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The Carcinogenic Effects of Acrylamide Formed During Cooking of Some Foods

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Abstract: Acrylamide is predominantly used as a monomer in the production of polyacrylamide and this polyacrylamide is widely used in many fields such as refining waste waters, wood pulp and paper manufacturing, processing crude, metal, mineral and asphalt production and molecular biology laboratory [1, 2]. Detection of acrylamide residues after carbohydrate-rich foods during cooking, frying and burning process at high temperatures, a high level of research activities have been allocated for find out is there any hazardous effects of acrylamide for human [3, 4]. Animal experiments performed with acrylamide have been shown that a variety of neoplastic developments observed in rats such as follicular thyroid tumors, scrotal mesotheliomas, mammary gland tumors, lung carcinomas, glial brain tumors, oral cavity papillomas and uterine adenocarcinomas [1, 5]. For this acrylamide has been classified by IARC [1] as a ‘‘Probably Human Carcinogen (Group 2A)’’. It was clearly exibited at the end of the experiments performing as an *in vivo* and *in vitro* that acrylamide and its main epoxide metabolite gliycidamide had genotoxic and clastogenic effects [6-10]. In this study it has been aimed to take in public attention into carcinogenic, genotoxic, neurotoxic and physiologic effects of this chemical such as neurotoxicity and reproductive toxicity. It has been concerned that more epidemiologic and experimental studies are necessary and more research activity should be allocated in order to make public conscious about possible carcinogenic effects at this substance.

Key words: Acrylamide · Harmful effects · Carcinogenic effects · Genotoxic effects

INTRODUCTION

Acrylamide is a monomer which has a molecular formula of C_3H_5NO (CH_2=CH-CONH_2) and has a molecular weight of 71.08 g, is colorless, odorless and is in crystalline form, can be solved in water with solvents such as acetone, ethanol, methanol and can be transformed into acrylic acid when hydrolyzed [1]. The acrylamide used in the production of polyacrylamide is also extremely used in the treatment of drinking and waste water, in paper production, in petroleum industry, in the production of mine, mineral, asphalt and in the treatment of land and soil. Moreover, it’s also commonly used as an additive in cosmetic industry, in electrophoresis used in molecular biology applications, in the production of photographic film, in the manufacturing of adhesive, varnish and dye and in the preparation of some alloys in dentistry [11].

At the beginning, while people are exposed to acrylamide by dermal or by inhalation exposure depending on its usage in industry, it was showed that this substance might be ingested by people after forming of acrylamide in carbohydrate-rich foods during frying, baking, burning or toasting at high temperatures determined by Stadler *et al*. [4]. As a result of these findings, to clarify the effects that may result due to acrylamide taking and to determine the probable risks that this substance may create became more of an issue.

Experimental studies carried out with rodents showed that acrylamide may have a carcinogenic effect on both female and male mice and rats. Different neoplastic structures were observed in animals such as follicular thyroid tumors, scrotal mesothelioma, breast gland tumors, lung carcinoma, glial brain tumors, oral cavity papilloma and uterus adenocarsinoma [1, 12]. As a result of epidemiological studies, the findings showing the carcinogenic effect of acrylamide in human occupationally exposed to acrylamide are insufficient. However, finding some tumors in animals which are treated with high dose of acrylamide in drinking water for long time increases the suspicions about this matter that it might have carcinogenic effect. The genotoxic effect of acrylamide

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Table 1: Acrylamide Levels in Some Processed Foods: (Rosen and Hellenas, 2002; Ono *et al.*, 2003; Mendel, 2003)

Foods	Level of Acrylamide (μg/kg=ppb)
Roasted almond	260
Roasted asparagus	143
Bread, cake and cookies	70-140
Beer and malt	30-70
Biscuit and cracker	30-3200
Breakfast cereals	30-134 6
Chocolate powder	15-90
Coffee powder	170-351
Corn chips and cornflakes	34-416
Toast	800-1200
Fish products	30-64
Gingersnap	90-1660
Red meat and fowl products	30-64
Hazelnut and hazelnutoil	64-457
Husky peanut	140
Boiled potatoes	48
Potatoe chips and flakes	170-3700
French fryings	200-12000
Deep frying potatoes	1270
Other types of potatoe appetizer	30-1915
Roasted soybean	25
Roasted sunflower seeds	66

in vitro cell cultures and its resemblance to chemicals such as vinyl carbamate and acrylonitrile being known as having carcinogenic properties caused acrylamide to be classified as "probably human carcinogen" (Group 2A) by IARC in 1994 [13].

In this study, some epidemiological and experimental studies carried out with acrylamide which is thought to have probable neoplastic effect are examined and it is aimed to inform public about the harmful effects that this matter may cause.

Acrylamide in Foods: While acrylamide and its polymerization product, polyacrylamide, have been used in many areas since 1950, determination of some Swedish researchers that acrylamide is formed by itself in fried and oven-dried foods, especially in potato chips and French fryings at a level of 30-2300 μg/kg [4] showed that acrylamide is transferred to human by nutritions and increased the interest of researchers for acrylamide. It was determined that acrylamide is present in high amounts in cereals and bread, husky foods such as almond, hazelnut or walnut and the oils obtained from them, baby foods, chocolate products, coffee and various dried foods (Table 1) [14-20]. Since acrylamide is not found in unfried or unboiled foods, this suggests that acrylamide may occur during heating of these foods at high temperatures. In the performed studies, observation of acrylamide formation in starchy foods as a result of heating at higher than 120°C revealed the result that temperatures higher than 100°C is enough for acrylamide formation. The researchers suggested that acrylamide is formed in higher amounts in foods in a state of extending the cooking periods of them [3, 4, 21-23]. The studies performed in order to understand how acrylamide is formed in fried or roasted foods showed that it occurs as a result of Maillard Reaction between carbonyl group of reduced sugars (glucose, fructose) during treatment with heat and amino group of amino acids (especially asparagine amino acid) [3, 4]. It was reported that acrylamide occurs at medium level (5-50 μg/kg) as a result of cooking foods rich in protein and at higher levels (150-4000 μg/kg) as a result of cooking foods rich in carbohydrate such as potatoes and cereals [16].

Emerging of acrylamide formation at high levels during cooking of vegetal foods such as potatoes and cereals was explained by the presence of asparagine amino acids in high amounts in these plants [14]. Because, it was showed in the studies that there is a structural relationship between asparagine amino acid and acrylamide and it was determined that three carbon atoms and one nitrogen atom of acrylamide come from asparagine [24]. Asparagine constitutes 14% of total free amino acids in wheat flour and 18% of total free amino acids in rye. 40% of total amino acid content of potatoes used in production of potato chips is asparagine [25].

The Factors Affecting the Formation of Acrylamide in Foods: The alteration in color of potatoes occurred during preparation of potato chips is an important parameter for the control of acrylamide content and this observed color change is an indication of Maillard Reaction which occurs depending on reduced sugar content, frying temperature and frying period [26, 27]. Some researchers reported that the acrylamide concentration that might occur in potatoe chips decreases approximately at a rate of 60% when sugar content of potatoes is decreased by boiling or blanching [28, 26]. On the other hand, pre-heating process made by microwave ovens creates a suitable medium for acrylamide formation [29].

At a definite temperature, cooking foods for long periods causes acrylamide formation in high amounts [4].

High temperature and long cooking periods increase the acrylamide level in potatoes that have lower surface rate than volume rate [30]. On the other hand the acrylamide content of coffees decreases with the extension of roasting period. Because, acrylamide starts to destroy as the heating period extends. Therefore, roasted dark coffees include less acrylamide than moderately roasted light color coffees. In addition to heating period and temperature, many factors such as pH level, water content and formerly boiling of foods also affect the acrylamide formation [31]. The addition of asparaginase enzyme which destroys free asparagine before cooking of foods also decreases the formation of acrylamide [24].

The acrylamide content in foods at the same time shows variations according to sorts of cereals, their processes and storages. Moreover, acrylamide formation is also different in potatoes which have different sugar contents [32]. The sugar content of potatoes is also affected with storage conditions as temperature and period. For example, storage above 8°C causes higher rate of sugar formation than the foods stored at 4°C. Free asparagine present in wheats which vary according to sorts of wheat and processing conditions is an important factor for acrylamide formation [31].

Acrylamide in Cigarette Smoke: The human beings not only take acrylamide with foods but also during smoking cigarettes. One of the components of cigarette smoke is acrylamide and acrylamide content in a normal cigarette is calculated as 1.1-2.34 μg per a cigarette [33]. The average acrylamide level in the urine of human are exposed to acrylamide by smoking is found four times higher than unsmoking people [34].

Harmful Effects of Acrylamide

Its Toxic Effects on Nervous and Reproductive Systems: The results of experimental studies and epidemiological findings show that acrylamide has toxic effect on nervous and reproductive systems [13, 35].

In the studies performed with cats, rats, mice, guinea pig and monkeys, it was determined that the disturbances such as weakness in skeleton muscles occurred with grip difficulties seen legs, anomaly in contraction of muscles, foot splay revealed as a result of neurotoxicity [36]. Moreover, it was shown in a study carried out with rats that the acrylamide caused impaired mobility, decreased grip strength, axonal degeneration in spinal cord and peripheral nerves [37].

It was concluded in epidemiological studies carried out with workers in acrylamide or polyacrylamide production factories and tunnel workers that the acrylamide has toxic effect also on the nervous system of human [35]. Neurological anomalies such as shaking of hands, pain and anomaly in touch feeling were determined in employees exposed to acrylamide via dermal or inhalation routes. Moreover, it was noticed that there were some complaints such as lack of appetite, sleeplessness, numbness and pins and needles in hands and feet, weakness in muscles, difficulty in gripping, lose of reflex in knees and feet, carelessness during walking, stumbling or falling down [38-42]. Peeling, redness and extreme sweating in hands were observed in the employees working on liquid acrylamide [38, 40].

Reproductive disturbances were observed in experimental animals applied high dose level of acrylamide and 2-5 mg/kg b.w./day was determined as a NOAEL (No Observed Adverse Effect Level) for reproductive toxicity [43-45].

It was proposed that the acrylamide caused a decrease in reproduction and birth rates, increase in abnormal number of sperms, decrease in number of sperms, disruption in epitel tissue of seminiferous tubes [46-48]. There was no evidence for acrylamide showing that it has a toxic effect on the reproduction of human [13].

There are also studies present showing that there was a relationship between nervous system toxicity and reproduction anomaly that acrylamide caused [47,49-51]. One of the theories suggested that acrylamide affected mating of animals and this was because of weakness in the back feet of the animals. Another theory was the effect of acrylamide on the functioning of motor protein of kinesin. This enzyme is present in nervous system and in other tissues as well as in the whip of the sperm. The degeneration in this protein decreased the fertilization by affecting the movement of the sperm [51, 44].

Finally, acrylamide decreases the testosterone and prolactin levels in blood serum [52] and affects negatively the development and movement of sperm causing testicular atrophia [53, 46].

Carcinogenic and Genotoxic Effects of Acrylamide: So many studies were carried out with rats and mice in order to search whether the acrylamide has cancer causing property or not. In a study performed with Fischer rats, it was determined that acrylamide caused testicular and thyroid tumors in male and development of breast fibroadenoma and thyroid tumors in female [12]. It was also reported that acrylamide increased DNA synthesis in target tissues such as thyroid, testicular mesothelium and adrenal medulla [54]. It was observed in experiments performed as *in vivo* and *in vitro* that acrylamide and glycidamide, the main metabolite of acrylamide, had genotoxic effect at high levels. In cell culture studies, it was seen that acrylamide and glycidamide caused breaks in chromosome and point mutations in chromosomes [5] and lead to chromosome aberation and mitotic disruption causing aneuploid in some test systems [6]. In a study carried out in order to understand whether the acrylamide causes aneuploid in mouse spermatocytes and rat bone marrow or not, it was shown that 120 mg/kg b.w. of acrylamide caused delay in cell cycle of both reproductive and somatic cells [7]. Moreover, it was determined that *i.p.* application of the same applied dose caused an increase the chromosome aberration in mouse bone marrow and a decrease in mitotic index [55, 56]. In many micronucleus studies performed both peripheral blood and bone marrow, the genotoxic effect of acrylamide was shown clearly. In the micronucleus studies carried out with mice and rats, it was concluded that acrylamide increased the micronucleus formation in mice [8-10, 57-64] and on the other hand, it didn't affect the micronucleus formation in rats [61,65,66]. In another study, it was shown that increasing the dose of applied acrylamide and changing the application route of acrylamide also increased the micronucleus formation in rat bone marrow [67].

Epidemiological Findings Obtained from Human: Determination of acrylamide presence in foods not long ago caused an increase in studies related with revealing the potential relationship between acrylamide intake with foods and cancer formation [35]. Most of the epidemiological studies related with whether the acrylamide in foods increases the risk of cancer were performed by Mucci *et al*. Whether there was a relationship between cancers seen in intestine, bladder, kidney and breast and the acrylamide intake with foods was searched in these studies [68-71]. In another study, on the other hand, the relationship between acrylamide intake depending on diet and oral cavity and pharynx, esophagus, intestine, rectum, larynx, breast, ovary and prostate cancers was evaluated [72]. As a result of data obtained from these epidemiological studies, a significant increase in terms of statistics was not determined between excess consumption of foods including high (300–1200 μg/kg) or medium level (30–299 μg/kg) acrylamide and catching risk for various types of cancers [35].

Hemoglobin Adducts as a Biological Indication of Acrylamide Intake: Both acrylamide and its metabolite, glycidamide, caused adducts showing that the acrylamide is metabolized in the body by reacting with hemoglobin and DNA. The hemoglobin adducts originated from acrylamide and glycidamide are formed as a result of a reaction between α-NH_2 group of valine amino acid present in N terminal part of hemoglobin and it is possible to determine these structures in the blood of human and animals exposed to acrylamide [16, 73, 74]. The acrylamide taken into the body can be metabolized by cytochrome P450 enzyme and an important part of it, approximately 50%, is converted into glycidamide [74-77]. These epoxides which are known as strong mutagen and carcinogen are considerably reactive and cause DNA adducts formations by interacting with DNA. These occurred DNA adducts, on the other hand, are mostly effective in cancer formation [78, 79]. Because, the formation of DNA adducts from carcinogenic chemicals or their reactive metabolites is considered as beginning step of carcinogenesis [80, 81, 2]. The DNA adducts from glycidamide are responsible for carcinogenicity, reproductive toxicity and mutagenicity originating from acrylamide [80, 82].

Epidemiological Studies Related with Hemoglobin Adducts: Determination of hemoglobin adducts from acrylamide in the blood of workers exposed to acrylamide by dermal or inhalation routes gives hints about intake mechanisms of acrylamide into the body [83, 42]. In a study performed in China, it was determined that there were hemoglobin adducts in the blood of 41 employees worked in acrylamide or polyacrylamide production for 0.1-8 years whereas hemoglobin adducts were not encountered in the blood of employees worked in the same region and not to expose to acrylamide. It was shown that the main route of intake of acrylamide into the body for these employees was dermal rather than inhalation [83].

Fuhr *et al.* [84] evaluated toxicokinetics of acrylamide in a study with 6 healthy volunteer young people after they ate red meat which included 0.94 mg acrylamide. 60.3% of dose of acrylamide taken was determined in urine sample after 72 hours from eating the meat and glycidamide was not encountered in the same sample. However, N-acetyl-S-(2-carbomoletil) cysteine and N-acetyl-S-(2-hydroxy-2-carbomolethyl) cysteine which are basic metabolites of acrylamide were determined in the rates of 50% and 5.9%, respectively. These results show that most of the acrylamide taken with foods was absorbed by the human. In the study conducted by Boettcher *et al.* [85], it was concluded that the acrylamide present in foods except the one in cigarettes is the main source of acrylamide taken by human.

DISCUSSION

In this study performed as a summary of literature, it was tried to point out the public opinion about possible harmful effects of acrylamide that it will cause in human. The evidences for adverse effects of acrylamide taken in depending on a diet are still inadequate and it's necessary to research more possible negative effects of acrylamide on the health of human beings. There is not so much thing known about the metabolism of this substance in the body, its storage way, its disposal and how to protect from its metabolites. The neurotoxic and carcinogenic effects of acrylamide and its metabolites are known partially and what sort of results they lead when they are taken in low dosages for long periods can be understood more with the studies. There are more than 200 studies which have been already carried out about genotoxicity, carcinogenicity, residues in the body, chemistry and biochemistry of acrylamide and its metabolites [86]. Achievement of these studies may provide a possibility for obtaining basic information about taking decisions related with the problems that might occur by acrylamide intake within diet.

It is necessary to decrease acrylamide formation in foods in order to minimize the negative effects that acrylamide taken with foods may cause. This can hardly be realized by decreasing either carbohydrate or asparagine content of foods. Moreover, since acrylamide is formed during cooking of carbohydrate-rich foods at high temperatures, adjusting the heating period and heating temperatures of foods for decreasing the acrylamide formation may minimize the damages of food-borne acrylamide.

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