	German (n=1085)	15-18 years (n=1085)	mean: 1.10 µg/kg b.w.	Toast 9 % Fried potatoes 6 %
BCS** 2012	Probabilistic dietary exposure to acrylamide based on the AA measured in samples of each food along with individual consumption data	Canada (n=32 088)	<1 year (n=279)	mean: 0.211 µg/kg b.w.	-
			1-3 years (n=2096)	mean: 0.609 µg/kg b.w.	French fries 30.0 % Snack chips 14.9 % Coffee 12.3 %
			4-8 years (n=3047)	mean: 0.597 µg/kg b.w.	
			9-13 years (n=3883)	mean: 0.442 µg/kg b.w.	
			14-18 years (n=4423)	mean: 0.356 µg/kg b.w.	
			19-30 years (n=3713)	mean: 0.288 µg/kg b.w.	
			31-50 years (n=5125)	mean: 0.248 µg/kg b.w.	
			51-70 years (n=5533)	mean: 0.187 µg/kg b.w.	
			≥71 years (n=3989)	mean: 0.157 µg/kg b.w.	
Sirot *et al.* 2012	Probabilistic approach on the basis of the weekly food consumption	French (n=336)	18-79 years (n=191)	mean: 0.43±0.33 µg/kg b.w.	French fries 44.8 % Coffee 29.5 % Biscuits 9.4 %
			3-17 years (n=145)	mean: 0.69±0.58 µg/kg b.w.	French fries 60.8 % Biscuits 18.8 % Cakes and other sweetened pastry 3.3 %

Konings *et al.* 2003	Probabilistic approach 'Monte Carlo Risk Analysis Programme'	Dutch (n=6250)	1-6 years (n, not given)	mean: 1.04 µg/kg b.w.	Crisps 40 % Dutch spiced cake 20 % Chips and comparable products 18 %
			7-18 years (n, not given)	mean: 0.71 µg/kg b.w.	Crisps 46 % Dutch spiced cake 23 % Chips and comparable products 11 %
			1-97 years (n=6250)	mean: 0.48 µg/kg b.w.	Crisps 31 % Chips and comparable products 21 % Dutch spiced cake 16 %
Dybing and Sanner 2003	Food Frequency Questionnaire (FFQ)	Norway (n=2672)	16-79 years (n=2672)	mean: 0.46-0.49 µg/kg b.w.	Coffee 28-28.6 % Potato crisps 17.6-17.4 % Soft bread 13.0-11.9 %
	Probabilistic approach based on UNGKOST 2000 data	Norway (n=6736)	9 years (n=2957)	mean: 0.32-0.36 µg/kg b.w.	Potato crisps, butter biscuits, sweet biscuits 55-65 %
			13 years (n=3779)	mean: 0.49-0.52 µg/kg b.w.	
Mojska and Gielecińska 2012	Probabilistic approach based on the theoretical number of food portions	Polish (n, not given)	6 month (n, not given)	mean: 17.46 µg/ person/day	Jarred baby food 56.7 % Follow-on formula 43.3 %
			7 month (n, not given)	mean: 20.87 µg/ person/day	Jarred baby food 52.7 % Follow-on formula 27.2 %
			8 month (n, not given)	mean: 21.65 µg/ person/day	Jarred baby food 50.8 % Follow-on formula 26.2 %
			9 month (n, not given)	mean: 29.06 µg/ person/day	Jarred baby food 37.9 % Follow-on formula 21.7 %
			10-12 months (n, not given)	mean: 38.05 µg/ person/day	Jarred baby food 55.2 % Infant cereals 18.2 %
Brantsæter *et al.* 2008	Probabilistic data based on the food frequency questionnaire (FFQ)	Norwegian, pregnant women (n=19, age 23-44)	n=19	median: 33.7 µg/person	Crispbread 10-22 % Potato crisps 14-16 % Bread 8-11 % Biscuits 5-10 % Breakfast cereals 6-8 %
	Probabilistic data based on the food diary (FD)		n=19	median: 28.5 µg/person	
	Probabilistic data based on the AA metabolite concentration in urine (non-smokers)		n=16	median: 20.3 µg/person	
	Probabilistic data based on the metabolite concentration in urine (smokers)		n=3	median: 91.1 µg/person	

* Federal Institute for Risk Assessment (BfR), ** Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch (BCS).

an average AA consumption in children aged 1-6 years was about 0.47 μg/kg b.w. per day and among children aged 7-18 years it was 0.34 μg/kg b.w. per day (Mojska and Gielecińska 2012). Maximum intake of acrylamide reaching 7.9 and 8.1 μg/kg b.w. per day was estimated in 13 years old Norwegian boys and girls respectively (Dybing and Sanner 2003). Food-related exposure of human populations to acrylamide together with methods used for estimation of exposure levels are listed in Table 2. Analysis of acrylamide studies is given in Figure 1.

Other kinds of exposure to acrylamide

Polyacrylamides are widely used in the industry for water treatment (as flocculator), component of mortars, adhesives, dyes or in the textile and cosmetic industries. Furthermore, polyacrylamide is used in laboratories, e.g. gel electrophoresis (Friedman 2003). AA is used for selective modification of protein's sulfhydryl groups (SH) and the electrophoretic separation of nucleic acids and proteins in the laboratories (Szczerbina 2005).

Initially, polyacrylamide added to fertilizers or used as coagulant for water treatment was claimed as the main source of AA (Szczerbina 2005, Żyżelewicz *et al.* 2010). Cosmetic, tobacco industry and plastics were indicated as the other sources of AA (Szczerbina 2005).

Occupational exposure mostly affects chemical plant workers, laboratory workers, construction industry workers, miners or workers of coal preparation plants (Pennisi *et al.* 2013). The most publicized incidences of exposure include exposure to ground water contaminated with acrylamide and N-methyloacrylamide in tunnel workers in Norway (Kjuus *et al.* 2004, Goffeng *et al.* 2008) or exposures to aqueous solution of acrylamide in workers of chemical manufactories in China (He *et al.* 1989). Typical symptoms of exposure are manifested by paresthesia of the extremities, muscle weakness, ataxia, increased sweating. Most of them result from peripheral neuropathy. In some cases impaired vision was diagnosed (Pennisi *et al.* 2013). Interestingly, similar neurological effects were indicated in laboratory studies in rats after 4 weeks of every third day exposures to doses of acrylamide as high as 40 mg/kg b.w. (Zhu *et al.* 2008).

High exposure to acrylamide occurs in tobacco smokers. Total amount of acrylamide in the smoke from a single cigarette is around 1 μg or higher. Cigarettes and other tobacco products like snuff, tobacco sticks or strips contain acrylamide in the range from below 100 to 367 ng/g (Moldoveanu and Gerardi 2011). Thus tobacco related acrylamide intake depends on the number of cigarettes smoked per day and/or the type of tobacco product consumed. Measurements of the hemoglobin adducts with acrylamide suggest several times higher exposure to AA in tobacco smokers than in non-smokers (Schettgen *et al.* 2004). Median value of acrylamide intake in smoking pregnant women was estimated in the level of 91.1 μg/day (Brantsæter *et al.* 2008).

Absorption, metabolism and distribution of acrylamide

There are three ways by which AA is transmitted into the body: digestive system, respiratory system (e.g. cigarette smoke) and skin absorption (e.g. cosmetics) (Carere 2006, Vesper *et al.* 2007). Irrespective of route, exposure to acrylamide rapidly occurs in blood plasma with a peak concentration of 60-90 min in rats. Its epoxide form occurs later. In rats, the peak concentration of GA is delayed by about 100 min in relation to AA plasma peak (Barber *et al.* 2001). Both AA and glycidamide may create adducts with hemoglobin following the reaction with sulfhydryl groups. The level of adducts is often used as indicator of exposure to acrylamide as their formation is proportional to the acrylamide dose ingested, inhaled or absorbed through the skin (Pingot *et al.* 2013, Tareke *et al.* 2008, Vikström *et al.* 2012). Relatively large concentrations of AA and GA are distributed into muscle and neural tissues (Barber *et al.* 2001). AA delivered by the oral route is metabolized in the liver. The biotransformation takes place with the cytochrome P450. As a result of acrylamide biotransformation, its epoxide form glycidamide (2,3-epoksypropan amide) is formed (Tareke *et al.* 2008). During the second phase of biotransformation, AA and glycidamide (GA) are coupled with reduced glutathione (GSH) by enzymes from the family of glutathione S-transferase (GST) which leads to formation the glutathione conjugates (Friedman 2003). The final products of the glutathione conjugates reaction are the derivatives of N-acetylcysteine excreted in urine (Pingot *et al.* 2013). As a result of reaction with GSH, AA and its derivatives lose their toxic properties and may be more easily excreted from the organism. Only about 50 % of the AA daily dose is depurated from the organism, mainly in the urine (EFSA 2008). The half-life of AA in human organism is 2-7 h which shows how

slowly this substance is being removed from the body (Sörgel *et al.* 2002).

Acrylamide and oxidative stress

Oxidative stress occurs when the rate of generation of free oxygen radicals (ROS) is larger than the rate of their neutralization. An excess of free radicals may cause oxidation of biological molecules namely lipid peroxidation, oxidation of enzymes and oxidation of DNA bases. This leads to damage to cell organelles, impaired cell metabolism, DNA fragmentation and cell death. Free radicals take part in pathogenesis of numerous diseases including diabetes, neurodegeneration, diseases of cardiovascular system, neoplasm formation (Rahman *et al.* 2012, Greń 2013). Under normal physiological conditions, the occurrence and metabolism of free radicals is controlled by antioxidative system which is composed of enzymatic and nonenzymatic antioxidants. Major enzymatic antioxidants include superoxide dismutase (SOD) catalyzing dismutation of superoxide anion to molecular oxygen and hydrogen peroxide; catalase (CAT) catalyzing the decomposition of hydrogen peroxide; glutathione peroxidase (GPx) catalyzing reduction of hydrogen peroxide accompanied by oxidation of the reduced glutathione (GSH). Nonenzymatic antioxidants like reduced glutathione, vitamins, thioredoxin, α-tocopherol etc., take part in neutralization of free radicals by donation of electrons (Lobo *et al.* 2010).

There is evidence to suggest increased generation of free radicals and hydroperoxides accompanied by lipid peroxidation in animals exposed to acrylamide (Prasad and Muralidhara 2012). Increased activity of SOD in blood plasma, liver, testes, kidneys and brain of acrylamide exposed rats hint at increased rate of formation of superoxide anion in the whole organism (Yousef and El-Demerdash 2006). Other studies indicated increased activity of GPx accompanied by depletion of GSH which suggest adaptation of the antioxidative system to increased H_2O_2 generation in different structures of neural system in rats (Zhu *et al.* 2008). In general, depletion of GSH is a common phenomenon in animals treated with acrylamide. The depletion of GSH results from higher rate of its consumption in reactions with hydrogen peroxide (Zhu *et al.* 2008, Kopańska *et al.* 2015) and conjugation with acrylamide and/or glycidamide in the phase II reactions catalyzed by glutathione s-transferase (Paulsson *et al.* 2005). Glutathione is a major cell antioxidant whose shortage may be an additional factor contributing to redox imbalance. Indeed, the data suggest that AA may overwhelm the antioxidative system and cause symptoms of oxidative stress. For instance Yousef and El-Demerdash (2006) indicated systemic increase of concentration of thiobarbituric acid reactive substances in rats orally exposed to acrylamide. In similar fashion, our team observed increased concentration of malondialdehyde in different brain areas of rats intraperitoneally injected with AA solutions, all to suggest redox imbalance and increased peroxidation of lipids (Kopańska *et al.* 2015). Moreover, Zhu *et al.* (2008) found decreased activity of SOD in neural system of rats after 10 weeks of exposure to acrylamide applied every third day. This effect probably resulted from oxidation of SOD by excessively generated superoxide ion.

All of these imply that acrylamide induces higher activity of antioxidative system, and that high doses of acrylamide applied for longer time period induce symptoms of oxidative stress. The data presented here were obtained in animal studies using the acrylamide doses in the range 0.5 μg to 40 mg/kg body weight. First symptoms of affected redox balance were found after 10 weeks of exposure to acrylamide doses of 25 μg/kg b.w. (Yousef and El-Demerdash 2006). Such doses are only several times higher than the maximum acrylamide doses possibly ingested with food by some human populations. Thus it is not clear whether people exposed to acrylamide concentrations typically occurring in food will experience affected regulation of redox reactions.

Genotoxicity and cytotoxicity of acrylamide

Oxidative imbalance induced by exposure to acrylamide may lead to cytotoxic and genotoxic effects. Free radicals may cause damage to mitochondria and other cell organelles. They induce apoptosis and cause oxidation of DNA bases, leading to fragmentation of the double strand. All of these may cause cell death or neoplastic transformation (Valko *et al.* 2004). The ROS related mechanism of cytotoxicity and/or mutations is attributed to all the factors capable of inducing oxidative stress. On the other hand, acrylamide is known to exhibit more specific effects on cells. AA was found to form 7-formamidoethyl adducts with guanine. The formation of adducts with other acid bases is also probable, although they show decreasing stability as

guanine > adenine > uracil (Solomon *et al.* 1985, Shelkovsky *et al.* 2002). The product of acrylamide biotransformation, glycidamide, shows higher affinity to acid bases of nucleic acids than acrylamide. Nonetheless, both compounds were found to form the strongest adducts with guanine at N-7 position (Atay *et al.* 2005). N7-dG-glycidamide is the main DNA adduct. It has large pro-mutagenic properties because of formation of G-T transversions during DNA replication (Besaratinia and Pfeifer 2004). In embryonic fibroblasts of transgenic Big Blue mice exposed *in vitro* to acrylamide dose of 320 μM, A-G transitions and G-C transversions were found (Besaratinia and Pfeifer 2003). In human lymphocytes, exposure to AA caused DNA strand to break, induced caspases-3 activity, and apoptosis. Moreover, AA was found to disrupt DNA repair (Blasiak *et al.* 2004).

The studies over genotoxicity of chronic doses of acrylamide indicated significant increase of glycidamide-DNA adducts in spermatocytes of mice exposed to doses of acrylamide as low as 0.01 μg per ml of drinking water given every day for 9-12 months. These animals also showed increased number of incidences of double-strand breaks in DNA of the germ cells (Nixon *et al.* 2012).

The genotoxic effects of acrylamide indicate that it may also play significant role in neoplastic transformation. The carcinogenic potency of acrylamide was proven by Friedman's *et al.* (1995) studies. The study was conducted in males and females of Fischer 344 rats. AA was administered in the drinking water, throughout the 106-week period, at the dose ranging from 0.1 to 0.3 mg/kg b.w. per day. The results indicated a significant increase in the frequency of thyroid follicular cell adenomas and adenocarcinomas in male rats from the high-dose group. Moreover, that group witnessed incidence of mesothelioma of the tunica of the testes. The females group saw a significant increase in the frequency of mammary gland fibroadenoma and adenocarcinomas. This study also reported the occurrence of the thyroid and mammary glands tumors after exposure to AA (Friedman *et al.* 1995). Another long term studies were performed in females of swiss-ICR mice. The specimens were exposed to AA doses going from 2.5 to 50.0 mg/kg b.w., administered orally, every second day. After 1-year observation, the development of skin tumor in mice exposed to the highest AA doses was observed (squamous cell papilloma and carcinoma). Moreover, incidences of lung cancer were noted (Bull *et al.* 1984).

The pro-oncogenic activity of acrylamide in humans is not evident. Epidemiological studies did not indicate any relation between exposure to acrylamide and cancer incidences in human (Marsh *et al.* 1999). On the other hand, the doses of acrylamide inducing genotoxic effects in animals well correspond with the AA doses ingested by high consumers of food containing acrylamide. This is why European Union classification of Carcinogens placed acrylamide in the second category, as carcinogen and mutagen (Szczerbina 2005). Moreover, International Agency for Research on Cancer classified AA as a potentially carcinogenic substance for people (IARC 1994).

Reproductive toxicity of acrylamide

As a low molecular weight compound easily dissolving in water, acrylamide passes through the placenta in animals and human organism. It was also found in breast milk of women. Thus it may have the influence on the normal prenatal and early postnatal development of infants (Sörgel *et al.* 2002). Nonetheless, the data on the risk of the harmful influence of acrylamide on the early development of human has not been assessed so far.

The food frequency questionnaire estimated medial acrylamide intake in pregnant women as 33.7 μg/day. The median excretion of acrylamide based on urine metabolites in this group of women was 11.2 μg/day (range: 3.3-75.6 μg/day). Assuming that about 55 % of acrylamide is depurated in urine as mercapturic acid metabolites, this would correspond to a median exposure of 20.3 μg/day (Brantsæter *et al.* 2008). According to the above calculation, the maximum exposure to acrylamide in pregnant women may be as high as 137.5 μg/day. It was estimated that about 50 % of dietary acrylamide may be transferred through the placental blood into the embryo (Sörgel *et al.* 2002). According to the questionnaire-based studies, the main dietary sources of acrylamide to pregnant women were potato crisps, crisp bread, biscuits, breakfast cereals and bakery products (Brantsæter *et al.* 2008).

The embryotoxic effects of acrylamide were studied in animal models. Exposure of pregnant females of rodents to AA doses ≥5 mg/kg b.w./day, administered orally, resulted in increased post-implantation loss of embryos and decreased number of live pups. Exposure of pregnant females to higher doses of AA (≤15 mg/kg

b.w./day) resulted in reduced pup weight and survival (NTP 2011). Interesting studies over acrylamide influence on embryonic and early postnatal development of rats were performed by El-Sayyad *et al.* (2011). In this study, pregnant females were orally exposed to high acrylamide doses of 30 mg/kg b.w. from day 6 of gestation until parturition and throughout lactation. The young pups derived from acrylamide exposed females had lower body size and weight and lower brain size in comparison to control animals. They also suffered from muscular dystrophy and ultrastructural changes in cerebral cortex.

The reproductive toxicity of acrylamide is also manifested by its influence on animal male infertility. According to Scientific Committee on Food (SCF 2002), the impaired fertility may involve affected sperm count and sperm motility parameters. The increased number of glycidamide-DNA adducts and fragmentation of DNA in germ cells of male mice exposed chronically to low AA doses was proved by Nixon *et al.* (2012). This suggests increased risk of DNA lesions in male reproductive material and their possible introduction into zygote. Indeed, it was found that exposure of male rats to acrylamide doses of 19 mg/kg for eight days and next mated to unexposed females led to reduced fertility rates and increased frequency of resorption of embryos (Sakamoto and Hashimoto 1986). Moreover, exposure of rats to acrylamide (dose of 100 ppm) resulted in disrupted mating performance, ejaculatory processes and subsequent transport of sperm (Zenick *et al.* 1986).

Neurotoxicity of acrylamide

The only toxic effects of acrylamide well documented in human were manifested by peripheral neuropathy related to occupational exposure (Pennisi *et al.* 2013). Symptoms of peripheral neuropathy were also described in animal studies. In monkeys, chronic oral exposure to AA doses of 10 mg/kg b.w./day for up to 12 weeks was associated with clinical signs of peripheral neuropathy like muscle weakness or ataxia of limbs (SCF 2002). In rats, neurotoxic AA effects were manifested by abnormal gait, shown as foot splay, ataxia and weakness of the hindlimb skeletal muscle. Complete paralysis of hindlimbs occurred after 10 weeks of AA administration in a dose of 40 mg/kg b.w. every second day. The behavioral effects were accompanied by serious alteration of electrophysiology of the sciatic nerve which may suggest alteration of the myelin capsule and/or altered activity of axolemmal Na/K-ATPase (Zhu *et al.* 2008).

In animal studies, toxic effects of acrylamide were also indicated in central nervous system. Rat pups born by mothers exposed to acrylamide (30 mg/kg b.w.) and fed with milk from lactating females exposed to acrylamide showed serious ultrastructural changes in cerebral cortex. They were manifested by massive increases of pyknotic neuronal cells separated by widened spaces, increased number of apoptotic cells, death of Purkinje cells and granular neuronal cells (El-Sayyad *et al.* 2011). It is reasonable to suggest that ultrastructural changes in brain may be followed by functional effects. Our team has indicated decreased activity of acetylcholinesterase, an enzyme playing regulatory function in cholinergic transmission, in cerebrum, cerebellum and medulla oblongata of mice exposed to acrylamide doses of 20 and 40 mg/kg for 24 h, 48 h and 8 days. This may hint at longer time of residence of acetylcholine in cholinergic synapses and higher excitation of cholinergic nerves engaged in memory formation, behavior, muscle controlling, controlling of autonomic functions etc. (Kopańska *et al.* 2015).

There are probably several mechanisms of acrylamide neurotoxicity. It is generally accepted that the most important one is related with conjugation of AA with cysteine residues of presynaptic membrane proteins engaged in neurotransmitter release. Consequently, the flow of nerve impulses may be inhibited, coupled with subsequent degeneration of neurons (LoPachin and Barber 2006, Pingot *et al.* 2013). Important role in neurotoxicity of acrylamide is probably played by oxidative stress. Zhu *et al.* (2008) indicated that peripheral neuropathy and altered electrophysiology of the sciatic nerve were accompanied by the symptoms of redox imbalance. Similarly, in our studies, the affected activity of acetylcholinesterase were accompanied by depletion of albumins and –SH group concentrations and elevated content of malondialdehyde in brain of mice exposed to AA which also suggest induction of redox imbalance (Kopańska *et al.* 2015).

The redox imbalance in brain of animals exposed to acrylamide is an important observation as free radicals are known to contribute to neurodegeneration. The increased level of malondialdehyde, the product of peroxidation of lipids, was found in erythrocytes, blood serum and neurofibrillary tangles in brains of Alzheimer's disease patients (Matveychuk *et al.* 2011). Assuming that neurodegeneration results from cumulative

damage to neuronal cells induced by free radicals (Praticò 2005), it may be reasonably established that food ingredients capable of inducing redox imbalance in brain may participate in etiology of neurodegenerative diseases. The relation between the consumption of acrylamide-rich food and risk of neurodegeneration has not been studied so far, although this seems to be an interesting problem for toxicological and epidemiological studies.

Conclusion

Acrylamide belongs to the most common toxins in human diet. It shows relatively high concentrations in asparagine rich foods processed at high temperature. Its mean consumption depending on the population and age of consumers usually reaches approximately 1 µg/kg body weight daily, although in high AA consumers its maximum intake may be above 8 µg/kg body weight per day. This corresponds with the AA doses inducing peroxidation of lipids and DNA lesions in long term animal studies. Genetic disorders may affect male fertility, embryonic and fetal development and neoplastic transformation. On the other hand, realistic data indicating the relation between consumption of AA rich food and health risk for human are still missing. The only well documented health disorders occurred as a result of occupational exposure and were manifested by peripheral neuropathy symptoms.

Conflict of Interest

There is no conflict of interest.

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